European Journal of Cancer

Editor-in-Chief: Alexander M.M. Eggermont
Institut Gustave Roussy
Villejuif, France

Editors:
- Preclinical Cancer Research: Ulrich Kerbel, Berlin, Germany
- Epidemiology and Prevention: Jan Willem Coebergh, Rotterdam, The Netherlands
- Drug Development: Jordi Rodon, Barcelona, Spain
- Tumour Immunotherapy: Aurélien Marabelle, Villejuif, France
- Breast Cancer: Giuseppe Curigliano, Milan, Italy
- Gastrointestinal Cancers: Volker Heinemann, Munich, Germany
- Genitourinary Cancers: Karim Fizazi, Villejuif, France
- Head and Neck Cancer: J.P. Machiels, Brussels, Belgium
- Hemato-Oncology: Roch Houot, Rennes, France
- Lung Cancer: Mary O'Brien, London, UK
- Gynaecological Cancers: Ignacio Vergote, Leuven, Belgium
- Rare Tumours: Stefan Sleijfer, Rotterdam, The Netherlands
- Melanoma: Dirk Schadendorf, Essen, Germany
- Neuro-Oncology: Martin van den Bent, Rotterdam, The Netherlands
- Paediatric Oncology: Rob Piepers, Utrecht, The Netherlands

Founding Editor: Henri Tagnon

Past Editors: Michael Peckham, London, UK; Hans-Jürg Senn, St Gallen, Switzerland; John Smyth, Edinburgh, UK

Editorial Office: Elsevier, The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK
Tel: +44 (0) 1865 845590, Email: ejcancer@elsevier.com

EDITORIAL BOARD

CLINICAL ONCOLOGY

<table>
<thead>
<tr>
<th>Country</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK</td>
<td>R. Baird (UK)</td>
</tr>
<tr>
<td>Denmark</td>
<td>N. Brüiner (Denmark)</td>
</tr>
<tr>
<td>UK</td>
<td>R. Califano (UK)</td>
</tr>
<tr>
<td>Spain</td>
<td>E. Calvo (Spain)</td>
</tr>
<tr>
<td>Portugal</td>
<td>F. Cardoso (Portugal)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>E. de Vries (The Netherlands)</td>
</tr>
<tr>
<td>USA</td>
<td>A. Dieker (USA)</td>
</tr>
<tr>
<td>Switzerland</td>
<td>R. Dimmer (Switzerland)</td>
</tr>
<tr>
<td>UK</td>
<td>S. Erridge (UK)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>M. Geelen (The Netherlands)</td>
</tr>
<tr>
<td>Belgium</td>
<td>B. Gevaert (Belgium)</td>
</tr>
<tr>
<td>Belgium</td>
<td>B. Hassan (Belgium)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Country</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Switzerland</td>
<td>J.C. Horiot (Switzerland)</td>
</tr>
<tr>
<td>Germany</td>
<td>D. Jäger (Germany)</td>
</tr>
<tr>
<td>Brazil</td>
<td>A. Katz (Brazil)</td>
</tr>
<tr>
<td>France</td>
<td>C. Le Tourneau (France)</td>
</tr>
<tr>
<td>France</td>
<td>Y. Loriot (France)</td>
</tr>
<tr>
<td>Taiwan</td>
<td>C.C. Lin (Taiwan)</td>
</tr>
<tr>
<td>UK</td>
<td>P. Loriot (UK)</td>
</tr>
<tr>
<td>France</td>
<td>C. Massard (France)</td>
</tr>
<tr>
<td>Australia</td>
<td>K. McDonald (Australia)</td>
</tr>
<tr>
<td>Belgium</td>
<td>P. Memel (Belgium)</td>
</tr>
<tr>
<td>Canada</td>
<td>A. Miller (Canada)</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>T. Mok (Hong Kong)</td>
</tr>
<tr>
<td>Korea</td>
<td>D. Nam (Korea)</td>
</tr>
<tr>
<td>Canada</td>
<td>J. Perry (Canada)</td>
</tr>
<tr>
<td>Canada</td>
<td>J. Ringash (Canada)</td>
</tr>
<tr>
<td>Germany</td>
<td>A. Rody (Germany)</td>
</tr>
<tr>
<td>Austria</td>
<td>M. Schmidinger (Austria)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>S. Sleijfer (The Netherlands)</td>
</tr>
<tr>
<td>Italy</td>
<td>S. Stacchiotti (Italy)</td>
</tr>
<tr>
<td>Italy</td>
<td>C. Sternberg (Italy)</td>
</tr>
<tr>
<td>Belgium</td>
<td>A. van Akkooi (The Netherlands)</td>
</tr>
<tr>
<td>Belgium</td>
<td>E. Van Cutsem (Belgium)</td>
</tr>
<tr>
<td>UK</td>
<td>G. Velikova (UK)</td>
</tr>
<tr>
<td>Canada</td>
<td>E. Waskowiak (Canada)</td>
</tr>
<tr>
<td>UK</td>
<td>T. Yap (UK)</td>
</tr>
</tbody>
</table>

BASIC SCIENCE, PRECLINICAL AND TRANSLATIONAL RESEARCH

<table>
<thead>
<tr>
<th>Country</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy</td>
<td>P. Allavena (Italy)</td>
</tr>
<tr>
<td>USA</td>
<td>J.M. Irish (USA)</td>
</tr>
<tr>
<td>UK</td>
<td>J. Anderson (UK)</td>
</tr>
<tr>
<td>Italy</td>
<td>M. Broggi (Italy)</td>
</tr>
<tr>
<td>Switzerland</td>
<td>C. Catapano (Switzerland)</td>
</tr>
<tr>
<td>France</td>
<td>C. Caux (France)</td>
</tr>
<tr>
<td>Spain</td>
<td>M. Esteller (Spain)</td>
</tr>
<tr>
<td>Italy</td>
<td>E. Garattini (Italy)</td>
</tr>
<tr>
<td>Italy</td>
<td>R. Giovannetti (Italy)</td>
</tr>
<tr>
<td>USA</td>
<td>J.M. Luria (USA)</td>
</tr>
<tr>
<td>USA</td>
<td>A.M. Meier (USA)</td>
</tr>
<tr>
<td>France</td>
<td>D. Olive (France)</td>
</tr>
<tr>
<td>Greece</td>
<td>A.G. Papavassiliou (Greece)</td>
</tr>
<tr>
<td>Israel</td>
<td>V. Roter (Israel)</td>
</tr>
<tr>
<td>UK</td>
<td>V. Sanz-Moreno (UK)</td>
</tr>
<tr>
<td>Canada</td>
<td>S. Singh (Canada)</td>
</tr>
<tr>
<td>Canada</td>
<td>J. Stagg (Canada)</td>
</tr>
<tr>
<td>UK</td>
<td>A. Virgós (UK)</td>
</tr>
<tr>
<td>USA</td>
<td>W. Weidert (USA)</td>
</tr>
<tr>
<td>UK</td>
<td>T. Yap (UK)</td>
</tr>
<tr>
<td>Italy</td>
<td>N. Zaffaroni (Italy)</td>
</tr>
</tbody>
</table>

EPIDEMIOLOGY AND PREVENTION

<table>
<thead>
<tr>
<th>Country</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>B. Armstrong (Australia)</td>
</tr>
<tr>
<td>France</td>
<td>D. Forman (France)</td>
</tr>
<tr>
<td>Australia</td>
<td>P. Antier (France)</td>
</tr>
<tr>
<td>France</td>
<td>A. Green (Australia)</td>
</tr>
<tr>
<td>UK</td>
<td>V. Bataille (UK)</td>
</tr>
<tr>
<td>Germany</td>
<td>K. Hemminki (Germany)</td>
</tr>
<tr>
<td>Denmark</td>
<td>J.M. Briers (Denmark)</td>
</tr>
<tr>
<td>Italy</td>
<td>C. Bosetti (Italy)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>I. Lansdorp-Vogelaar (The Netherlands)</td>
</tr>
<tr>
<td>Denmark</td>
<td>L.A. Kemeny (The Netherlands)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>H. Brenner (Germany)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>E. Lyng (Denmark)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>M. Maynadier (France)</td>
</tr>
<tr>
<td>France</td>
<td>S. Franceschi (France)</td>
</tr>
<tr>
<td>Argentina</td>
<td>G. Chantada (Argentina)</td>
</tr>
<tr>
<td>France</td>
<td>P. Peeters (The Netherlands)</td>
</tr>
<tr>
<td>Spain</td>
<td>S. Sanjose (Spain)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>M.K. Schmoll (The Netherlands)</td>
</tr>
<tr>
<td>France</td>
<td>I. Stojanovska-Ivanovska (France)</td>
</tr>
<tr>
<td>Denmark</td>
<td>H. Storm (Denmark)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>L.V. van den Poll-Franse (The Netherlands)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>H.M. Verkooijen (The Netherlands)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>E. de Vries (The Netherlands)</td>
</tr>
<tr>
<td>Italy</td>
<td>R. Zacetti (Italy)</td>
</tr>
</tbody>
</table>

PAEDIATRIC ONCOLOGY

<table>
<thead>
<tr>
<th>Country</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>C. Bergeron (France)</td>
</tr>
<tr>
<td>Argentina</td>
<td>G. Chantada (Argentina)</td>
</tr>
<tr>
<td>France</td>
<td>A. Biondi (Italy)</td>
</tr>
<tr>
<td>France</td>
<td>F. Doz (France)</td>
</tr>
<tr>
<td>Canada</td>
<td>B. Boutef (Canada)</td>
</tr>
<tr>
<td>Italy</td>
<td>A. Ferranti (Italy)</td>
</tr>
<tr>
<td>USA</td>
<td>M. Cairo (USA)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>M.A. Groothuis (The Netherlands)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>H. Caron (The Netherlands)</td>
</tr>
<tr>
<td>USA</td>
<td>K. Pritchard-Jones (UK)</td>
</tr>
<tr>
<td>Canada</td>
<td>L. Sung (Canada)</td>
</tr>
<tr>
<td>Italy</td>
<td>M. van den Heuvel-Eibrink (The Netherlands)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>M. van Noesel (The Netherlands)</td>
</tr>
</tbody>
</table>
Peer Review Policy for the *European Journal of Cancer (EJC)*

The practice of peer review is to ensure that only good science is published. It is an objective process at the heart of good scholarly publishing and is carried out by all reputable scientific journals. Our reviewers therefore play a vital role in maintaining the high standards of the *European Journal of Cancer (EJC)* and all manuscripts are peer reviewed following the procedure outlined below.

**Initial manuscript evaluation**

The Editors first evaluate all manuscripts. In some circum- stances it is entirely feasible for an exceptional manuscript to be accepted at this stage. Those rejected at this stage are insufficiently original, have serious scientific flaws, have poor grammar or English language, or are outside the aims and scope of the journal. Those that meet the minimum criteria are passed on to experts for review.

Authors of manuscripts rejected at this stage will be informed within 2 weeks of receipt.

**Type of Peer Review**

The EJC employs single blind review, where the reviewer remains anonymous to the authors throughout the process.

**How the reviewer is selected**

Reviewers are matched to the paper according to their expertise. Our reviewer database contains reviewer contact details together with their subject areas of interest, and this is constantly being updated.

**Reviewer reports**

Reviewers are asked to evaluate whether the manuscript:

- Is original
- Is methodologically sound
- Follows appropriate ethical guidelines
- Has results which are clearly presented and support the conclusions
- Correctly references previous relevant work

Reviewers are not expected to correct or copyedit manuscripts. Language correction is not part of the peer review process. Reviewers are requested to refrain from giving their personal opinion in the “Reviewer blind comments to Author” section of their review on whether or not the paper should be published. Personal opinions can be expressed in the “Reviewer confidential comments to Editor” section.

**How long does the peer review process take?**

Typically the manuscript will be reviewed within 2-8 weeks. Should the reviewers’ reports contradict one another or a report is unnecessarily delayed a further expert opinion will be sought. Revised manuscripts are usually returned to the Editors within 3 weeks and the Editors may request further advice from the reviewers at this time. The Editors may request more than one revision of a manuscript.

**Final report**

A final decision to accept or reject the manuscript will be sent to the author along with any recommendations made by the reviewers, and may include verbatim comments by the reviewers.

**Editor’s Decision is final**

Reviewers advise the Editors, who are responsible for the final decision to accept or reject the article.

**Special Issues / Conference Proceedings**

Special issues and/or conference proceedings may have different peer review procedures involving, for example, Guest Editors, conference organisers or scientific committees. Authors contributing to these projects may receive full details of the peer review process on request from the editorial office.

**Becoming a Reviewer for the EJC**

If you are not currently a reviewer for the EJC but would like to be considered as a reviewer for this Journal, please contact the editorial office by e-mail at ejcancer@elsevier.com, and provide your contact details. If your request is approved and you are added to the online reviewer database you will receive a confirmatory email, asking you to add details on your field of expertise, in the format of subject classifications.
Contents

Late Breaking Abstracts

Friday, 16 November 2018
Plenary Session 10
New Drugs in Clinical Trials: The Therapeutics of the Future e3

Oral Abstracts

Tuesday, 13 November 2018
Plenary Session 1
Should we be conducting more Genomics Driven, Multi-Arm Clinical Trials? e15

Wednesday, 14 November 2018
Plenary Session 2
Proffered papers e15

Thursday, 15 November 2018
Plenary Session 6
Proffered Papers e17

Poster Abstracts

Wednesday, 14 November 2018
Posters in the Spotlight Session
Poster in the Spotlight I e23

Thursday, 15 November 2018
Posters in the Spotlight session
Poster in the Spotlight II e24

Tuesday, 13 November 2018
Poster Session
DNA Repair Modulation e25
Molecular Targeted Agents – PART I e35
Oncolytic viruses e55
Vaccination e56

Wednesday, 14 November 2018
Poster Session
Adoptive Cell Transfer therapy e56
Clinical Methodology e57
Combinatorial Chemistry e60
Cytotoxics e60
Drug Screening e66
Drug Synthesis e72
Immunecheckpoints e72
New Therapies with Pleiotropic Activity e82
Thursday, 15 November 2018
Poster Session
   Animal Models e83
   Chemoprevention e87
   Drug Delivery e89
   Drug Design e92
   Other e95
   Preclinical Models e97
   Radiation Interactive Agents e112
   Regulatory Affairs e112

Friday, 16 November 2018
Poster Session
   Drug Resistance and Modifiers e112
   Epigenetic modulators e118
   Molecular Targeted Agents – PART II e121
   Paediatric Oncology e145

Author Index e149
Late Breaking Abstracts
Background: The majority of human malignant tumors are resistant or only partially respond to chemotherapy (C/T) or immunotherapy (I/T) such as immune checkpoint inhibitors (ICIs). Irrespective of the treatments, efficient tumor-cell killing requires amplification of inflammatory signaling, which however is tightly regulated by various "checkpoint" mechanisms evolved by epithelial cells to prevent excessive tissue damage induced by virus and immune attack. Novel approaches to disable theses conserved and cell-intrinsic inflammation checkpoints may provide breakthrough and "tumor-agnostic" strategies to circumvent the innate treatment-resistance to unleash the full potential of C/T and I/T in treatment-refractory malignant tumors, such as triple-negative breast cancer (TNBC) and glioblastoma multiform (GBM).

Material and Methods: We designed and conducted integrated genomic and proteomic cancer profiling combined with molecular and functional studies to identify conserved anti-inflammatory pathways that mediate innate and cell-intrinsic resistance to C/T and I/T agents. Preclinical studies were used to validate a gene therapy strategy to disable the inflammation-checkpoint identified from this process.

Results: We uncovered that the cytotoxic and immunogenic death induced by C/T and I/T agents is constrained by repression of a toll-like receptor-2 (TLR-2)/TLR-3 and NF-kB-induced interferon regulatory factor-1 (IRF-1) and interferon (IFN)-gamma anti-viral response program in various malignant tumor cells. Loss and gain of function studies implicated that co-repressor-2 (N-CoR2) co-translocated with NF-kB p50 into cell nuclei in response to therapy, wherein it serves as an epigenetic checkpoint of this inflammation program by mediating a histone deacetylase-dependent chromatin remodeling and repression of a specific panel of pro-inflammatory and pro-apoptotic genes. Thus, high N-CoR2 expression predicts treatment refractoriness and poor prognosis in neoadjuvant or adjuvant treated breast cancer patients. Blockade of the epigenetic checkpoint function of N-CoR2 by a small decoy of N-CoR2 hyper-sensitized malignant cells to assorted C/T agents, death ligands, and IFN-gamma. Consistently, intratumoral delivery of the N-CoR2 co-repressor blockade dramatically potentiated systemic C/T and ICI therapies, including anti-PD-1 and anti-CTLA-4 antibodies, and IFN-gamma. Consistently, intratumoral delivery of the N-CoR2 checkpoint blockade dramatically potentiated systemic C/T and ICI therapies, including anti-PD-1 and anti-CTLA-4 antibodies, and IFN-gamma. Consistently, intratumoral delivery of the N-CoR2 checkpoint blockade dramatically potentiated systemic C/T and ICI therapies, including anti-PD-1 and anti-CTLA-4 antibodies, and IFN-gamma.

Conclusion: Our findings suggest that malignant tumors can access intrinsically conserved anti-inflammatory mechanisms that enable them to escape from C/T and I/T. As such, strategies that can override this defense program may constitute novel anti-tumor gene therapies that may be applied to overcome resistance in treatment-refractory tumors and improve patient prognosis.

Conflict of interest: Ownership: K. Tsai is a co-founder of Stempodia Corporation, Ltd. and has ownership interest in the company. Corporate-sponsored Research: The development of the N-CoR2 checkpoint blockade described in this study was sponsored by Stempodia Corporation, Ltd.
High-grade serious ovarian cancer (HGSOC), characterized by defects in DNA damage repair and high levels of genomic instability and DNA replication stress, is the most lethal gynecological malignancy in the United States. The current treatment options for HGSOC are limited and new approaches are needed. Checkpoint kinase 1 (Chk1) is a key kinase that regulates the cell cycle, DNA damage and the replication stress response through the modulation of cell cycle checkpoints and replication origin firing, and has emerged as an attractive target for anti-cancer therapy. Prexasertib (LY2606368), an ATP-competitive inhibitor of Chk1, has demonstrated single-agent activity in vitro and in vivo across a variety of tumor histologies. Prexasertib is being evaluated in a Phase 2 trial sponsored by NCI (NCT02203513). Preliminary results showed activity in patients with wild-type BRCA HGSOC (29% partial response in the intention-to-treat population). To understand the mechanism underlying prexasertib’s activity in HGSOC and to identify potential biomarkers that may predict response or future treatment regimens, we investigated the anti-tumor activity of prexasertib in ovarian cancer cell lines and tumors. Prexasertib cell proliferation in 22 ovarian cancer cell lines (including 11 HGSOC with IC50 values ranging from 0.6 nM to 46 nM, and induced DNA damage (as measured by pChk1 (S345), RPA32 (S4/S8) and hG2AX) in multiple HGSOC cell lines. Prexasertib treatment induced DNA damage and cell death (as measured by gH2AX, activated Caspase 3/7, TUNEL and decreased cell counts) in HGSOC cells in vitro, and resulted in tumor growth inhibition of 108.6% (i.e. 8.6% regression) and 157% (i.e. 57% regression) in platinum-resistant HGSOC OV-90 and Cov504 xenograft tumors in vivo, respectively. The anti-tumor effect of prexasertib was also assessed in 39 HGSOC PDX, and resulted in complete tumor regression in 38% (15/39) models, partial tumor regression/stasis in 36% (14/39) models and no benefit in 26% (10/39) models. Potential biomarkers of prexasertib were characterized in ovarian cancer cell lines and PDX tumors. Ovarian cancer cells with high protein expression levels of cyclin E1 and high phosphorylation levels of H2AX and RPA32 were sensitive to prexasertib treatment. siRNA knock down of cyclin E1 decreased the phosphorylation of H2AX and desensitized ovarian cancer cell response to prexasertib. HGSOC PDX tumors with high RNA protein levels of cyclin E1p15, low RNA protein levels of cyclin D1/CDC6 and high phosphorylation of H2AX were sensitive to prexasertib treatment. Taken together, these data support further clinical development of prexasertib in the treatment of HGSOC (including an ongoing Phase 2 study (NCT03414815)), and provide a patient selection hypothesis to identify the subsets of HGSOC that respond to prexasertib monotherapy.

No conflict of interest

Poster Clinical-stage monitoring of adaptive tumor resistance to PD-1 checkpoint blockade

M. Smalley1, B. Shanthappa2, H. Gertij3, M. Lawson2, B. Ulagathanan4, A. Thayakumar5, L. Maciejko1, P. Radhakrishnan6, M. Bawiski7, S. Thiyagarajan8, B. Majumder6, K. Gopinath7, G. Babu8, A. Goldman1.1Eli Lilly and Company, Oncology Research, Indianapolis, USA; 2Mitra Biotech RxDx, Cancer Biology, Woburn, USA; 3Mitra Biotech RxDx, Histopathology, Woburn, USA; 4Mitra Biotech RxDx, Research & Development, Bangalore, India; 5Kidwai Memorial Institute of Oncology, Research & Development, Bangalore, India; 6Kidwai Memorial Institute of Oncology, Department of Medical Oncology, Bangalore, India; 7Kidwai Memorial Institute of Oncology, Department of Medical Oncology, Bangalore, India

Background: Immune checkpoint inhibitors revolutionized cancer immunotherapy, yet clinical success remains highly variable and often patient-specific. Mechanisms of resistance that contribute to clinical failure of checkpoint inhibitors are still being understood. We previously discovered a mechanism of adaptive cancer therapy resistance using tissue from treatment-refractory patients and a clinically-trained algorithm, which led to a novel combination of kinase inhibitors and conventional drugs that prolonged survival.

Methods: Here, using biopsies from patients prior to therapy and/or on treatment, combined with a clinically trained algorithm, we studied mechanisms of resistance to immune checkpoint inhibitors. First, we demonstrate preservation of lymphocyte lineage differentiation, spatial organization of...
immune cells using multispectral imaging, and tumor-immune biology using flow cytometry and immunohistochemistry, ex-vivo.

**Results:** We determined that CD4+ T-helper lineages, especially Th1 and Th2, were induced under drug pressure, which associated to improved survival. Conversely, a subset of patient samples were determined to resist treatment, which, paradoxically, associated to increased OX40 and nF77 signaling. On-treatment biopsies were studied by RNA transcriptionics, which match to clinical response, buttressed the role of these immune cells in tumor response.

**Conclusions:** While these findings highlight the now-obvious need to profile efficacy of immunotherapy at the individual patient level, they also shed light on recent clinical trial evidence suggesting that sequencing immunotherapies, particular PD-1 inhibitors and OX40 agonists, can provide better therapeutic outcomes.

**Conflict of interest:** Corporate-sponsored Research: Munisha Smalley, Basavaraja U Shanthappa, Hans Gertje, Mark Lawson, Baranedharan Uluganathan, Allen Thayakumar, Laura Maciejko, Padhma Radhakrishnan, Manjusha Biswas, Saravanan Thiyagarajan, Biswanath Majumder and Aaron Goldman are all employed and funded by Mira Biotech RxDx.

**8LBA (PB-209)**

**Poster**

**CPI-818: a selective interleukin-2-inducible T-cell kinase (ITK) inhibitor has clinical activity in dogs with spontaneous T-cell lymphoma**

D. Thamm1, K. Weishaar1, S. Das1, A. Madriaga2, C. Hill2, F. Flicker2, B. Wolfe3, P. Ng4, A. Hotsen5, R. Miller5, J. Buggy6, J. Janc6, 1Colorado State University, Department of Clinical Sciences, Fort Collins, USA, 2Corvus Pharmaceuticals, None, Burlingame, CA, USA

**Background:** ITK is a non receptor tyrosine kinase that plays a key role in T-cell receptor (TCR) signaling. In malignant T cells, TCR signaling is maintained, and inhibition of ITK may provide therapeutic benefit. Non-Hodgkin lymphoma (NHL), the most common hematopoietic tumor of dogs, is an aggressive and highly metastatic disease. Approximately 30% of lymphomas are of the T cell immunophenotype, and most correlate closely with human intermediate-to high-grade NHL with peripheral T cell lymphoma being the most common histotype. CPI-818 is an irreversible small molecule inhibitor of ITK that is shown to have a high degree of selectivity for ITK over RLK or Bruton’s tyrosine kinase (BTK), allowing for assessment of the impact of selective ITK inhibition on malignant T cells. Given that ITK activation may require coordinated signaling through the TCR, conventional murine xenograft models may not accurately recapitulate the microenvironment necessary for modeling the effect of ITK inhibition on lymphoma progression. For this reason, clinical, pharmacokinetic and pharmacodynamic evaluation of CPI-818 was performed.

**Materials and Methods:** To assess the potential of CPI-818 to treat T cell lymphoproliferative disorders, the safety and efficacy of CPI-818 in client-owned dogs with spontaneously-occurring T cell lymphoma was evaluated. This trial was performed with Institutional Animal Care and Use Committees approval and signed owner consent. CPI-818 was given orally at a dose of 20 mg/kg BID for from 2 weeks to 5 months. Owner history, physical examination and clinicopathologic examination was performed weekly for the first 4 weeks, then every other week thereafter. Irreversible inhibition of ITK in peripheral blood lymphocytes was measured using a labeled competitive probe to assess ITK occupancy. Tumor biopsies were obtained prior to treatment, 2 weeks following treatment initiation and at the time of relapse, and Affy-based gene expression profiling and pathway analysis was performed.

**Results:** Three animals have been treated to date: 1 with peripheral T cell lymphoma (PTCL) and 2 with cutaneous T cell lymphoma (CTCL). Full ITK occupancy in peripheral blood was confirmed using the probe assay in all 3 dogs. Evidence of anti-tumor activity was observed in all dogs including complete and partial responses. CPI-818 was well tolerated with no change in normal lymphocyte counts. Gene expression pathways significantly upregulated following CP-818 exposure included those associated with cytokine signaling and interferon response.

**Conclusions:** CPI-818 is a novel and selective ITK inhibitor that blocks TCR driven signaling. Evidence of in vivo ITK inhibition and clinical anti-tumor activity with excellent tolerability was shown in client-owned dogs with spontaneous T cell lymphoma.

**Conflict of interest:** Ownership: Author Thamm has common stock ownership in Corvus Pharmaceuticals, Inc. Authors Madriaga, Hill, Flicker, Wolfer, Ng, Hotsen, Miller, Buggy and Janc are employees and/or shareholders of Corvus Pharmaceuticals, Inc. Corporate-sponsored Research: The research presented herein was funded by Corvus Pharmaceuticals, Inc.

**7LBA (PB-210)**

**Poster**

**Cytoplastic FLIP(S) and nuclear FLIP(L) mediate resistance of castrate-resistant prostate cancer to apoptosis induced by IAP antagonists**

C. Mccann1, N. Crawford1, J. Majkul1, C. Holohan1, C. Armstrong1, P. Maxweall1, C. W. Ong1, M. Labonte-Wilson1, S. Mcdaide1, D. J. Waugh1, D. B. Longley1, 1Queens University Belfast, Centre for Cancer Research and Cell Biology, Belfast, United Kingdom

**Background:** Expression of tumor necrosis factor-α (TNFa) in the serum of prostate cancer patients is associated with poorer outcome and progression to castrate-resistant (CRPC) disease. TNFa promotes the activity of NFkB, which regulates a number of anti-apoptotic and pro-inflammatory genes, including those encoding the inhibitor of apoptosis proteins (IAPs); however, in the presence of IAP antagonists, TNFa can induce cell death.

**Materials and Methods:** We used IAP antagonists (TL32711) to prime CRPC cell lines (PC3, DU145 and VCaP) for cell death induced by recombinant or macrophage-derived TNFa, and splice form-specific FLIP siRNA or the class-I selective histone-deacetylase (HDAC) inhibitor Entinostat as a means of overcoming resistance to IAP antagonist therapy. The combination of HDAC inhibitor along with IAP antagonist was well tolerated and effectively retarded tumour growth in a murine xenograft model.

**Results:** In the presence of recombinant or macrophage-derived TNFa, we found that IAP antagonists triggered degradation of cIAP1 and induced formation of Complex-Ib, consisting of caspase-8, FADD and RIPK1 in CRPC models; however, no, or modest levels of apoptosis were induced. This resistance was found to be mediated by both the long (L) and short (S) splice forms of the caspase-8 inhibitor, FLIP, another NFkB-regulated protein frequently overexpressed in CRPC. By decreasing FLIP expression at the post-transcriptional level in PC3 and DU145 cells (but not VCaP), the Class-I histone deacetylase (HDAC) inhibitor Entinostat promoted IAP antagonist-induced cell death in these models in a manner dependent on RIPK1, FADD and Caspase-8. Of note, Entinostat primarily targeted the nuclear rather than cytoplasmic pool of FLIP(L). While the cytoplastic pool of FLIP(L) was highly stable, the nuclear pool was more labile and regulated by the Class-I HDAC target Ku70, which we have previously shown regulates FLIP stability. The efficacy of IAP antagonist (TL32711) and Entinostat combination and their effects on cIAP1 and FLIP respectively were confirmed in-vivo.

**Conclusions:** This study highlights the therapeutic potential for targeting IAPs and FLIP in pro-inflammatory CRPC.

**6LBA (PB-212)**

**Poster**

**Targeting epigenetic crosstalk as a therapeutic strategy for EZH2-aberrant solid tumors**

X. Huang1, J. Yan1, M. Zhang1, Y. Wang1, M. Tan2, J. Ding1, M. Geng1, 1Shanghai Institute of Materia Medica, Division of Antitumor Pharmacology, Shanghai, China, 2Shanghai Institute of Materia Medica, Chemical Proteomics Center, Shanghai, China

**Background:** Mutations or aberrant upregulation of EZH2 occur frequently in human cancers, yet clinical benefits of EZH2 inhibitor (EZH2i) remain unsatisfactory and limited to certain hematological malignancies.

**Material and Methods:** We profile global posttranslational histone modification changes across a large panel of cancer cell lines with various sensitivities to EZH2i.

**Results:** We report here oncogenic transcriptional reprogramming mediated by MIL1’s interaction with the p300/CBP complex, which directs H3K27me loss to reciprocal H3K27ac gain and restricts EZH2i response. Concurrent inhibition of H3K27me and H3K27ac results in transcriptional repression and MAPK pathway dependency in cancer subsets. In preclinical models encompassing a broad spectrum of EZH2-aberrant solid tumors, a combination of EZH2i and BRD4 inhibitors, or a triple-combination including MAPK inhibition display robust efficacy with very tolerable toxicity.

**Conclusions:** Our results suggest an attractive precision treatment strategy for EZH2-aberrant tumors on the basis of tumor-intrinsic MIL1 expression and concurrent inhibition of epigenetic crosstalk and feedback MAPK activation.

**No conflict of interest**
9LBA (PB-211)  Poster
Preclinical studies with models from the National Cancer Institute’s Patient-Derived Models Repository (PDMR)
1Frederick National Laboratory for Cancer Research, NCI Patient Derived Models Repository, Frederick, USA; 2National Cancer Institute, Biological Testing Branch, Developmental Therapeutics Program, Bethesda, USA; 3Frederick National Laboratory for Cancer Research, Biological Testing Branch, Developmental Therapeutics Program, Frederick, USA; 4Frederick National Laboratory for Cancer Research, Molecular Characterization Laboratory, Frederick, USA; 5National Cancer Institute, Division of Cancer Treatment and Diagnosis, Bethesda, USA

Background: The National Cancer Institute (NCI) has developed a Patient-Derived Models Repository (PDMR) comprised of quality-controlled, early-passage, clinically-annotated patient-derived tumor xenografts (PDXs), in vitro tumor cell cultures (PDCs), cancer associated fibrobasts (CAF’s), and patient-derived organoids (PDOs). NCI has focused on generating models to complement existing PDX collections and address unmet needs in the preclinical model space. These models are offered to the extramural community for research use (https://pdm.cancer.gov), along with clinical annotation and molecular information (whole exome sequence, RNASeq), via a publicly accessible database. Currently, over 185 PDX models, 50 PDC models, and 100 CAF models are available for distribution to the US research community. Additional models, including PDOs, will be released as they pass final quality control standards. As part of its rare cancer initiative, the NCI is also targeting the collection of infrequently-observed tumor histologies to advance both biological investigations and drug development efforts for understudied malignancies.

Materials and Methods and Results: Over the past two years, NCI has performed a screening study on over 60 PDX models in NSF mice using 5-7 standard-of-care agents to (1) characterize drug response in the Repository’s PDX models, and (2) determine the capacity for drug screening studies by the PDMR. The standard of care agents selected for this pilot study were paclitaxel, carboplatin, 5-FU, and gemcitabine; two targeted agents, erlotinib and vemurafenib, were included for comparative studies and all were used at human-relevant doses. This effort has generated over 380 model x drug data sets to date with an overall 8% response rate. PDX models on this study were treated with all drugs irrespective of the clinical match to standard-of-care. In addition, the PDMR is beginning an initiative to screen rare cancer PDX models with 40–50 drug combinations to identify previously untested therapeutic agent combinations that can be moved to early phase clinical studies. The PDMR contains over 80 models from rare cancers (as defined by the SWOG DART Study), including mesothelioma, osteosarcoma, and Merkel cell, Hurlte cell, and salivary gland cancers that will be enrolled in this study.

Conclusions: These studies are the first preclinical characterization of PDMR models using standard of care agents. The planned rare tumor PDX screening study will provide details on how model stability through passages will be assessed and small-scale screening to preclinical efficacy study design are integrated. Funded by NCI Contract No. HHSN281200800001E

No conflict of interest

10LBA (PB-213)  Poster
A phase 1 study of the safety, pharmacokinetics and pharmacodynamics of AST-008, a TL9 agonist Spherical Nucleic Acid, in healthy subjects
W. Daniel1, U. Lorch2, S. Coates3, S. Mux1, 1Exicure Inc., Clinical Development, Skokie, USA; 2Richmond Pharmacology, Clinical Development, London, United Kingdom; 3Exicure Inc, Clinical Development, Skokie, USA

Background: Exicure develops spherical nucleic acid (SNA) constructs, which are 3-dimensional arrangements of oligonucleotides where the nucleic acids are densely packed and radially oriented around a nanoparticle. SNAs have properties that are distinct from the ‘linear’ nucleic acids (i.e., nucleic acids not arranged in the SNA format), which include, most importantly, increased cellular uptake compared to linear nucleic acids.

AST-008 is an SNA configuration of a toll-like receptor 9 (TLR9) agonist oligonucleotide, designed to trigger anti-tumour immune responses in patients with cancer. AST-008 is intended to be administered intratumorally in combination with checkpoint inhibitors for the treatment of solid tumours.

AST-008 has potent antitumor activity as a monotherapy and synergizes with standard-of-care PD-1 antibody therapy in several preclinical tumour models. A variety of nonclinical toxicology, safety pharmacology and toxicokinetic studies were performed in Sprague-Dawley rats and cynomolgus monkeys prior to initiating clinical trials.

Materials and Methods: AST-008 has been evaluated in a Phase 1a study (Protocol number: AST-008-101). The safety, tolerability, pharmacokinetics and pharmacodynamics of single ascending doses of AST-008 were studied in healthy volunteers. Four dose levels of AST-008 were evaluated in four cohorts. Each cohort included four volunteers, and all received a single dose of AST-008.

Results: The results indicated that AST-008 was safe and well tolerated after a single subcutaneous injection. No serious adverse events or dose limiting toxicity were reported. The most common adverse events observed were flu-like symptoms, injection site reactions, and reversible, short-lived leukopenia and neutropenia. AST-008 induced an innate immune response after administration to healthy volunteers. Cytokine and chemokine analysis indicated that a Th1-type immune response was elicited. For the four subjects receiving the trial’s top dose of about 20 µg/kg of AST-008, initial analyses suggest that the average fold-increase above baseline for these cytokines is approximately as follows: IFN-gamma: 3 fold; IL-6: 57 fold; IL-12: 2 fold; IP-10: 32 fold; and MCP-1: 6 fold. In addition, at this dose, AST-008 elicited 9.5 fold and 3.5 fold increases in the fraction of activated T cells and natural killer (NK) cells, respectively, compared to baseline. CD69 was the activation marker for the T and NK cells. Pharmacokinetic analysis revealed that AST-008 was not detected in any sample.

Conclusions: AST-008 elicited no serious adverse events or dose limiting toxicity at the doses tested. AST-008 is a potent innate immune activator and exhibits pharmacodynamic properties that are expected to result in anti-tumour effects in patients with cancer. Preparation of a Phase 1b/2 study of AST-008 in combination with a checkpoint inhibitor in cancer patients is ongoing.

Conflict of interest: Ownership: WD and SM are employees and optionholders of Exicure Inc.

11LBA (PB-214)  Poster
BAL101553, a novel microtubule-targeting tumor checkpoint controller, in combination with eribulin leads to increased cures in a TNBC xenograft model
N. Forster-Gross1, F. Bachmann3, P. Mcsheehy3, H.A. Lane1, 1Basilea Pharmaceutica International Ltd., Research - Cancer Biology, Basel, Switzerland

Background: BAL101553 (B535) is a highly soluble pro-drug of the synthetic small molecule BAL27862 (B682) that induces tumor cell death by modulating the spindle assembly checkpoint through microtubule (MT) depolymerization. Oral and IV administered B535 has activity in diverse tumor models resistant to conventional agents, and is currently in phase 1 clinical evaluation in advanced solid tumor patients. Eribulin (Eri) is approved for the treatment of advanced metastatic breast cancer patients, including TNBC.

Materials and Methods: The coefficient of drug interaction (CDI) was evaluated by measuring anti-clonogenic potential in soft agar or induction of apoptosis for Eri combined with B682. A CDI of < 1 = synergistic effects; ≤ 0.7 = statistically significant effect. Effects on MT stability were tested by immunoblotting of soluble vs. cytoskeletal-associated tubulin. MDA-MB-231 TNBC xenografts were treated alone or concomitantly with Eri (0.1–0.5 mg/kg, IV, 3xqw) and B535 (15–25 mg/kg, po, qd). Tumor growth/body weights were monitored. Animals with regressed tumors without tumor regrowth were assessed for residual tumor cells by histological examination of the implantation site. Compound levels in plasma were determined after multiple-dosing by LC-MS/MS in non-tumor bearing mice of the same strain.

Results: Combinations of B535 with 5 tumor lines (inc. MDA-MB-231) in soft agar at discrete IC50 fractions (B682 at 1x, 0.5x, 0.25x; Eri at 0.2x, 0.1x, 0.03x) gave CDIs < 1 in ~75% of the combination scenarios. CDIs ≤ 0.7 were most frequently observed with IC50 concentrations of B682. B682/ Eri concentrations below IC50 in H460- nd Jurkat-GFP cells were highly synergistic in inducing apoptosis (mean CDIs of 0.44 and 0.58, resp.). MT stability assays with MDA-MB-231 cells (B682/Eri at IC50 concentrations) confirmed a strong shift of cellular MTs to the soluble fraction with the combination, indicating distorting effects of enhanced destabilization activity. In MDA-MB-231 xenografts, B535 alone at MTD (25 mg/kg) induced tumor-stasis and Eri alone induced stasis (0.1 mg/kg) or dose-dependent regressions (0.25–0.5 mg/kg) with tumor growth retardation in most animals after dosing cessation. Strikingly, combinations were associated with significant increases in cures in three independent experiments. In a representative experiment, the percentage of cures animals with Eri at 0.1 or 0.5 mg/kg was 0% or 30%.
respectively, whereas combination with sub-MTD B553 increased cure rates to 50% (0.1 mg/kg Eri + 15 mg/kg B553) and 70–80% (0.4 mg/kg Eri + 15 mg/kg B553). Mouse PK analysis showed no change in plasma exposure to either agent, supporting mechanistic synergy between Eri and B553.

**Conclusions:** B862 combined with Eri caused synergistic tumor cell death in vitro, associated with profound effects on microtubule stability. Increased cure rates in a TNBC xenograft model suggest a potential for clinical exploration.

**Conflict of interest:** Ownership: Stock (or option) holder of Basilea Pharmaceutica. Corporate-sponsored Research: Employee of Basilea Pharmaceutica International

12LBA (PB-215) Poster

**TLR2 inhibition for cancer therapy, a novel approach with Tomaralimab a humanised IgG4 antibody**

B. Keogh1, P. Mogurik1, T. Kearney1, A. Alvero2, G. Mo2, S. Gupta2, G. Garcia-Manero2, Y. Wei, B. Jenkins3, R. Miller1, M. Reilly1. Opsona Therapeutics Ltd, Tara Street, Dublin, Ireland; 2Yale University, School of Medicine, Connecticut, USA; 3MD Anderson Cancer Center, Department of Pathology, Texas, USA; 4MD Anderson Cancer Center, Department of Leukemia, Texas, USA; 5Hudson Institute, Monash University, Victoria, Australia

**Background:** TLR ligands and TLRs are a group of innate sensors that are key in the activation of immune responses. TLRs signal via adaptor molecules that result in the activation of NF-kB or AP-1 leading to the secretion of inflammatory cytokines. Inappropriate TLR2 activation is implicated in the development of a range of tumours. Opsona has developed a fully humanised monoclonal antibody Tomaralimab (OPN-305) against TLR2 that has potential as a novel clinical candidate in a range of cancers.

**Materials and Methods:** Apoptosis and proliferation assays were used to assess the effect of Tomaralimab on cancer cell lines. The efficacy of Tomaralimab was tested in genetic, orthotopic and xenograft mouse models with standard of care (SOC) or as a monotherapy. Tomaralimab is being assessed in a Phase I clinical trial in HMA-failure low risk myelodysplasia syndrome (MDS) patients as a monotherapy with potential for HMA add-back therapy in combination.

**Results:** Animal models show that Tomaralimab therapy has potential benefits in pancreatic, gastric, melanoma and ovarian cancer demonstrated by a decrease in tumour volume (TV), an increase in overall survival (OS), or both. In both genetic and xenograft models of gastric cancer, Tomaralimab alone displayed in vivo activity and human ex vivo analysis of CD34+ cells demonstrated a direct apoptotic effect on tumour cells. In an orthotopic mouse model of pancreatic cancer, treatment with SOC drugs in the presence of Tomaralimab resulted in smaller tumours and increased OS compared to single drug treatment groups that correlated with tumour penetration of Tomaralimab and increased CD8+ T cells. The effect of combination therapy with anti-PD1 and Tomaralimab was investigated in a B16 melanoma model where a decrease in TV was observed with combination therapy. Tomaralimab therapy was also assessed in recurrent paclitaxel-resistant ovarian cancer where it significantly delayed the regrowth of tumours via a decrease in intra-tumoural myeloid-derived suppressor cells (MDSCs). Ex vivo analysis of CD34+ cells from MDS patients showed that Tomaralimab may be of therapeutic benefit in haematological tumours as TLR2 inhibition led to a significant increase in differentiation. Tomaralimab is currently in a Phase II study in low-intermediate risk MDS following hypomethylating agent (HMA) failure (NCT02363491). Recruted patients have typically failed at least three prior therapies. Despite this, a clinically meaningful ORR of 50% has been observed.

**Conclusions:** In summary, Tomaralimab/combination benefits in a range of animal models of solid organ tumours as well as in a clinical study of MDS suggests a potential broad applicability of this novel treatment through multiple mechanisms. To date, clinical benefit had been seen with Tomaralimab therapy in heavily pre-treated lower risk MDS patients that have failed HMA therapy.

**Conflict of interest:** Other Substantive Relationships: Opsona Therapeutics Ltd have consultancy and collaborative agreements with academic partners representing human tumor models (provided by CRL DRS Freiburg). No conflict of interest

14LBA (PB-219) Poster

**Determination of ATP and inosine levels in tumor and tumor-free flank of PDX mice by freely-moving in vivo microdialysis**

M. Heins1, L. Wleicht2, S. Gornia3, G. Flik1, A. Rassouli3. 1Charles River Laboratories, Discovery, Groningen, Netherlands; 2Charles River Laboratories, Discovery, Freiburg, Germany; 3Charles River Laboratories, Discovery, South San Francisco, USA

The tumor microenvironment (TME) constitutes unique surroundings where cancer cells communicate with one another and with the host immune system by releasing a multitude of factors. Information about the physiological state of the TME can help in gaining a broader understanding of cancer biology and developing novel cancer therapeutics. Previous work has demonstrated the ability to use in vivo microdialysis to monitor the release of adenosine and its metabolites from murine cancer models (abstract number 253). To further these findings, we used in vivo microdialysis to measure biomarkers in the patient-derived xenograft (PDX) cancer model. By using human tumor tissue models, we aim to bridge the gap between rodent models and human tumors to help develop better cancer therapeutics.

The current set of studies used microdialysis in different PDX lines representing human tumor models (provided by CRL DRS Freiburg). Xenograft growth was monitored and upon a tumor volume >400 mm³, the animals underwent surgical implantation of microdialysis probes in the tumor and the tumor-free flank. The implanted probes were perfused with dialysate fluid and samples from both flanks were continuously collected over several hours. Levels of ATP and inosine were quantified by EIA and LC-MS/MS analysis, respectively. Analysis of microdialysate samples from tumor and tumor-free flank demonstrated differences in analyte levels between the two sampling sites within one animal indicating that the levels of ATP and inosine measured in PDX tumors is unique to the tumor microenvironment. In addition, the various
Background: AMHRII mediates regression of Mullerian-Hormone-Receptor II (AMHRII): safety and hints of activity in Granulosa Cell Tumors (GCT)

Methods: AMHRII expression was evaluated on FFPE GCT samples by IHC and PCR. In the phase 1 GM102 clinical study, 6 GCT patients (pts) were treated in the dose escalation cohorts and 15 in the GCT expansion cohort at 15 mg/kg every two weeks (q2w). Safety, pharmacodynamics (circulating immune cells markers [ICOS, CD14, CD16, CD64, CD69]), anti-tumor activity by RECIST 1.1 criteria and change in Tumor Growth Rate (TGR = % change in tumor volume/month pre-treatment vs. after 2 cycles) were assessed.

Results: GM102 RNA was found as overexpressed (x20) in the 13 GCT samples tested as compared to normal tissue. In the clinical trial out of 21 GCT treated pts, 12 are still ongoing, with 2–10 single agent GM102 cycles received. No dose limiting toxicity up to 10 mg/kg every week and 15 mg/kg q2w was observed. Toxicities were mostly grade 1–2 (fatigue) and for one pt, grade 3 anorexia and weight loss. One pt achieved partial response per independent imaging review after 6 cycles (~33%); tumor size decreased under GM102 in 4/17 (24%) evaluable pts; TGR decreased in 8/12 (66%) evaluated patients per independent imaging review. Effects of GM102 on circulating T cells, total and CD16+ monocytes, neutrophils are under evaluation and preliminary results support an expansion to normal of the classical monocytes proportion.

Conclusions: In this orphan disease with high unmet need, and in these patients having exhausted all therapeutic options, GM102, a targeted immunotherapy, showed hints of activity and an excellent safety profile, that paved the way to further development in this subset of ovarian cancers.

Conflict of interest: A. Leary: AZ, Gritstone, Gamamabs, Clovis, Meros, Roche, Pfizer. I. Ray Coquard: Roche, AZ, Pharamar, Clovis, Tesaro, Lilly, MSD, BMS, Pfizer. A. Floquet Lab ROCHE, TESARO, ASTRA ZENECA, PHARMA MAR; P. Cassier Novartis, Roche/Gentech, Blueprint Medicines, Amgen, Lilly, Blueprint Medicines, Bayer, AstraZeneca, Ceigene, Plexikon, Abbvie, Bristol-Myers Squibb, Merck Serono, Merck Sharp & Dohme, Taiho Pharmaceutical. Toray Industries, Transgene, E. Kalbacher; A. Coste Gamamabs; C. Dumontet Roche/Glycami et Roche France Janssen, Janssen, Astra; J.M. Barret Gamamabs’s employee; F. Lémée Gamamabs’s employee; I. Tabah Fish Gamamabs’s employee; J.F. Prost Gamamabs’s employee, Servier.

16LBA (PB-216) Poster
BAL101553, a novel microdialysis-based tumor tumor point cytokine counter, synergizes with gencitabine providing cures in a PDX-pancreatic model

H. Lane1, P. Mcsheehy1, F. Bachmann1, Basilea Pharmaceutica International Ltd., Research - Cancer Biology, Basel, Switzerland

Background: BAL101553 is a highly soluble pro-drug of the synthetic small molecule BAL27662 (B862) that retains activity in paclitaxel-refractory tumor models; inducing tumor cell death through microtubule depolymerization and activation of the spindle assembly checkpoint. B553 may be administered orally or IV and is currently in Phase 1/2a clinical evaluation in advanced solid tumor patients. Gencitabine (GEM) is approved for the treatment of pancreatic ductal adenocarcinoma (PDAC) in combination with Nab-paclitaxel. Here, we investigated the activity profile of B553 in PDAC models, including combination with GEM in a mouse model.

Methods: The anti-proliferative activity of B862 was evaluated in human patient-derived tumors (PDX) grown in soft agar. Concentrations inhibiting 50% cell growth were calculated. For in vivo studies, the PDAC-PDX model, PAXF1657, was grown s.c. to 100 mm in NMRI nude mice, before treatment began. Tumor growth/body weights (BW) were monitored. Preliminary experiments identified efficacious doses and schedules that were tolerated using B553 or GEM alone or in combination. Animals with progressed tumors without tumor regrowth were assessed for residual tumor cells by histological examination. Plasma PK of monotherapy and combinations was determined by LC-MS/MS in non-tumor bearing mice of the same strain.

Results: In the PDX clonogenic assay, the median IC50 was 111μM across 26 PDAC models. PAXF1657 (IC50 = 66μM) was selected for in vivo testing. Tumor-bearing mice were treated with GEM at 40 mg/kg, IV, qw (equivalent to the clinical-dose in mouse) and B553 at 8 or 10 mg/kg, po, qd (equivalent to approx. half the mouse-MTD). After 3-weeks treatment, when vehicle-treated tumors began to be cycled, B553 showed dose-dependent inhibition (T/C = 0.49, 0.32, 0.28 for 8, 10, 16 mg/kg, qd, respectively) GEM showed stasis (T/C = 0.03) and combinations of GEM with 8 or 10 mg/kg B553 caused regression. Continued combination treatment and GEM- monotherapy for an additional 3-weeks, resulted in complete regressions in the combination groups, as compared to maintenance of stasis in the GEM group. Strikingly, upon treatment cessation (6-weeks) tumors in the GEM- monotherapy group regrew but 40–80% of animals in the combination groups showed complete regressions; confirmed as cures by histopathological analysis. All mice in the combination-groups survived, had an average less BW-gain than the monotherapy groups but no BW-losses. PK analyses after multiple-dosing alone or in combination, showed no change in plasma exposure of either GEM or B862 (or B553) indicating that the strong synergy was not due to a drug-drug interaction.

Conclusion: B553 combined with GEM caused a well-tolerated and synergistic anticancer effect, leading to up to 80% cures in a pancreatic-PDX model. This data suggests a potential for clinical exploration.

Conflict of interest: Ownership: Stock (or option) holder of Basilea Pharmaceutica. Corporate-sponsored Research: Employee of Basilea Pharmaceutica International.

17LBA (PB-220) Poster
First-in-class dual inhibitors of CD73 and A2AR for effective suppression of the adenosine signaling pathway to improve anti-tumor immunity

C. Dinesh1, N. Gowda1, S. Panigrahi1, G. Priyadarshini1, J. Mani1, M. Goyal4, S. Ds1, K. Narayanam1, K. Nellore1, T. Anthony9, S. Samadjar1, M. Ramachandra1, Aurigene Discovery Technologies Ltd, Medicinal Chemistry, Bangalore, India; 2Aurigene Discovery Technologies Ltd, Cell and Molecular Biology, Bangalore, India; 3Aurigene Discovery Technologies Ltd, Computational Chemistry, Bangalore, India; 4Aurigene Discovery Technologies Ltd, Biochemistry, Bangalore, India; 5Aurigene Discovery Technologies Ltd, Pharmacokinetics, Bangalore, India; 6Aurigene Discovery Technologies Ltd, Preclinical Biology, Bangalore, India

Adenosine is a potent immunosuppressor, which is essential for maintaining tissue homeostasis and preventing an overzealous immune response during inflammation and infection. However, adenosine generated within the tumor
microenvironment by the action of ecto-nucleotidases including CD73
hampers the immune reaction towards cancer cells by signaling through
adenosine receptors such as high affinity A2AR expressed on immune
cells. Inhibitions of either adenosine generation or signalling by inhibiting
CD73 or A2AR have been shown to be effective therapeutic approaches.

Recent studies have also shown that the co-blockade of CD73 and A2AR
results in a more pronounced anti-tumor activity than blockade of either,
likely due to increased CD73 expression upon A2AR inhibition and compensatory
activity of other adenosine receptors such as A2BR. In view of this, we sought
to discover and develop small molecule inhibitors that dualy target CD73 and
A2AR with oral bioavailability for ease of administration and use in
combination with other anti-cancer therapies.

Here, we report a structure-guided drug design-based approach for
identification of dual inhibitors targeting CD73 and A2AR. Molecular
modelling of A2AR and CD73 with several de novo designed hits greatly
aided in the identification of dual inhibitors that exhibit potent inhibition
of both A2AR and CD73 in respective biochemical and cellular assays. High potency
translated into resuce of NECA or AMP induced repression of IFN-
A2AR with oral bioavalability for ease of administration and use in
combination with other antitumor reponse in syngeneic mouse models of cancer is in progress. To
the best of our knowledge, this is the first report of a small molecule agent
dually targeting CD73 and A2AR for potential use in cancer immunotherapy.

No conflict of interest

18LBA (PB-221)  Poster
Targeting HIF2α with an RNAi therapeutic for the treatment of clear cell renal cell carcinoma
Arrowhead Pharmaceuticals, Chemistry, Madison, USA;
Arrowhead Pharmaceuticals, Biology, Madison, USA;
Arrowhead Pharmaceuticals, Toxicology, Madison, USA;
Arrowhead Pharmaceuticals, Chief Operating Officer, Pasadena, USA;
Arrowhead Pharmaceuticals, Senior Vice President, Madison, USA

Background: Approximately 80%–90% of clear cell renal cell carcinoma (ccRCC) tumors express an inactive mutant form of the von Hippel-Lindau protein (pVHL). This functional loss of pVHL leads to the accumulation of (ccRCC) tumors express an inactive mutant form of the von Hippel-Lindau transcription factor is

HIF2RNAi molecules using the TRiM™ delivery platform can be used to deliver a RNAi therapeutic selectively targeting HIF2α for the treatment of ccRCC. This represents a novel therapeutic approach either as a monotherapy or in combination with other therapies in seeking better tolerated and/or more effective treatment for ccRCC.

Conflict of interest: Ownership: We are employees and/or shareholders of Arrowhead Pharmaceuticals Inc.

19LBA (PB-222)  Poster
Exploiting synthetic lethality to target 9p21 locus deleted bladder cancer
P. Gasperi1, D. Prandi1, T. Fedrizzi1, V. Adams1, M. Pancher1, F. Lorenzin1, B.M. Flattas2,3, F. Demichelis1,3. 1CIBIO, University of Trento, Povo, Italy; 2Division of Hematology and Medical Oncology, Weill Cornell Medicine, New York, USA; 3Englander Institute for Precision Medicine-, New York-Presbyterian Hospital, Weill Cornell Medicine, New York, USA

Background: The growing accumulation of large-scale patient-derived data sets allows for data-driven approaches to exploit somatic aberrations to identify vulnerabilities for cancer treatment. Mutual exclusivity of two aberrant genes might suggest that their co-occurrence is not tolerated and leads to cell death (synthetic lethality, SL). We have adopted this approach to identify new SL in bladder cancer, a disease with limited treatment options. The identification of SL pairs can lead to the identification of novel therapeutic targets and effective drug combinations.

Material and Methods: 1. Mutual exclusivity analysis. An ad hoc approach to perform large SL computational analyses of thousands of patients’ pan-cancer genomic data, named SPICE (ERC-CoG 648670), was applied to all TCGA datasets including the bladder cancer cohort of 412 patients.
2. Drug screening: A high content drug screening was performed on cells exposed to a battery of 2,400 compounds, from Anticancer compound (Selleck®) and MicroSource Spectrum® collections with and without a CDK4–6 inhibitor. Cells were fixed and stained. Fluorescence images were acquired with the Opera® High Content Imaging System.
3. Genome Wide CRISPR screening. We adopted the Gecko v2 library, a 2 vectors library system with over 120,000 unique gRNAs for gene knock-out human genome.
4. Cell line. The bladder cancer cell line HT1197 was selected because of wild type status of RB1 gene and 9p21 locus.

Results: SPICE nominated several mutually exclusive pairs including signal related to the recurrent homogygous deletion (HD) of CDKN2A/B/MTAP at the 9p21 locus. This event has a frequency of 22% in Bladder Cancer. With the purpose of developing rational treatment strategies, we used CRISPR-Cas9 to generate isogenic clones of HT1197 cells lacking the entire 9p21 locus that spans 213 kb leading to the co-deletion of CDKN2A/B/MTAP (termed 3KO). 3KO cell clones proliferate faster than their wild type counterparts do. Gene set enrichment analysis performed on RNAseq data of the 3KO and WT clones showed significant differences in DNA damage bypass (FDR 3.043 e-04) and cell cycle (FDR 2.625 e-08) genes. A multi-parameter drug screening designed to exposed specific pharmaceutical sensitivities/vulnerabilities of the 3KO clones nominated 75 compounds of therapeutic potential as single agents and 76 compounds specifically active only when combined with a CDK4-6 inhibitor. Genome wide CRISPR screening will follow on single cell 3KO clones to: (i) comprehensively test the in silico nominated SL partners of CDKN2A/B/MTAP; (ii) potentially discover additional partners; (iii) nominate potential therapeutic targets for combination treatments of urothelial carcinoma characterized by 9p21 locus HD.

Discussion: This work is a proof of concept of data-driven discovery of potential molecular targets within and across cancer types.

No conflict of interest

20LBA (PB-223)  Poster
Targeting FGFR4 for rhabdomyosarcoma immunotherapy
1Leidos Biomedical Research, Inc., Biomedical Informatics and Data Science, Bethesda, USA; 2National Cancer Institute, Genetics Branch, Bethesda, USA; 3Seattle Children’s Hospital, Ben Towne Center for Childhood Cancer Research, Seattle, USA

Background: Despite multimodal therapy including surgery, radiation and aggressive chemotherapies, relapsed refractory or metastatic rhabdomyo-
sarcoma (RMS) remains a lethal disease with no significant improvement in outcome over several decades of clinical trials and therefore novel therapies

Conclusions: We demonstrate that the TRiM™ delivery platform can be used to deliver a RNAi therapeutic selectively targeting HIF2α for the treatment of ccRCC. This represents a novel therapeutic approach either as a monotherapy or in combination with other therapies in seeking better tolerated and/or more effective treatment for ccRCC.

Conflict of interest: Ownership: We are employees and/or shareholders of Arrowhead Pharmaceuticals Inc.
Material and Methods: Using monoclonal antibody technologies and a yeast display B-Cell library, we generated fifteen binders against FGFR4. Those binders were engineered into human IgG2a or scFvFc and were produced in vitro. We further characterized these binders by using FACS and ELISA for its FGFR4 specificity. Octet was used to measure the binding affinity against FGFR4. For those lead hits selected, they were made into different format of therapeutic including Chimeric Antibody Receptor (CAR) and Antibody Drug Conjugated (ADC). We then performed in vitro killing assays and in vivo xenograft model to determine the efficacy of these therapeutics in killing FGFR4 expressing RMS cells.

Results: 3A11 and M410 were two lead hits that highly specific to FGFR4. The IgG2a (3A11) and scFvFc (M410) version of our lead hits were successfully produced in vitro and retained its FGFR4 specificity with a binding affinity at nanomolar range. By ELISA, these binders showed dose dependent binding to FGFR4 protein but not to other FGFR family members. 3A11 was found internalized upon binding to FGFR4 positive cell lines, and therefore were made into ADC. We conjugated 3A11 with a cytotoxic drug pyrrolobenzodiazepine (PBD) through glycan modification and CLICK chemistry. When cultured RH30 (a RMS cell line expresses FGFR4) in the presence of the 3A11 PBD for 72 hours, dose-dependent cytotoxicity was observed, suggesting that 3A11 could deliver a cytotoxic payload to FGFR4 positive cells. M410 was made into CAR construct and T cells transduced with this construct were found highly potent in inducing gamma interferon, TNF alpha and cytotoxicity when the FGFR4-CART are co-cultured with RMS cells. Preliminary in vivo testing found them to be effective in eliminating RMS cells in a xenograft model.

Conclusions: Here our data showed that we had successfully generated binders that were specific to FGFR4. The CAR and ADC that we developed from these binders were able to kill FGFR4 positive target cells. Our data thus far suggested that the FGFR4 ADC and CAR we developed may provide effective immune therapy for RMS with high FGFR4 expression.

No conflict of interest

22LBA (PB-225) Poster

Utilizing novel oncolytic vaccinia virus for selective expression of immunotherapeutic proteins in metastatic tumors


The Ottawa Hospital Research Institute, Centre for Cancer Therapeutics, Ottawa, Canada; Leeds University, Leeds Institute of Cancer and Pathology, Leeds, United Kingdom; Thoracic Research, Translational Immunotherapeutics, London, United Kingdom; Turnstone Biologics, Research and Development, Ottawa, Canada

The treatment paradigm for patients with metastatic cancer has evolved rapidly with the approval of agents targeting CTLA-4 and PD-1 immune checkpoints. Despite the profound impact these agents have had, they are minimally effective in the majority of cancer patients. Rational combinations of complementary immune modulating agents have thus far not lead to clear patient benefit and newer technologies which are better able to safely combine multiple modes of action could well prove to be vital.

Oncolytic viruses (OVs) have the capacity to be the ideal therapeutic partner for immune checkpoint therapies in several ways. First on their own OVs can “heat-up” immunologically “cold” tumors by initiating a pro-inflammatory infection within the tumor microenvironment (TME). Second, some OVs can be engineered to strategically express one or more immune modulatory genes cultured tumors (a RMS cell line expresses FGFR4). In the presence of the 3A11 PBD for 72 hours, dose-dependent cytotoxicity was observed, suggesting that 3A11 could deliver a cytotoxic payload to FGFR4 positive cells. M410 was made into CAR construct and T cells transduced with this construct were found highly potent in inducing gamma interferon, TNF alpha and cytotoxicity when the FGFR4-CART are co-cultured with RMS cells. Preliminary in vivo testing found them to be effective in eliminating RMS cells in a xenograft model.

Conclusions: Here our data showed that we had successfully generated binders that were specific to FGFR4. The CAR and ADC that we developed from these binders were able to kill FGFR4 positive target cells. Our data thus far suggested that the FGFR4 ADC and CAR we developed may provide effective immune therapy for RMS with high FGFR4 expression.

No conflict of interest
25LBA (PB-228) Poster

TRAIL receptor activation overcomes resistance to trastuzumab in HER2 positive breast cancer cells

E. Díaz-Rodríguez1, J. Pérez-Peña1, C. Rios-Lucí1, J. Arríbas2, A. Ocaña3, A. Pandiella4. 1Centro de Investigación del Cáncer, Laboratorio oncológico, Valencia, Spain; 2Vall d’Hebron Institute of Oncology, Barcelona, Spain; 3Center of Studies and Biomedical Research, Albacete, Spain

Background: During the last few years and thanks to the development of new targeted therapies, the prognosis of patients diagnosed with breast cancer overexpressing HER2 has clearly improved. Nonetheless, it is frequent to find patients in which after a time of response to these therapies, tumors progress due to the development of resistances. Identifying the mechanisms leading to those resistances as well as designing new therapeutic strategies in that context represents nowadays an important challenge in the oncology clinic.

Materials and Methods: A new model of in vitro resistance to trastuzumab was generated in the laboratory based on the continuous culture with this drug. Besides through functional genomic and proteomic profiling, a panel of genes and cellular functions deregulated in trastuzumab-resistant cells were identified, and the expression of some key genes validated by qRT-PCR. The functional deregulation of cell death pathways was confirmed in proliferation assays (MTT), as well as apoptotic cell death induction by flow cytometry after treatment with the identified death ligand. Final target validation was carried out by retroviral or lentiviral transduction designed to induce gain- or loss-of-function of the preferred proteins.

Results: The trastuzumab-resistant cell line was generated and characterized. In fact, using genomic and proteomic approaches we have identified a deregulation in cell death pathways in trastuzumab resistant cells. More precisely, an increase in the cell death receptors DR4 and DR5, which participate in TRAIL apoptotic signaling, was detected in the resistant population both at the RNA and protein levels. At the same time, a decrease in other inhibitory components of the pathway, such as FLIP, was detected. This combination produces a more efficient assembly of the functional complex DISC (death-inducing signaling complex) and its downstream signaling in the trastuzumab-resistant cells that is translated in an increased apoptotic response to the death factor TRAIL as demonstrated in proliferation, cytometry and biochemical analyses. Moreover, the importance of all these processes was validated in a gain or loss of function experiments. Finally, this cell death pathway is not only altered in our model system, but in also in other HER2+ breast cancer cell lines primarily sensitive to trastuzumab, opening the possibility of using this pathway in therapy.

Conclusions: Taken together our data identify a vulnerability of trastuzumab-resistant cells, which are more sensitive to TRAIL induced cell death. Such vulnerability could be exploited and used to design new targeted therapies in that context.

No conflict of interest

26LBA (PB-229) Poster

[10]-gingerol in combination with doxorubicin inhibits tumor growth and metastasis in syngeneic and xenograft orthotopic breast cancer models

A. Baptista Moreno Martin1, R. Tomás1, M. de Araújo Naves1, A. Ellen Graminha1, L. Luna-Dulcey1, R. Handerson Gomes Teles1, M.R. Cominetti1. 1Federal University of Sao Carlos, Gerontology, Sao Carlos, Brazil; 2Chemist, Biochemistry, Sao Paulo, Brazil

Background: Currently, there is no effective therapy for triple negative breast cancer (TNBC) and most treatments are very aggressive and present undesired side effects. TNBC corresponds to 15–20% of all breast cancers and frequently relapses within 3 years. Therefore, new drugs that could be used in the adjuvant treatment are needed. The aim of this work was to study the chemosensitizing effects of [10]-gingerol (10G) in combination with standard chemotherapy (doxorubicin - DOX) against TNBC in vivo.

Material and Methods: For the orthotopic syngeneic spontaneous breast cancer metastasis model, 4T1Br (1*105cells) cells were injected into the mammary fat pad (IMFP) of Balb/C mice. When tumors became palpable, mice were treated as described above, tumor growth and body weight were also monitored. The mice were harvested 35 days post cell injection for tissues collection (Approved by ethics committee: 35283031016). Regarding to the orthotopic xenograft breast cancer model, MDA-MB-231 HMTL6 cells were injected IMFP (1*106 cells). When tumors became palpable, mice were treated as described above, tumor growth and body weight were also monitored. The mice were harvested 35 days post cell injection for tissues collection (Approved by ethics committee: 4805090418).

Results: Concerning the orthotopic model, 10G in combination with DOX reduced primary tumor growth and metastases to lung, spine and femur. 10G also reduced DOX toxicity as observed by attenuation of weight loss and liver toxicity. Notably, in the xenograft model, tumor growth was inhibited only when 10G and DOX were combined.

Conclusion: [10]-gingerol had synergetic and additive anticancer effects in combination with doxorubicin in vivo. Furthermore, 10G demonstrated not only to be a non-toxic compound for the host, but was also able to reduce doxorubicin undesired side effects.

Financial support: FAPESP, CAPES, CNPq.

No conflict of interest

27LBA (PB-230) Poster

Radiation-induced R-loop generation and abscopal effect in vitro and in vivo NSCLC models

A. Teseli1, C. Arienti1, L. Strigarì2, G. Bossì1, S. Santì1, M. Zanoni1, S. Pignatta1, A. Zamagni1, A. Sarnefì1, A. Romeo1, M. Bonafè2. 1Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori I.R.S.T, Department of Medical Oncology and Oncohaematology, Meldola, Italy; 2Regina Elena National Cancer Institute, Department of Medical Physics, Roma, Italy; 3Institute of Molecular Genetics CNR- Rizzoli Orthopaedic Institute, SC Laboratory of Musculoskeletal Cell Biology, Bologna, Italy; 4Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori I.R.S.T, Medical Physics Unit, Meldola, Italy; 5Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori I.R.S.T, Radiotherapy Unit, Meldola, Italy

Background: Radiation therapy is one of the most common treatments for primary and advanced cancer in place of or in combination with surgery or chemotherapy. Traditionally, radiobiological explanation of radiation tumor cell kill was mostly represented by a direct cytotoxic effect, including the induction of irreparable DNA damage, leading to cell death. Over the last decade, a significant growing body of evidence has shown that radiotherapy has immune modulatory effects either locally on both tumor cells and within the tumor microenvironment (bystander effect) and at metastatic lesions distant from irradiation site (abscopal effect). In this context, we aim to explore the role of the cytoplasmic and extracellular vesicles–mediated release of RNA–DNA hybrid structures (R-loops) by tumor cells after exposure to specific RT doses.

Material and Methods: All the experiments were conducted on NSCLC A549 cell line and its derived p53 knocked-down clone A549sip53. In vitro irradiation experiments were performed using the same device used to irradiate cancer patients. The cell survival was evaluated by clonogenic assay. DNA damage was evaluated by microarray analysis and comet assay. Extracellular vesicles (EVs) were isolated by size exclusion chromatography: immune-precipitation and ultracentrifugation. R-loop expression analysis was performed by confocal microscopy and dot blot analysis. Gene expression levels of the immune-related molecule were evaluated by Real-Time PCR Assays.

CD-1 nude mice were xenografted with A549 cells for in vivo experiments.

Results: We observed either in A549 and A549sip53 cells, an increase in cytoplasmic RNA:DNA hybrid production following the exposure to different irradiation doses, ranging from 5 Gy to 10 Gy. We also observed a decrease in R-Loop expression after exposure of the cells to 20 Gy dose. We showed the presence of R-Loop only in EVs produced by the A549 cells exposed to specific irradiation doses. We also observed that only the EVs produced by the irradiated A549 cells induce in the non-irradiated cell line a decrease in their clonogenic capacity. Furthermore, the exposure of A549 cells to nonnucleoside RT inhibitor Efavirenz (EFV) before irradiation treatment significantly reduced the R-Loop generation. Finally, we observed a significant effect on tumor growth inhibition in the unirradiated contralateral mass of wt-p53 tumors only in the 20 Gy-irradiation group.

Conclusions: We showed that only specific irradiation dose induced the abscopal effect in a murine p53-sip53-bearing xenograft model. Made in vitro and in vivo data strongly suggest that such effect was mediated by RNA-DNA hybrid structures vehiculated by extracellular vesicles. The results obtained could represent a scientific rationale for the use of innovative radiotherapy chemotherapy schemes.

No conflict of interest
Effect of food on the pharmacokinetics of the weel inhibitor adavosertib (AZD1775) in patients with advanced solid tumors

M. Nágárdí1, K. So2, M.L. Ah-See2, M. Vermunt2, F. Thistelthwaite3, M. Labots4, P. Roxburgh6, A. Ravaud2, M. Camponië5, L. Valkenburg-van Iersel7, L. Ottesen7, G. Muganda1,2

*Quantitative Clinical Pharmacology, Early Clinical Development, IMED Biotech Unit, AstraZeneca, Gaithersburg, USA, 2Late Phase Development, GMD, AstraZeneca, Cambridge, UK, 3The Netherlands Cancer Institute-Antoni van Leeuwenhoek, Amsterdam, The Netherlands, 4The Christie NHS Foundation Trust and University of Manchester, Manchester, UK, 5VU University Medical Center, Amsterdam, The Netherlands, 6Beaeston West of Scotland Cancer Centre, Glasgow, UK, 7Groupe Hospitalier Saint André, Bordeaux, France, 8ICO – Site René, Saint Herblain, France, 9Maastricht University Medical Center, Maastricht, The Netherlands, 10Quantitative Clinical Pharmacology, Early Clinical Development, IMED Biotech Unit, AstraZeneca, Boston, MA, USA

**Background:** Adavosertib is an orally active, first in class, small molecule reversible inhibitor of WEE1 kinase that is being developed either as a monotherapy or in combination with olaparib or chemotherapy or durvalumab. Here we report the effect of a high-fat meal on the pharmacokinetics of adavosertib to support dosing recommendations in future clinical studies.

**Methods:** This was an open-label, randomized, 2-period crossover study in 31 patients with advanced solid tumours. In the fasted treatment, patients were fasted overnight for at least 10 hours before and 4 hours after administration of 300 mg adavosertib. In the fed treatment, patients were fasted overnight and consumed a high-fat meal before the administration of adavosertib. Pharmacokinetic and safety assessments were obtained for up to 72 hours post-dose in each treatment period, and the treatments were separated with at least a 5-day washout period. Geometric mean ratios and 90% confidence intervals (CIs) of PK parameters were calculated based on a mixed effects model with fixed effects for sequence, period, and treatment and subject nested within sequence as a random effect.

**Results:** Compared with the fasted state, the high-fat meal decreased the adavosertib maximum plasma concentration by 16%, the systemic exposure (area under the adavosertib plasma concentration-time curve) by 6%, and delayed the time to maximum plasma concentration by 2 hours. The 90% CIs of the geometric mean treatment ratios for AUCs were fully contained within the no effect limits of 0.8–1.25 while that of geometric mean treatment ratio for Cmax was not entirely contained within the no-effect limits. Overall, 25 (83.3%) patients in the fed treatment and 23 (79.3%) patients in the fasted treatment reported any adverse event (AE). Causally related AEs (as assessed by the Investigator) were reported by 16 (53.3%) patients in the fed treatment and 14 (48.3%) patients in the fasted treatment. AEs Grade 3 or 4 were reported by 1 (3.3%) patient in the fed treatment and 3 (10.3%) patients in the fasted treatment. Of these, 2 AEs were deemed causally-related to Adavosertib (hypokalaemia and diarrhoea). Three patients experienced a SAE. In 2 patients, the SAEs were reported in the fasted period (Urosepsis, CTCAE grade 3). None of the SAEs were related to Adavosertib (hypokalaemia and diarrhoea). Three patients experienced a SAE. In 2 patients, the SAEs were reported in the fasted period (Pyrexia, CTCAE grade 1; Hydronephrosis, CTCAE grade 1) & in 1 patient in the fed period (Urosepsis, CTCAE grade 3). None of the SAEs were related to Adavosertib (hypokalaemia and diarrhoea). Three patients experienced a SAE. In 2 patients, the SAEs were reported in the fasted period (Pyrexia, CTCAE grade 1; Hydronephrosis, CTCAE grade 1) & in 1 patient in the fed period (Urosepsis, CTCAE grade 3). None of the SAEs were related to Adavosertib (hypokalaemia and diarrhoea).

**Conclusions:** Administration of adavosertib 300 mg as a single oral dose following a high-fat meal did not have a clinically relevant effect on systemic exposure. Therefore, the results from this study suggest that adavosertib can be administered without regard to meals. No new safety signals with the administration of adavosertib were identified in this study.

**Conflict of interest:** Employee of AstraZeneca and owns stock.
Oral Abstracts
PLenary session 1

should we be conducting more genomics driven, multi-arm clinical trials?

1 AZD5363 in patients (pts) with tumors with AKT mutations: NCI-MATCH Subprotocol EAY131-Y, a trial of the ECOG-ACRIN Cancer Research Group (EAY131-Y)

1 Columbia University Medical Center, Medicine, New York, USA; 2 Dana-Farber Cancer Institute, Harvard T.H. Chan School of Public Health, Boston, USA; 3 Washington University School of Medicine, Gynecologic Oncology, St. Louis, USA; 4 Genentech/Genentech Research Institute, Medicine, Scottsdale, USA; 5 Thomas Jefferson University Hospital, Medicine, Philadelphia, USA; 6 National Cancer Institute, Division of Cancer Treatment and Diagnosis, Bethesda, USA; 7 Dana Farber Cancer Institute, ECOG-ACRIN Biostatistics Center, Boston, USA;
Molecular Characterization and assay development laboratory, Leidos, Frederick, USA; 8 MD Anderson Cancer Center, Pathology, Houston, USA; 9 University of Pennsylvania, Medicine, Philadelphia, USA; 10 UT Southwestern, neurology, Dallas, USA; 11 Massachusetts General Hospital, Medicine, Boston, USA

Background: In the NCI-MATCH Trial, pts receive agents that target genetic abnormalities in their tumors. In sub-protocol E131-Y, pts with AKT1 E17K mutant metastatic tumors received the pan-AKT inhibitor AZD5363 (capivasertib).

Methods: Pts received AZD5363 480 mg orally twice daily for 4 days on 3 days off weekly in a 28-day cycle until progression or unacceptable toxicity. Concurrent fulvestrant or aromatase inhibitor was allowed in pts with hormone receptor+ (HR+)/HER2− breast cancer (BC), if their last metastatic regimen included these hormonal therapies (AZD5363: 400 mg). Pts with well-controlled diabetes were eligible, unless baseline fasting glucose >160 mg/dL, or >2 oral hypoglycemics, or on insulin. Tumor assessments were repeated every 2 cycles. Except for FDA approved rapalogs, no prior PI3K/AKT inhibitors were allowed. The primary endpoint was objective response rate (ORR). Secondary endpoints included progression-free survival (PFS) and toxicity.

Results: Of the 35 pts enrolled between July 2016-August 2017, 30 were female (86%); 27 were Caucasian (77%), 5 were African American (14%), and 4 were Hispanic (11%); and 11 had a performance status of 0 (31%). The majority had >3 lines of prior metastatic treatment (tx) [19/35 (54%)]. The most prevalent cancers were breast [n = 18; 52%; 5 HR+/HER2− and 3 HR− HER2−] and endometrial adenocarcinoma (n = 8; 23%; 6 endometrial and 2 ovarian). The partial response (PR) rate was 23% (n = 8) [90% confidence interval (CI): 12%−38%]. Of those with PR, 6 pts had HR+/HER2− BC, 1 endometrioid adenocarcinoma, and 1 leiomyosarcoma. The best confirmed responses for others included 16 with stable disease (46%), 2 with progressive disease (PD; 6%), 8 not evaluable (23%), and 1 with missing data (6%). The estimated 6-month PFS rate was 52% (90% CI: 39%−70%). The most common reason for tx discontinuation was PD (54%), followed by adverse events (25%), irs, of causality. Most common grade 1-2 adverse events (AEs) included diarrhea (49%), fatigue (40%), nausea (31%), rash (26%), proteinuria (26%); liver function test (LFT) increase (26%), and hyperglycemia (23%). Grade 3 AEs included hyperglycemia (20%), rash (11%), and diabetes (9%) LFT increase (9%); vomiting (9%); nausea (3%); and proteinuria (3%), with 1 grade 4 hyperglycemia event. Within cycle 1, at least 17/35 pts (49%) required a dose modification. At this time, 4 pts remain on study, at months 9, 17, 18, and 21 months.

Conclusions: AZD5363 demonstrated clinically significant activity in tumors with AKT1 E17K mutations with an ORR of 25%. E131-Y met its primary endpoint. Toxicities, including hyperglycemia, fatigue, gastrointestinal AEs and skin abnormalities, should be carefully managed. Tissue and plasma sample analyses are planned. Further studies are warranted with AZD5363 in patients with AKT1 E17K mutations. Clinical Trial NCT02465060.

Conflict of interest: Ownership: Kevin Kalinsky (Spouse – employee at Novartis, currently: Array BioPharma), Advisory Board: Kevin Kalinsky (Lilly, Amgen, AstraZeneca, bioTheranostics, Pfizer, Novartis, Eisai, Ipsen) Fangxi Hong (Merck), Other Substantive Relationships: Speakers’ Bureau: Kevin Kalinsky (Lilly) Speakers’ Bureau: Carolyn McCourt (Genentech).

Wednesday, 14 November 2018 14:30–16:00

PLenary session 2

Proffered papers

2 Oral

MammaPrint high/Hi2 risk class as a pre-specified biomarker of response to nine different targeted agents plus standard neoadjuvant therapy for ~1000 breast cancer patients in the I-SPY 2 TRIAL

L.J. van’t Veer1, D. Wolf1, C. Yau1, A. Glas2, L. Brown-Swigart3, S. Asare4, G. Hirst5, I. Investigators6, N. Hytton7, F. Symmans2, D. Berry4, A. Demichele8, D. Yee9, L. Esserman1
1 University California San Francisco, Cancer Center, San Francisco, USA; 2 Agency BV, Bioinformatics, Amsterdam, Netherlands; 3 Quantumleap Health, Trial Network, San Francisco, USA; 4 MD Anderson, Cancer Center, Houston, USA; 5 University Pennsylvania, Medicine, Philadelphia, USA; 6 University Minnesota, Cancer Center, Minneapolis, USA

Background: Further stratification of the 70-gene MammaPrint™ signature into “high” (MP1) and “ultra-high” (MP2) risk groups may help predict overall chemo-sensitivity. In I-SPY 2, patients were classified as MP1 or MP2, with MP1 defined as trial MP_score <-0.154 (numerical clinical test MP < −0.604). MP1/MP2 was added to HR and HER2 to define the 8 cancer subtypes used in the I-SPY 2 adaptive randomization engine. Here, we assess the performance of MP1/MP2 class as a biomarker of response in the first 9 experimental arms of the trial and in controls (Ct).

Methods: 986 patients were considered in this analysis. Treatment regimens included paclitaxel alone (or with trastuzumab (H) in HER2+) (Ct), or in combination with investigational agents including: veliparib/carboplatin (VC), neratinib (N): MK2206; Ganitumab; Ganetespib; AMG386; TDM1/ pertuzumab(P); H+P; and Pembrolizumab. We assessed association between MP1/2 and response in the whole population and within each arm using a logistic model. This analysis was adjusted for HR status, HER2 status, and treatment arm as covariates, and within receptor subtypes. This analysis does not adjust for multiplicities of other biomarkers.

Results: 51% (503/986) of I-SPY 2 patients were MP1, and 49% (483/986) MP2 class (84%), and HR+HER2− and HR+HER2+ mostly MP1 (72% and 85%, respectively). Across all arms combined, MP2 associated with pCR (OR = 2.62; p = 3.52E−12), and also in a model adjusting for treatment arm, HR, and HER2 status (p = 2.43; p = 1.31E−06).

Evaluated within treatment arms, MP2 associated with pCR in half the arms (VC, N, ganitumab, H/P and pembrolizumab) in a model adjusting for treatment arm, HR, and HER2 status (p = 2.43; p = 1.31E−06).

Conclusion: Further stratification of the 70-gene prognostic signature into “high” MP1 and “ultra-high” MP2 risk groups predicts chemo-sensitivity in early breast cancer to a variety of agents/combos and may guide treatment prioritization of targeted agents.

Conflict of interest: Ownership: Co-founder, board member, stock holder Agency BV.

3 Oral

blueprint basal subtype predicts neoadjuvant therapy response in ~400 HR+HER2− patients across 8 arms in the I-SPY 2 TRIAL

L.J. van’t Veer1, D. Wolf1, C. Yau1, Z. Zhu2, A. Glas2, W. Audenh2, L. Brown-Swigart3, S. Asare4, G. Hirst5, I. Investigators6, A. Demichele8, D. Yee9, L. Esserman1
1 University of California, Cancer Center, San Francisco, USA; 2 Agency BV, Bioinformatics, Amsterdam, Netherlands; 3 Agendia BV, Medical Oncology, Irvine, USA; 4 Quantumleap Health, Trial network, USA; 5 University Pennsylvania, Medicine, Philadelphia, USA; 6 University Minnesota, Cancer Center, Minneapolis, USA

Background: The 80-gene BluePrint (BP) signature can be used to classify breast cancers based on their expression profiles into 3 subtypes: Luminal, Basal, and HER2+. Some breast cancer subtypes exhibit unique chemotherapeutic and endocrine responses, and breast cancer subtyping is associated with improved treatment outcomes through prioritization of targeted agents. Basal subtype breast cancer cases display a marked worse prognosis in comparison to luminal cases. The 80-gene BluePrint (BP) signature can be used to classify breast cancers based on their expression profiles into 3 subtypes: Luminal, Basal, and HER2+. Some breast cancer subtypes exhibit unique chemotherapeutic and endocrine responses, and breast cancer subtyping is associated with improved treatment outcomes through prioritization of targeted agents. Basal subtype breast cancer cases display a marked worse prognosis in comparison to luminal cases.

Conflict of interest: BluePrint is owned by Agendia BV. The 80-gene BluePrint (BP) signature can be used to classify breast cancers based on their expression profiles into 3 subtypes: Luminal, Basal, and HER2+. Some breast cancer subtypes exhibit unique chemotherapeutic and endocrine responses, and breast cancer subtyping is associated with improved treatment outcomes through prioritization of targeted agents. Basal subtype breast cancer cases display a marked worse prognosis in comparison to luminal cases.
HER2, and Basal; previous studies show BP subtypes predict response to neoadjuvant therapy. I-SPY 2 is a multicenter Phase 2 platform trial evaluating novel agent/combinations added to standard chemotherapy within Hormone Receptor (HR)/HER2/MammaPrint (MP) defined signatures. We have previously observed that, in the I-SPY 2 TRIAL, HR+HER2+ "BP HER2-type" patients are more likely to respond to HER2-targeted agents/combinations than HR+HER2+ "BP Luminal-type" patients. In this study, we evaluated BP subtype as a predictor of response among HR+HER2+ patients in the I-SPY 2 TRIAL.

Methods: The Blueprint signature was assigned to apply subtypes for 981 I-SPY 2 patients, 375 of whom are HR+HER2+ (and randomized to one of 7 experimental therapy or control arms). We compared the expression levels of ER, PR, and basal-type keratins (KRT5/14/17) between BP subtypes using a Wilcoxon rank sum test. Association between BP subtypes and MP (High1/High2) class are used in I-SPY2 randomization; pathologic complete response (pCR) was evaluated with Fisher's Exact test and logistic regression adjusting by treatment arm. A Bayesian hierarchical logistic modeling estimated pCR rates as a function of BP subtypes within treatment arms. Our statistics are descriptive rather than inferential and do not adjust for multiplicities of biomarkers outside this study.

Results: While the majority of HR+HER2+ patients (266/375) are BP Luminal, 29% (108/375) are BP basal-type. As expected, HR+HER2+ BP Luminal patients have higher ER/PR and lower basal keratins expression levels, when compared to HR+HER2+ BP Basal patients (p < 0.0001). In addition, BP subtype is associated with MP class, where 77% HR+HER2+ BP Basal patients are MP High2 class, compared to only 9% of HR+HER2+ BP Luminal patients. Across all arms, HR+HER2+ BP Basal-type patients is more likely to achieve a pCR when compared to BP luminal patients (Odds Ratio: 4.41, p < 0.0001), with similar association observed in a treatment-arm adjusted model (Odds Ratio: 4.98, p < 0.0001). Within treatment arms, the estimated pCR rates among HR+HER2+ BP Basal patients ranged from 29%–51% in Arm A compared to 7%–17% in HR+HER2+ BP Luminal patients. Conclusion: Our findings suggest that the BP basal signature identifies a subset of HR+HER2+ patients who are more likely to respond to neoadjuvant chemotherapy. The overlap between BP-basal and MP class suggests that different predictive signatures may identify similar sets of responsive patients. These findings will aid in guiding prioritization of targeted agents when the goal is to optimize the chance of pCR for patients.
Results: 18 patients have been enrolled with the MTD of IPIrD determined to be 1200 mg using this schedule. Currently, an additional cohort of 5 patients is being entered at the MTD to confirm the pharmacokinetics and to measure the %IPIrD DNA cellular incorporation in tumor biopsies at Day 8 and weekly in circulating granulocytes.

No conflict of interest

7 Oral

Activity and safety of ibrutinib and durvalumab in patients with relapsed or refractory follicular lymphoma (FL) or diffuse large B-cell lymphoma (DLBCL)

A. Herrera,1 A. Goy,2 A. Mehta,3 R. Ramchandren,4 J. Page,5 J. Svoboda,6 S. Guan,7 J. Hill,7 K. Kwee,8 E. Liu,9 T. Phillips10.1. City of Hope, Department of Hematology/Hematopoietic Cell Transplantation, Duarte, CA, USA; 2. Hackensack University Medical Center, John Theurer Cancer Center, Hackensack, NJ, USA; 3. University of Alabama, Division of Hematology & Oncology, Birmingham, AL, USA; 4. Karmanos Cancer Institute, Department of Hematology/Oncology, Detroit, MI, USA; 5. Swedish Cancer Institute, Center for Blood Disorders and Stem Cell Transplantation, Seattle, WA, USA; 6. University of Pennsylvania, Department of Medicine, Philadelphia, PA, USA; 7. Pharmacyclics LLC, an AbbVie Company, Department of Biostatistics, Sunnyvale, CA, USA; 8. Pharmacyclics LLC, an AbbVie Company, Department of Translational Medicine, Sunnyvale, CA, USA; 9. Pharmacyclics LLC, an AbbVie Company, Department of Clinical Science, Sunnyvale, CA, USA; 10. University of Michigan, Rogel Cancer Center, Ann Arbor, MI, USA

Background: Ibrutinib (ibr), a first-in-class, once-daily BTK inhibitor, is approved in the US and EU for the treatment of various B-cell malignancies. Durvalumab (durv) is an anti-PD-L1 antibody, anti-PD-1/PD-L1 agents restore antitumor immune responses, and some have shown promising activity in FL and DLBCL. Data in murine lymphoma models suggest ibr and durv may have synergistic activity. This phase 1b/2, multicenter, open-label study evaluated ibr plus durv in patients (pts) ≥18 y with relapsed/refractory FL (grade 1, 2, or 3A) or DLBCL. Material and methods: Pts were treated with once-daily 560 mg ibr PO plus 10 mg/kg durv IV every 2 wk in 28-day cycles (durv max 12 cycles). The phase 2 primary endpoint was ORR. Secondary endpoints were duration of response (DOR), PFS, OS, and safety. Results: Sixty-one pts with FL (n = 27), GCB DLBCL (n = 16), non-GCB DLBCL (n = 16), or unspecified DLBCL (n = 2) were treated. The median age was 60 y (range: 22–82); median time from diagnosis was 26 mo; 51% had ECOG PS 0; 52% had refractory disease; 85% had ≥2 prior regimens. ORR was 26% in response-evaluable pts (Table). A pre-specified interim analysis was performed on the first 19 evaluable FL pts and 9 evaluable DLBCL pts; efficacy stopping rules were met (≤5/19 FL responders; ≤19 DLBCL responders). All pts were discontinued from the study, with a median treatment duration of 3.8 mo (range: 0.1–23.2). The majority of pts discontinued ibr (66%) and durv (50%) due to progressive disease. The

Table (abstract 7): Best Response and Survival

<table>
<thead>
<tr>
<th>FL</th>
<th>GCB DLBCL</th>
<th>Non-GCB DLBCL</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response-evaluable pts, n</td>
<td>27</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>ORR, n (%)</td>
<td>7 (26)</td>
<td>2 (14)</td>
<td>6 (40)</td>
</tr>
<tr>
<td>CR</td>
<td>1 (4)</td>
<td>1 (7)</td>
<td>3 (33)</td>
</tr>
<tr>
<td>PR</td>
<td>6 (22)</td>
<td>1 (7)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Stable disease, n (%)</td>
<td>14 (52)</td>
<td>3 (21)</td>
<td>0</td>
</tr>
<tr>
<td>Median DOR, mo (95% CI)</td>
<td>11.3 (6.3–NR)</td>
<td>NR (5.5–NR)</td>
<td>NR (2.5–NR)</td>
</tr>
<tr>
<td>All pts, n</td>
<td>27</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Median PFS, mo (95% CI)</td>
<td>10.2 (4.7–13.1)</td>
<td>2.9 (1.2–5.1)</td>
<td>4.1 (1.2–10.1)</td>
</tr>
<tr>
<td>Median OS, mo (95% CI)</td>
<td>5.5 (3.2–13.2)</td>
<td>7.3 (1.8–NR)</td>
<td>18.1 (7.8–NR)</td>
</tr>
</tbody>
</table>
most frequent grade 3–4 treatment-emergent AEs were neutropenia (FL: 4/27 pts [26%], dermatitis{x: -126.7875} 4 [15%]; 3 [9%]; rash [2 (7%); 4 [12%]; fatigue [1 (4%); 4 [12%]; atrial fibrillation [1 (4%); 3 (9%)], and peripheral edema [1 (4%); 3 [9%]). Immune-related AEs were reported for 12/21 (58%) pts. No treatment-related grade 5 AEs were reported. PD-L1 expression was performed in 28 pts (FL: n = 15; GCB DLBCL: n = 5) with baseline tumor samples. PD-L1 expression in tumor or immune cells in the tumor microenvironment did not show a relationship with response.

Conclusion: Combination of 560 mg ibr once-daily and 10 mg/kg durr every 2 wk demonstrated only moderate activity in FL and GCB DLBCL but activity in non-GCB DLBCL as expected with single-agent ibr. Longer follow-up and a better understanding of tumor biology are needed to determine if PD-L1 inhibition provides additional benefit.


8 Oral
Phase III study of the AZAR antagonist NIR178 (PBF-509) combined with the anti-PD-1 monoclonal antibody spartalizumab in patients with advanced NSCLC


Background: Adenosine 2A receptor (ADAR) dampens immune responses when bound to extracellular adenosine. Adenosine in the tumor microenvironment can suppress T- and NK-cell function via AZAR. NIR178 (PBF-509), an oral small-molecule AZAR antagonist that selectively inhibits ADAR, has shown an 11% response rate as a single agent in a recent Phase I trial. Since combined immunotherapies may enhance antitumor efficacy, this Phase III trial evaluated NIR178 combined with the anti-PD-1 monoclonal antibody spartalizumab (PDR001) in patients (pts) with relapsed/refractory advanced NSCLC (NCT02403353).

Materials and Methods: Pts (ECOG PS 0–1) had received 1 ≥ prior line of therapy; EGFR/ALK-mutant pts had failed prior tyrosine kinase inhibitor therapy. Objectives: primary – determine MTD of NIR178 in combination with spartalizumab; Celgene, Johnson & Johnson, Takeda, and Pharmaceuticals LLC, an AbbVie company.

Results: An oral 20 March 2018 (data cut-off), 25 pts had been treated: median age 64.0 (range 49–85) years; 60% male; 76% ECOG PS 0; 1/125 (44%) pts received prior anti-PD-L1 therapy. A total of 22/25 (88%) pts had discontinued (due to progression [n = 12], death [n = 4], unrelated to study treatment [adverse events [n = 4]; withdrawal of consent [n = 2]; other [n = 2]); 1 partial response (DL 1A; PD-L1 TPS unknown), both ongoing in anti-PD-1/1A-naive pts; stable disease was reported in 6/14 (43%) anti-PD-1/1A-naive pts and in 7/11 (64%) anti-PD-1/1A-pretreated pts.

Conclusion: NIR178 in combination with spartalizumab was well tolerated; AEs were manageable and there were no Gr 4 treatment-related AEs. The MTD was determined as NIR178 240 mg BID + spartalizumab 400 mg Q4W. Clinical benefit was observed in immunotherapy-exposed and -naïve pts.

Abstracts, 30th EORTC-NCI-AACR Symposium

Plenary Session 6 (Thursday, 15 November 2018) e18

European Journal of Cancer 103S1 (2018) e13–e20

response criteria (irRC) and pharmacodynamic (PD) assessment using FACS analyses for assessment of immune cell subsets in peripheral blood.

**Results:** At data cut-off (19 June '18), 9 patients with metastatic solid tumours (pancreatic = 3; colorectal = 2; uterine adenosarcoma = 1; squamous cell carcinoma = 1; endometrial carcinoma = 1; adenocortical carcinoma = 1) were enrolled in the escalation phase (at 25 mg, 3 at 50 mg). Maximum tolerated dose (MTD) was determined to be 25 mg due to two dose-limiting toxicities (DLTs) in the 50 mg cohort (hypoammonemia/multi-organ failure/death and infusion reaction/pulmonary oedema). Thus, the RD for the expansion phase is 25 mg. Thus far, of the 6 patients enrolled at this dose, only 1 patient discontinued treatment due to a DLT (pneumonitis). Most frequent drug-related adverse events were fatigue (7 events in 4 subjects), anaemia (3 in 2), and transaminitis (2 in 2). Severe events were common in the 25 mg cohort; however, there was only one severe event in the 25 mg cohort (pneumonitis). Of the 5 subjects available for evaluation of anti-tumour activity, one with micrometastable stable (MSS) micrRC had a partial response (PR) with an 86% reduction in the size of target lesions at the 26-week scan. Evidence of DC stimulation, NK cell and/or T cell activation was observed in peripheral blood of some patients treated with the combination.

**Conclusions:** A 25 mg dose of pixatimod was sufficiently well tolerated in combination with nivolumab and thus is expected to be used for the nivolumab expansion cohort. Preliminary activity in MSS micrRC warrants further study. Latest safety, efficacy and PD data will be presented.

**Conflict of interest:** Corporate-sponsored Research: Zucero Therapeutics Ltd are co-funding the study. Other Substantive Relationships: DH, EH and KD are consultants or employees of Zucero Therapeutics Ltd.

### 11 Oral

**ADV15155: Phase 1 study of prexasertib (LY2606368), a CHK1/2 inhibitor, in pediatric patients with recurrent or refractory solid tumors, including CNS tumors: a report from the children's oncology group phase 1/pilot consortium**

C. Wetmore1, T. Cash2, X. Li3, C. Minard2, E. Fox2, B. Weigel2, Children's Oncology Group. 1Phoenix Children's Hospital/University of Arizona, Hematology/Oncology/Bone Marrow Transplantation, Phoenix, USA; 2Children's Healthcare of Atlanta/Emory University, Aflac Cancer and Blood Disorders Center, Atlanta, USA; 3Children's Oncology Group, Biostatistics, Monrovia, USA; 4Baylor College of Medicine, Biostatistics, Houston, USA; 5Children's Hospital of Philadelphia, Oncology, Philadelphia, USA; 6University of Minnesota/Masonic Cancer Center, Hematology/Oncology/BMT, Minneapolis, USA

**Background:** Checkpoint kinase proteins 1 (CHK1) and 2 (CHK2) are activated in response to DNA damage or replication stress inhibition. CHK1 abrogates the DNA damage response checkpoint, allowing cells that have sustained DNA damage to prematurely enter mitosis and undergo mitotic catastrophe due to incompletely replicated chromosomes. Prexasertib is a novel, second generation, selective dual inhibitor of CHK1 and CHK2 that was designed as a first-in-pediatrics phase 1 study of prexasertib to determine the maximum tolerated dose (MTD), establish a recommended phase 2 dose (RP2D), and to describe the dose-limiting toxicities (DLTs) in pediatric patients with recurrent or refractory solid or CNS tumors.

**Materials and Methods:** Prexasertib was administered intravenously on days 1 and 15 of a 28-day cycle. Four dose levels, 80, 100, 125, and 150 mg/m², were evaluated using a rolling-six design. Samples for pharmacokinetic (PK) analysis were obtained during cycle 1. Pharmacodynamic (PD) analyses including immunohistochemistry (IHC) of CHK1 and gammaH2AX in peripheral blood mononuclear cells, and IHC of CHK1/2 expression and IHC or sequencing for TP53 deletions or mutations in gammaH2AX in peripheral blood mononuclear cells, and IHC of CHK1/2 gene expression signature was observed in PBMCs after 3 h. Consistent with covalent binding of SY-1365 to CDK7, occupancy, dose-dependent transcriptional downregulation of the SY-1365 target, SY-1365 gene expression signature was observed in PBMCs pre/post-treatment. Clinical activity was evaluated by RECIST 1.1.

**Results:** Twenty-four patients enrolled; all were eligible. Four patients were ineligible due to not having received the required percentage of study drug. The median age was 13 years (range: 2–20) with 21 males and 3 females. All patients received at least one prior treatment regimen, and all had radiologic or clinical evidence of progressive disease prior to enrollment. Eleven patients had solid tumors [hodgdomsarcoma (N = 4), carcinoma (N = 3), Ewing sarcoma (N = 1), osteosarcoma (N = 1), non-hodgdomsarcoma soft tissue sarcoma (N = 1), Wilms tumor (N = 1)] and 13 patients had CNS tumors [glioblastoma multiforme high-grade glioma (N = 4), ependymoma (N = 3), primitive neuroectodermal tumor (N = 2), medulloblastoma (N = 1), choroid plexus carcinoma (N = 1), oligodendroglioma (N = 1), pineocytoma (N = 1)]. There were no cycle 1 DLTs observed. Common grade 3/4 toxicities were all hematologic in nature except for one patient (5%) who experienced a grade 3 increase in alanine aminotransferase (ALT). Neutropenia occurred in all patients during cycle 1, 20/20 (10%) with grade 3 and 18/20 (90%) with grade 4. Additional cycle 1 hematologic toxicity included anaemia (10% grade 3) lymphopenia (20% grade 3, 5% grade 4), and thrombocytopenia (10% grade 3, 15% grade 4).

**Conclusions:** While the MTD of prexasertib was not defined by this study, the 150 mg/m² administered intravenously on day 1 and 15 of a 28 day cycle was tolerable and exceeds the adult RP2D (105 mg/m²). Hematologic toxicity was reversible. PK and PD will be used to determine if 150 mg/m² is the RP2D in children and adolescents with solid tumors including CNS tumors.

**Conflict of interest:** Advisory Board: Data safety monitoring committee member for Helsinn, SA for supportive care therapy (EF). Corporate-sponsored Research: Eli Lilly support of a Phase I clinical trial (aliserterib) currently underway (CW).

**11 Oral**

**Prexasertib is a novel, second generation, selective dual inhibitor of CHK1/2 in pediatric patients with recurrent or refractory solid tumors, including CNS tumors: a report from the children’s oncology group phase 1/pilot consortium**

D. Juric1, K.P. Papadopoulos1, A. Tolcher2, K.T. Do3, D. Orlando1, W. Zamboni1, G. Hodgson1, E. di Tomaso1, K. Stephens1, D. Roth1, G.I. Shapiro1. 1Massachusetts General Hospital, Hematology/Oncology, Boston, USA; 2South Texas Accelerated Research Therapeutics, Medical Oncology, San Antonio, USA; 3Dana-Farber Cancer Institute, Medical Oncology, Boston, USA; 4Syros Pharmaceuticals, Inc., Clinical Research and Development, Cambridge, USA

**Background:** SY-1365 is a selective, potent, covalent inhibitor of CDK7, a cyclin dependent kinase implicated in cancers with transcriptional dependence, including breast and ovarian cancers. Preclinical models of SY-1365, including analyses of CDK7 target occupancy and effects on gene expression, provided an approach to evaluate mechanistic effects of CDK7 inhibition in a phase 1 dose escalation study.

**Methods:** Pts with advanced solid tumors who had sustained standard of care treatment options received SY-1365 IV once or twice weekly for 3 or 4 weeks per cycle. Safety including cycle 1 DLTs was evaluated. Plasma PK was assessed on initial and repeat exposure at multiple Cycle 1 timepoints. Target CDK7 occupancy was determined by nanoString in PBMCs and optional paired tumor biopsies using a Meso Scale Discovery (MSD) immunoassay. An SY-1365-responsive gene expression signature was established by NanoString in PBMCs pre/post-treatment. Clinical activity was evaluated by RECIST 1.1.

**Results:** As of May 9, 2018, 19 pts were enrolled (5 ovarian, 2 breast, 2 pancreatic and 10 other). The majority were female (63%) and median age was 62 (range 26–87). Dosing ranged from 2 to 64 mg/m², with a median treatment time of 38 days (range 3–127). Adverse events were generally low grade and reversible; frequently reported (≥15%) AEs including headache (42%), GI (vomiting (42%), nausea (26%), diarrhea (16%)), hypotension (28%), fatigue (21%), cough, dyspnea, anemia and hyponaglycerinaemia (each 16%). Notably, AEs of myelosuppression were not reported to date. Headache was reported only at the highest dose levels, including one DLT of reversible Gr 3 headache at 64 mg/m². There were no treatment-related SAEs or deaths. SD was observed in 4 of 9 evaluable patients. A linear relationship was observed between SY-1365 dose, plasma Cmax and AUC. SY-1365 plasma concentrations were similar on days 1 and 15. SY-1365 plasma elimination t½ was 16.5±2.7 h. Dose-dependent target occupancy in PBMCs was observed, with maximum occupancy after 3 h. CDK7 occupancy remained above 50% at 72 h at the highest dose levels tested, consistent with covalent binding of SY-1365. CDK7 occupancy was also observed in tumor tissue, consistent with PBMCs. Consistent with CDK7 occupancy, dose-dependent transcriptional downregulation of the SY-1365 gene expression signature was observed in PBMCs after 3 h.

**Conclusions:** SY-1365 is a first-in-class selective and potent inhibitor of CDK7 in p11 clinical development (NCT03134638). Doses ranging from 2 to 64 mg/m² demonstrated linear PK without accumulation. Proof of mechanism was established based on CDK7 target occupancy and dose-dependent modulation of gene expression in PBMCs at tolerable doses where AEs were predominantly low grade, reversible and generally manageable. Updated data will be presented.

Poster Abstracts
Wednesday, 14 November 2018 12:50–13:30
POSTERS IN THE SPOTLIGHT SESSION
Poster in the Spotlight I

19 (PB-199)  Poster Spotlight
Discovery and functional characterization of small molecule inhibitors of SWI/SNF ATPase activity in BRG1/SMARCA4-Deficient non-small cell lung cancers

Members of the ATP-dependent SWI/SNF chromatin remodeling complexes are among the most frequently mutated genes across various cancers informing critical roles of their dysregulation toward the malignant state. While elucidating the mechanisms of SWI/SNF mutations remains a significant area of investigation, recent studies have also revealed an important role for the remaining SWI/SNF activity in supporting the growth of SWI/SNF-mutant cancers. In particular, the discovery of synthetic lethality between BRM/SMARCA2 and BRG1/SMARCA4, the highly homologous and mutually exclusive catalytic subunits of SWI/SNF complexes, has driven great interest in pursuing the therapeutic targeting of BRG1 in BRG1-mutant deficient cancers. We report for the first time the discovery and functional characterization of allosteric small molecule dual inhibitors of BRM and BRG1 ATPase activity, BRM011 and its structurally related analogs display cellular activity in modulating BRM-dependent gene expression and inducing growth inhibition in BRG1-mutant lung cancer models. Genome wide assessments show that BRM011 treatment induces specific changes in chromatin accessibility and gene expression profiles similar to genetic depletion of BRM. Overall, these studies not only elucidate the previously unexplored feasibility of chemically modulating the enzymatic activity of such a complex and unprecedented target, but also provide fundamental tools for further dissecting SWI/SNF function in cancers, normal tissues and other disease contexts.

No conflict of interest

20 (PB-200)  Poster Spotlight
Phase I dose expansion results from a multicenter, open-label study of the MET inhibitor capmatinib (INC280) in adult patients with MET-dysregulated advanced NSCLC
1. Novartis Institutes for BioMedical Research, Oncology, East Hanover, USA; 2. Novartis Institutes for BioMedical Research, Protein Sciences, Emeryville, USA; 3. Novartis Institutes for BioMedical Research, Preclinical Safety, Basel, Switzerland; 4. Netherlands Cancer Institute, Division of BioMedical Research, PreClinical Safety, Amsterdam, The Netherlands; 5. Novartis Pharmaceuticals Corporation, Novartis Oncology, East Hanover, USA; 6. Seoul National University Hospital, Biomedical Research Institute, Seoul, South Korea.

Background: MET dysregulation (exon 14 skipping mutation or gene amplification) occurs in 3–4% of NSCLC; concurrent MET amplification (median gene copy number [GCN] ≥10) has been reported in 15% of cases with MET mutation. MET dysregulation has been described as a negative prognostic factor and predictor of poor response to standard systemic therapies. In the dose escalation part (NCT01324479), capmatinib, a potent and selective oral MET inhibitor, was well tolerated with a manageable safety profile; RP2D 400 mg BID tablets or 600 mg BID capsules. We present primary data from combined expansion groups of patients (pts) with advanced MET-dysregulated NSCLC.

Materials and Methods: Eligible pts (aged ≥18 years; ECOG PS ≤2) with MET-dysregulated advanced NSCLC, defined as either, (1) MET IHC 2+ or 3+; or (2) MET copy number GCN ≥150, or MET exon 14 skipping mutations (median gene copy number [GCN] ≥10) has been reported in 15% of cases with MET mutation. MET dysregulation has been described as a negative prognostic factor and predictor of poor response to standard systemic therapies. In the dose escalation part (NCT01324479), capmatinib, a potent and selective oral MET inhibitor, was well tolerated with a manageable safety profile; RP2D 400 mg BID tablets or 600 mg BID capsules. We present primary data from combined expansion groups of patients (pts) with advanced MET-dysregulated NSCLC.

Conclusions: Capmatinib showed promising antitumor activity in pts with heavily pretreated, MET-dysregulated advanced NSCLC. Clinical benefit was particularly prominent if MET copy number ≥3+ or H-score ≥6. PFS was 3.0, 3.8, 3.9, and 18.6 months (all censored) for pts with MET CCN ≥6, PFS was 3.0, 3.8, 3.9, and 18.6 months (all censored) for pts with MET GCN ≥6 (n = 37) there was 1 CR and 10 PRs (ORR 29.7%; DCR 62.2%). In pts with MET GCN ≥6 (n = 15) there was 1 CR and 3 PRs (ORR 53.3%; DCR 80.0%). A response to capmatinib (CR or PR) was reported in 4 pts with retrospectively identified MET exon 14 skipping mutations were present, highlighting the key role of precise biomarker selection. A pivotal Phase II study (NCT02414139) is ongoing to confirm the efficacy of capmatinib in MET-dysregulated and MET-amplified advanced NSCLC.


21 (PB-201) Poster Spotlight Utility of a selective SHP2-inhibitor in KRAS-mutant cancer
H. Hao1, H. Wang1, C. Liu1, S. Kovats2, R. Velazquez2, H. Lu1, B. Pant1, J. I. de, M. Fleming1, M. Shirley1, M. Lamarche2, S. Moody1, S. Silver-Brown1, G. Caponigro1, T. Abrams1, P. Hammerman1, J. Williams1, J. Engelman1, S. Goldoni1, M. Mohseni1, 1Novartis Institute of Biomedical Research, Oncology, Cambridge, USA; 2Novartis Institute of Biomedical Research, Global Discovery Chemistry, Cambridge, USA

Background: RTK-feedback reactivation of the MAPK pathway by MEK inhibition in Kras-mutant cancers can be effectively blocked by targeting SHP2, suggesting that SHP2 inhibition alone can alter activity of mutant KRas. The in vivo utility of the selective allosteric SHP2 inhibitor, SHP099, has made it possible to further interrogate single agent activity of SHP2 within the Kras mutant context.

Methods: Efficacy of Kras mutant models to SHP099 and RTK pathway inhibitors (Lapatinib, BGJ398, INC280) was evaluated in vitro using CellTiter-Glo, under both monolayer conditions and in matrigel or ultra-low inhibitors (Lapatanib, BGJ398, INC280) was evaluated in vitro using CellTiter-Glo, under both monolayer conditions and in matrigel or ultra-low attachment plates. RNAseq was performed in vitro from SHP099 or Trametinib treated for 19 hours in the Kras-mutant, MiaPaca-2 cell line. In vivo efficacy of SHP099 in Kras-mutant xenograft models: implantation of subcutaneous or orthotopically was performed at 50–100 mg/kg, daily for >10 days, during which caliper measurements and bodyweights were collected twice weekly. At the end of treatment, tumors were collected and evaluated for DUSP6 and phospho-ERK. Genetic depletion of SHP2 by CRISPR or by shRNA was evaluated in vitro in 2D, 3D and in vivo in the KRAS-mutant cell line, MiaPaca-2. Phenotypic rescue of SHP099 efficacy was performed by overexpression of constitutive active SOS1 (SOS1-F).

Results: Inhibition of SHP2 in KRAS mutant cancers is efficacious across variants (G12C, G12D, G12V, G13D, and Q61H). Sensitivity of KRAS-driven cancers towards the allosteric SHP2-inhibitor, SHP099, has revealed a dependency for mutant KRAS on upstream signaling. We further demonstrate that efficacy is tumor cell autonomous as genetic deletion of SHP2 recapitulated SHP099 activity. Mechanistically, overexpression of constitutive active SOS1 rescues SHP2 inhibition highlighting the dependency of mutant KRAS, and the downstream signaling, on SHP2. Interestingly, cell lines harboring KRAS mutations with low intrinsic GTP hydrolysis, i.e., KRAS(G12S), also display in vivo efficacy upon SHP099 treatment with concomitant downregulation of many growth factors and suppression of pERK in vitro.

Conclusions: Taken together, these data support that mutant KRAS depends on upstream signaling from SHP2 and support a potential opportunity for treatment of KRas-mutant cancers.

No conflict of interest

22 (PB-202) Poster Spotlight PLX3984 selectively disrupts BRAF-dimers and RAS-independent BRAF mutant-driven signaling
Z. Yang1, Y. Gao1, W. Su1, R. Yang2, N. Na1, Z. Yang3, C. Zhang3, A. Rymarc1, A. Tao1, N. Torres1, R. Mogriskin1, H. Zhao1, Q. Chang1, B. Qeriqi1, E. de Stanchina1, M. Barbacid2, G. Bollag1, N. Rosen1.
1Memorial Sloan Kettering Cancer Center, Molecular Pharmacology, New York, USA; 2Memorial Sloan Kettering Cancer Center, Department of Medicine, New York, USA; 3Flexion Inc., Berkeley, USA; 4New York University, NYU Langone Medical Center, New York, USA; 5Centro Nacional de Investigaciones Oncologicas CNIO, Molecular Oncology Programme, Madrid, Spain

Background: BRAF mutants and fusions are expressed in ~8% of human tumors. Most all known activating BRAF mutants and fusions signal as RAS-independent, constitutive, active dimers with the single exception of BRAF V600 alleles which function as active monomers in cells with low levels of RAS activation. Current RAF inhibitors potently inhibit BRAF V600 mutant monomers but their ability to inhibit RAF dimers is limited by their induction of receptor cooperativity when bound to one of the proteins in the dimer.

Material and Methods: We tested the activity of PLX3984 and six other RAS inhibitors in four cell lines, in which the ERK signaling is driven by mutant BRAF monomer, mutant BRAF dimers, or WT BRAF dimers activated by IRT or mutant RAS. We determined the activity of PLX3984 against the second protomer of the BRAF dimers of which the other protomer was occupied by LGX818. The effects of PLX3984 on the formation of different RAS-dependent WT RAF homo- or heterodimers and RAS-independent mutant RAF homo- or heterodimers were determined by co-IP assays. We also studied the mechanism of the different properties of PLX3984 against BRAF or CRAF dimers based on the structural data. Last, we tested the activity of PLX3984 in a panel of tumor cell lines and PDX models driven by RAS-dependent or -independent BRAF mutants.

Results: We show here that PLX3984 inhibits signaling driven by mutant BRAF dimers by specifically disrupting BRAF-containing dimers, including BRAF-BRAF homodimer and BRAF-CRAF heterodimers, but not CRAF homodimers or ARAF-containing dimers. The differences in the amino acid residues situated at the N-terminus of the kinase domain between the RAF isoforms appear to be responsible for this differential vulnerability. This feature of PLX3984 allows it to inhibit both activating BRAF monomers (V600 mutant alleles) and dimers, but paradoxically activate CRAF homodimers by binding to one of the two CRAF protomers. Thus, this drug has minimal effects on ERK signaling in wild type RAF cells, in which it disrupts and inhibits BRAF dimers, but activates CRAF homodimers. In contrast, in tumors driven by activated mutant BRAF dimers or fusion BRAF dimers, CRAF homodimer levels are vanishingly low because of feedback inhibition of RAS signaling. In these tumors, ERK signaling is dominantly driven by mutant BRAF dimers which are disrupted by this drug; thus, the signaling and tumor growth can be effectively inhibited by the drug.

Conclusions: PLX3984 is the first drug that selectively disrupts and therefore inhibits BRAF-containing dimers as well as BRAF V600 monomers in tumors at doses that have almost no effect on WT dimers in normal cells.


Thursday, 15 November 2018 13:00–13:30

POSTERS IN THE SPOTLIGHT SESSION
Poster in the Spotlight II

23 (PB-203) Poster Spotlight Analysis of cell-free plasma DNA to identify tumors with microsatellite instability and exceptionally high tumor mutation burden in patients treated with PD-1 blockade
A. Georgiadis1, J. N. Durham2,3,4, L. Kefer1, B. R. Bartlett1, M. Zielonka1, D. Murphy1, J. R. White1, S. Lu1, E. Vermeer1, F. Ruan1, D. Riley1, R. A. Andersen2,5, S. V. Angiuoli1, S. Jones1, V. E. Velculescu1, D. T. Le2,3,4, L. A. Diaz Jr.1, M. Sausen1, 1Personal Genome Diagnostics, Research and Development, Baltimore, USA; 2Johns Hopkins, Bloomberg-Kimmel Institute for Cancer Immunotherapy, Baltimore, USA; 3Johns Hopkins, Sidney Kimmel Comprehensive Cancer Center, Baltimore, USA; 4Johns Hopkins, Swim Across America Laboratory, Baltimore, USA; 5Johns Hopkins, Ludwig Center and Howard Hughes Medical Institute, Baltimore, USA; 6Johns Hopkins, Department of Pathology, Baltimore, USA; 7Memorial Sloan-Kettering Cancer Center, Division of Solid Tumor Oncology, New York, USA

Background: Microsatellite instability (MSI) and tumor mutation burden (TMB) are promising pan-tumor biomarkers used to select patients for treatment with immune checkpoint blockade; however, real-time sequencing of unsegregable or metastatic solid tumors is often challenging. Here we report a plasma-based next generation sequencing (NGS) approach using a 98 kb targeted panel of 58 genes for the detection of MSI and exceptionally high TMB in cancer patients with advanced disease.

Material and Methods: Our approach utilized a hybrid-capture-based, targeted NGS analysis of a pan-cancer gene panel, designed at Personal Genomics Diagnostics (PGDx), including targeted mononucleotide repeat loci for MSI detection. We developed a multifactorial error correction method to detect sequence alterations in plasma, and used a novel peak finding
algorithm to identify rare MSI frameshift alleles in cell-free DNA (cfDNA). To demonstrate its capacity to predict response to immune checkpoint blockade using baseline plasma, 16 samples from patients treated with PD-1 blockade therapy, both with or without mismatch repair deficiency, were analyzed for the presence of MSI and exceptionally high TMB. In addition, serially collected plasma was used to assess molecular remission and circulating tumor DNA (ctDNA) dynamics in patients during PD-1 blockade treatment.

Results: Using the error correction and peak finding approaches developed by PGxD to detect rare mutations in plasma derived cfDNA, we demonstrated a per-patient specificity of 99.4% (162/163) and 100% (163/163), respectively for MSI and exceptionally high TMB. In the PD-1 blockade treated patient cohort, MSI and TMB status demonstrated the capability to predict progression-free survival using baseline plasma samples ($n = 16$, $p = 0.01$ and $0.004$, respectively). Additionally, we analyzed longitudinal cfDNA samples for reduction in protein biomarkers, and analyzed cfDNA for MSI mutant allele fraction, and mutational burden to develop a prognostic signature for patients who achieved a durable response to PD-1 blockade ($p = 0.01$, $p = 0.032$, $p = 0.013$, respectively).

Conclusions: Here we have described the development of a method for simultaneous detection of MSI and exceptionally high TMB directly from cfDNA. These data provide feasibility for pan-cancer screening and monitoring of patients who exhibit these biomarkers and may respond to immune checkpoint blockade.

Conflict of interest: Board of Directors: Luis Diaz and Victor Velculescu are on the Board of Directors for Personal Genome Diagnostics. Other Substantive Relationships: All authors affiliated with Personal Genome Diagnostics are employees of Personal Genome Diagnostics.

24 (PB-204) Poster Spotlight
Chromatin destabilization by CBL0137 and panobinostat leads to complete tumour regression of childhood neuroblastoma in immunocompetent transgenic mice

L. Xiao1, J. Murray1, A. Ehtedaie2, C. Somers1, C. Mayo1, L. Gambile3, R. E. Ethridge4, G. Eden2, S. Allison1, S. Sarraf1, A. Koscielny1, D. Ziegler1, M. Henderson1, N. Issaeva1, K. Gurvov1, A. Gudkov3, M. Haber1, M. Norris1, 1Children’s Cancer Institute, Molecular Diagnostics, Sydney, Australia; 2Children’s Cancer Institute, Targeted Therapy, Sydney, Australia; 3Children’s Cancer Institute, Experimental Therapeutics, Sydney, Australia; 4Yale Cancer Centre, Yale School of Medicine, Division of Otolaryngology, Surgery Department, New Haven, Australia; 5Roswell Park Cancer Institute, Department of Cell Stress Biology, Buffalo, USA

Background: Neuroblastoma is the most common extracranial solid tumour in children. High-risk neuroblastomas, which have less than 50% survival rates, are frequently characterised by amplification of the oncogene MYCN. Using the highly clinically relevant Th-MYCN transgenic neuroblastoma mouse model representative of high-risk neuroblastoma, we have previously found that CBL0137, which is a safe, non-DNA damaging drug currently in phase I trials for adult cancers, significantly extended survival rates of these mice in a dose-dependent manner. CBL0137 belongs to a novel class of anti-neoplastic agents called curaxins, which can induce chromatin damage leading to chromatin destabilization, cell-cycle arrest, and activation of interferon (IFN) pathways.

Material and Methods: Drug synergy in vitro was determined by colony and cytotoxicity assays. Animal models included the Th-MYCN transgenic neuroblastoma model, and xenograft models for mixed lineage leukaemia (MLL)-rearranged leukaemia and diffuse intrinsic pontine glioma (DIPG). Histone eviction was visualised in cells transduced with fluorescence-tagged histone 1 protein. DNA damage repair inhibition was quantified by pulsed-field gel electrophoresis. RNA-seq was used to determine gene expression in Th-MYCN murine and human CBL0137 and panobinostat treatment. Combination of CBL0137 and panobinostat produced strong synergy to reduce cell viability and clonogenicity in neuroblastoma cells in vitro. More strikingly, the combination eradicated established neuroblastoma in 100% Th-MYCN mice tested, which is the most significant result we have ever obtained in this aggressive neuroblastoma model. Mechanistic studies show that panobinostat markedly enhanced chromatin destabilization induced by CBL0137, resulting in histone eviction and DNA repair suppression. Furthermore, the combination elicited a rapid and robust induction of interferon-mediated responses, increasing expression of interferon-induced genes, such as Ifit1, Ifit3, and Ifi3lb, by more than 200 fold, strongly indicating that CBL0137/panobinostat may activate anti-tumour immunity. Using preclinical xenograft models of two other aggressive paediatric tumours, DIPG and MLL-rearranged leukaemia, we also demonstrated that CBL0137 and panobinostat synergised strongly to slow tumour progression and significantly extend survival.

Conclusions: Our studies have identified CBL0137 and panobinostat as a highly effective drug combination for neuroblastoma and other aggressive paediatric malignancies. This combination likely halts tumour growth through a two-pronged attack: chromatin destabilisation and activation of interferon pathways. Our results will greatly facilitate clinical development of effective and non-toxic therapies for childhood cancer.

Conflict of interest: Ownership: Cleveland BioLabs stock ownership.

25 (PB-205) Poster Spotlight
MAPKAPK5 inhibition suppresses YAP-driven tumorigenesis

M. H. Kim1, J. Seo2, H. Hwang1, S. K. Kim3, J. Kim1. 1Yonsei University College of Medicine, Division of Medical Oncology, Department of Internal Medicine, Seoul, Korea; 2KAIST, Graduate School of Medical Science and Engineering, Daejeon, Korea; 3Yonsei University College of Medicine, Department of Pathology, Seoul, Korea

Background: Recent evidence suggests that the Hippo pathway effector, YAP, critically involves tumorigenesis in human malignancies. The oncogenic YAP activation is induced by various molecular mechanisms, including loss of LATS1/2, the negative regulators of YAP. We aimed to find therapeutic targets for tumors with LATS1/2 loss by RNA interference (RNAi) screening.

Materials and Methods: We deleted LATS1/2 alleles in human RPE1 cells by CRISPR-Cas9, and the cells were subjected to an image-based kinase RNAi library screening for identifying YAP downregulating hits. The influence of target kinase activity on nuclear localization, protein stability, and tumorigenic potential of YAP was investigated.

Results: The LATS1/2 knockout in RPE1 cells caused nuclear localization and transcriptional activation of YAP. In contrast to wild-type, LATS1/2-null RPE1 cells showed robust cell proliferation on matrigel culture and tumor formation on xenograft model. Our RNAi screening firstly found that inhibition of MAPKAPK5 (MK5) provokes YAP degradation and cytoplasmic retention. MK5 physically interacted with YAP and prevented its pro-tumorigenic degradation. The MK5 particularly involved $CK1_{\alpha_{i}i}$-dependent YAP degradation pathway that appears independent from LATS1/2 activity. The MK5-dependent gene signature, derived by RNA-seq of MK5 knockdown experiment, correlated with poor prognosis of YAP-driven cancers, malignant mesothelioma (MM) and uveal melanoma (UVM), in TCGA database. The MK5 depletion significantly suppressed growth of LATS1/2-null RPE1 tumor xenograft, as well as proliferation of MM and UVM cell lines.

Conclusions: These results propose MK5 as a novel component of YAP protein stability regulation. We suggest that MK5 inhibition is a promising therapeutic strategy for YAP-driven tumors.

No conflict of interest
CP sensitive OC xenografts. CP was dosed 50 mg/kg IV once weekly (QW). SY-1365 was dosed 30 mg/kg IV IV. 4-24 h after CP. Tumor growth inhibition (TGI) was determined by comparing the average change in tumor volume in drug- versus vehicle-treated mice on the last dosing day.

**Results:** Treatment of OC cell lines in vitro with varying concentrations of SY-1365 and CP revealed synergistic interactions in all 3 cell lines. In vivo, SY-1365 and CP each induced measurable TGI in OC xenografts as single agents at sub-maximal therapeutic doses, whereas regressions were observed when the same doses of both agents were used in combination (Table).

To explore a potential mechanistic basis for synergy, RNA expression levels of HR genes BRCA1/2, ATM, ATR, and RAD51, important for sensing and repairing double strand breaks created by CP, were assessed at 0, 6, and 16 hours (h) after treatment with 50 nM SY-1365. Relative to 0 h, lower RNA expression was evident for most genes by 6 h; by 16 h all genes were down regulated in all cell lines (average 2.2-fold; range 1.1–4.6). Similar mechanistic analyses in OC xenograft models are ongoing.

**Conclusions:** SY-1365 is synergistic with CP in OC cell lines in vitro and enhances CP activity in OC xenografts in vivo. SY-1365 transcriptionally downregulates key mediators of the HR pathway, including BRCA1/2, in OC cell lines in vitro. Taken together these results suggest that SY-1365 may impede DNA damage responses and DNA repair in OC patients, and support the potential for combination strategies aimed at exploiting this mechanism of action. SY-1365 is currently being assessed in a phase 1 trial in adult patients with advanced solid tumors (NCT03154638) with a planned expansion cohort in OC to explore SY-1365 in combination with carboplatin.

**Conflict of interest:** Ownership: All authors are employees and share-holders in Syros Pharmaceuticals.

---

**51 (PB-002) Poster Phase II trial of pembrolizumab in patients with solid tumors functionally competent or deficient for the Fanconi Anemia repair pathway**

M. Villalona1, J.P. Diaz1, W. Duan2, Z. Diaz1, E.D. Schroeder1, S. Aparo1, F. Vargas Madueno1, A. Alonso1, S. Cuitiva1, F. Albrecht1, S. Venkatappa1, V. Guardiola1, M. Troner1, G. Walker3, G. Narasimhan4.

**Background:** Based on the activity of immune checkpoint inhibitors (ICI) in mismatch repair deficient tumors we are evaluating the interplay between homologous recombination (HR) repair deficiency, and solid tumor response to ICI using an all-inclusive functional triple stain (FANCD2/DAPK/IK67) immunofluorescence assay of the Fanconi Anemia pathway (FATS1).

**Material and Methods:** We are conducting a phase 2 trial (NCT03274661) of pembrolizumab (PEM) in patients with metastatic solid tumors progressing on standard of care therapy and for whom PEM does not have an FDA approved indication. FATS1 is performed in all patients to evaluate if it is a useful patient selection biomarker. Patients with microsatellite instability (MSI) high tumors are not eligible. The primary objective is to evaluate objective response rate (ORR, CR+PR) by Immune Response Criterial (no PD). Regional variation functionally competent and functionally deficient (FATS1 negative) tumors, with the exploratory hypothesis that patients with FATS1 negative tumors will have better clinical outcome. Secondary objectives are 20-week progression free survival and overall survival. Exploratory objectives include evaluation of mutation load, stool analyses for microbiome composition (before and after treatment), and alterations in HR repair genes. We utilized a two-stage phase II trial design to detect an ORR ≥20% in the whole population tested vs. the null hypothesis that the true ORR ≤5%. At least 2 of the first 20 evaluable patients should have an objective response in order to proceed to full accrual of 39 evaluable patients. Better outcomes in the FATS1 negative group would support a biomarker selection population approach.

**Results:** Starting Nov 2017 29 patients (23F, 6M; median age 60[36–83] have enrolled. The median number of prior regimens was 3 [0–7]. Primary Dx include ovarian/fallopian (11), endometrial (4), colorectal (3), cervix (2), pancreatic (2), breast, esophagus, small-cell lung, oral cavity, GI- neuroendocrine, small bowel and thymic carcinoma (1 each). No unexpected toxicities have occurred. Response evaluation for the first stage was completed with 2PR, 1SD, 20PD among 20 patients. FATS1 analysis in this first batch of patients showed 11 positive, 5 negative. Functional tissue required (no k67) tumors. 1PR, 5SD, 5PD occurred among the 11 FATS1(-) and 1PR, 2SD, 1PD among the 4 FATS1(-) tumors. Given the rapid pace of accrual, complete clinical data and the rest of correlative will be available at the meeting.

**Conclusions:** Meaningful antitumor activity was observed with PEM in non MSI-high malignancies with no current FDA approved indications. Evaluation of FATS1 as a predictive biomarker needs full accrual to the trial. If suggestive, further testing of the combination of ICI plus a PARP inhibitor versus individual agents in FATS1 (-) selected population is planned.

**Conflict of interest:** Corporate-sponsored Research: We receive a grant to support clinical trials costs and free pembrolizumab from Merck (manufacturer of pembrolizumab).
S5 (PB-004) Poster
Investigating the interaction of the ATR inhibitor, AZD6738, with platinum chemotherapy.

S. Hall 1, A. Lau 2, E. Dean 3, E. Martin 4, C. Huins 5, C. Otley 5, G. Veal 5, Y. Drew 5
1 Northern Institute for Cancer Research, Paul and Gordon Building, Newcastle-upon-Tyne, United Kingdom; 2 Bioscience, Onconova, IMED Biotech Unit, AstraZeneca, Cambridge, United Kingdom; 3 Early Clinical Development, IMED Biotech Unit, AstraZeneca, Cambridge, United Kingdom; 4 Drug Safety and Metabolism, IMED Biotech Unit, AstraZeneca, Cambridge, United Kingdom; 5 Department of Earth Sciences, Durham University, Durham, United Kingdom

Background: Drug combinations involving conventional chemotherapy with inhibitors of the DNA damage response may be therapeutically challenging due to potential overlapping toxicities, such as myelosuppression, and determination of the optimal sequence and schedule. AZD6738, an oral ATR inhibitor, has entered early phase clinical trials in combination with anti-cancer drugs, including carboplatin (NCT02264678). An understanding of the mechanism of interaction of AZD6738 with platinum (Pt) chemotherapy at a molecular level may be beneficial to guide future trial design and clinical use, in terms of maximising efficacy whilst minimising toxicity. In particular, quantification of Pt-DNA adducts formed in the presence of AZD6738 monotherapy would provide a novel insight into the repair and recovery of DNA following combination treatment.

Material and Methods: Sulphorhodamine B (SRB) colorimetric assays were performed to assess the growth inhibitory effect of AZD6738 alone, as well as in combination with cisplatin and carboplatin in a panel of non-small cell lung (H460, H23) and breast (MCF7, HCC1806, MDA-MB-436, MDA-MB-468) cancer cell lines. Data were evaluated by median effect analysis using CalcuSyn software (Biosoft, Cambridge, UK). Time-dependent analyses of Pt-DNA adduction formation in cells treated with cisplatin (5 μM) and AZD6738 (1.5 μM) were measured over varying incubation times using inductively-coupled plasma mass spectrometry (ICP-MS).

Results: AZD6738 in combination with cisplatin was synergistic in all the cell lines investigated. Synergy was also observed with AZD6738 and carboplatin in H23, HCC1806 and MDA-MB-468 cell lines. Co-incubation of H460 cells with AZD6738 and cisplatin (4–24 hours), compared to cisplatin alone, did not identify any significant differences in Pt-DNA adduction formation. Varying the sequencing of AZD6738 in relation to cisplatin and maintaining exposure to AZD6738 following cisplatin administration did not affect the total number of Pt-DNA adducts, assessed in H460 cells.

Conclusion: Preliminary data suggests that the in vitro synergy observed between AZD6738 and cisplatin does not involve an interaction at the level of Pt-DNA adduction formation. Work is ongoing and we will present further time-courses of Pt-DNA adduction formation in additional cell lines using a lower dose range of cisplatin to investigate if this is a consistent effect.

Conflict of interest: Advisory Board: Y.D. sits on an advisory board for AstraZeneca and has received research grant funding from AstraZeneca for projects unrelated to AZD6738. Corporate-sponsored Research: S.H. is funded by a joint Cancer Research UK and AstraZeneca grant. Other Substantive Relationships: A.L., E.D., E.M., are employees of AstraZeneca as stated in author affiliations.

S5 (PB-006) Poster
Inhibition of dUTPase induces a state of nucleotide pool and DNA repair imbalance that sensitises triple negative breast cancer cells to standard of care chemotherapies

C. Davidson 1, C. Knowlson 1, K. Savage 1, R. Wilson 1, K. Mulligan 2, P. Wilson 2, P. Johnston 1, R. Ladner 1, M. LaBonte Wilson 1
1 Queen’s University Belfast, Centre for Cancer Research and Cell Biology, Belfast, United Kingdom; 2 CV6 Therapeutics, Belfast, United Kingdom

Background: Triple negative breast cancer (TNBC) makes up 15% of all breast cancer cases and is associated with a poor prognosis due to a high rate of lymph node involvement and early visceral metastasis at the time of diagnosis. TNBC treatment is also limited by a lack of effective therapeutic options. Advances in treatment that have translated to significant improvements in outcome have been painstakingly incremental, despite advances in molecular profiling and subsequent TNBC subtyping. We previously identified the enzyme deoxyuridine triphosphate nucleotidohydrolase (dUTPase) as a critical gatekeeper that protects tumour DNA from the misincorporation of uracil following exposure to standard of care therapies that target thymidylate synthase (TS) such as 5-FU and pemetrexed. We hypothesised that targeting dUTPase and inducing uracil misincorporation during the repair of DNA damage induced by both TS-targeted therapies such as 5-fluorouracil and non-TS-targeted therapies such as the anthracyclines, represents a future clinical strategy to significantly improve clinical outcome in TNBC.

Material and Methods: Inhibition of dUTPase (DUTI) was carried out using SMARTpool siRNA or small molecule inhibition (CV6-530). Drug response was assessed by growth inhibition and clonogenic assays. DNA damage was assessed by Western blot, foci detection, and flow cytometry.

Results: DUTI by both siRNA and treatment with CV6-530 significantly enhanced cancer cell death in TNBC cell lines treated with FUDR (5-FU active metabolite) and epirubicin as shown by the colony formation assay. This sensitisation to FUDR correlated with a significant increase in growth inhibition and an increase in magnitude and persistence of DNA damage in combination-treated cells. DUTI plus epirubicin treatment did not increase absolute DNA damage above that of epirubicin alone, but significantly increased the persistence of un repaired DNA double-strand breaks. These data suggest that imbalanced nucleotide pools (TPP/dUTP) following DUTI induction with the repair of DNA damage induced by FUDR and epirubicin. Consistent with this, silencing of uracil DNA glycosylase (UNG), the primary enzyme involved in excising misincorporated uracil, resulted in enhanced sensitisation to FUDR and epirubicin when combined with DUTI, indicating that uracil-DNA misincorporation is a contributory mechanism.

Conclusion: These results provide clear rationale for the future application of dUTPase inhibitors in combination with fluoropyrimidines for TNBC. In addition, these results indicate that repair of anthracycline-induced DNA damage requires dUTPase to prevent uracil misincorporation at the site of repair and provides compelling evidence that dUTPase inhibitors represent a future clinical strategy to significantly enhance the antitumor activity of anthracycline-based combinations in TNBC.

No conflict of interest
Talazoparib and decitabine: a promising combination for BRCA-mutated cancers treatment

Background: BRCA genes (e.g. BRCA1 and BRCA2) are known to play a major role in tumorigenesis. These genes are key mediators of DNA damage repair response including the homologous recombination repair (HRR) pathway. To leverage impaired DNA repair in tumors, a new class of drugs, the poly (ADP-ribose) polymerase inhibitors (PARPi), were developed and olaparib, niraparib, rucaparib have already been approved as single agents. Efforts to combine these agents with cytotoxic agents showed increased efficacy but overlapping toxicities renders combination therapy difficult to tolerate. Furthermore, the short duration of responses in diseases like breast cancer has further inspired the search for combinations with other agents including epigenetic modifiers.

To overcome those hurdles, we explored synergistic interactions between PARPi and DNA Methytransferases inhibitors (DNMTi) such as decitabine (a global DNA methyltransferase inhibitor). This cytidine analog, when incorporated in newly synthesized DNA strands (during DNA replication phase (S phase), will trigger a covalent protein-DNA complex formation (a global DNA methyltransferase inhibitor). This cytidine analog, when included in vitro, will trigger a covalent protein-DNA complex formation.

Materials and Methods: cDNA was extracted from plasma samples and targeted NGS was performed on a panel of cancer-related genes, including all exons of BRCA1, BRCA2, and TP53. BRCA reversion mutations were defined as those mutations predicted to restore the BRCA open reading frame and were confirmed by NGS of available matched pretreatment tumour biopsies. Platinum status was classified based on time to progression of the most recent platinum-based treatment. Progression-free survival (PFS) was defined as the time from the first dose of rucaparib to investigator-assessed disease progression or death from any cause.

Results: We sequenced 112 pretreatment cDNA samples and detected somatic TP53 mutations in 96% (107/112) of samples, indicating that shedding of neoplastic DNA is frequent in pts with relapsed HGOC. Of these cases, primary deleterious BRCA mutations (germline or somatic) were detected in 97 cDNA samples. BRCA reversion mutations were identified in pretreatment cDNA from 16% (2/11) of platinum-refractory and 13% (5/38) of platinum-sensitive cancers compared with only 2% (1/48) of platinum-sensitive cancers (P = 0.049). Pts without BRCA reversion mutations detected in pretreatment cDNA had significantly longer PFS with rucaparib than those with reversion mutations (hazard ratio [HR], 0.12; 95% confidence interval [CI], 0.05–0.26; P < 0.02) (Table 1). Within the platinum-resistant/refractory subgroup, pts without BRCA reversion mutations had significantly longer PFS with rucaparib than those with reversion mutations (HR, 0.16; 95% CI, 0.07–0.42; P = 0.0001), with a median PFS of 7.3 and 1.7 months, respectively. To study acquired resistance, we sequenced 78 postprogression cDNA samples and identified 8 additional pts with BRCA reversion mutations not found in pretreatment cDNA. Sequencing of cDNA also detected multiple BRCA reversion mutations not detected in tumour biopsies, highlighting the ability to capture multilocal heterogeneity.

Conclusions: BRCA reversion mutations are detected in cDNA from platinum-refractory refractory HGOC and are associated with decreased clinical benefit from rucaparib treatment.

Conflict of interest: Advisory Board: Iain A. McNeish has served on advisory boards for Clovis Oncology, Tesaro, Takeda and AstraZeneca. Amit M. Oza has served on steering committees for AstraZeneca, Clovis Oncology, and Tesaro. Ana Oaknin has served on advisory boards for Roche, AstraZeneca, PharmaMar, Clovis Oncology, and Tesaro. Isabelle Ray-Coquard has served on an advisory board for AstraZeneca, Pharmacmar, and Roche. Anna V. Tinker has served on an advisory board for AstraZeneca, Rucaparib, and Roche. James D. Brenton has been an advisor for Novartis, and has served on advisory boards for AstraZeneca, Roche, Genentech, Janssen, OncogenMed, Millennium, Merck, Clovis Oncology, Esperance, Tesaro, GammaMabs, Pfizer, Genmab, Gralabis, Bayer, and AbbVie. James D. Brenton has been an advisor for Novartis, and has served on advisory boards for AstraZeneca, Roche, Genentech, Janssen, OncogenMed, Millennium, Merck, Clovis Oncology, Esperance, Tesaro, GammaMabs, Pfizer, Genmab, Gralabis, Bayer, and AbbVie. James D. Brenton has been an advisor for Novartis, and has served on advisory boards for AstraZeneca, Roche, Genentech, Janssen, OncogenMed, Millennium, Merck, Clovis Oncology, Esperance, Tesaro, GammaMabs, Pfizer, Genmab, Gralabis, Bayer, and AbbVie. Other Substantive Relationships: Kevin K. Lin, Jeff Isaacson, Lara Maloney, Heidi Giordano, and Thomas C. Hofsieck are employees of a speakE Oubrey and may own stock or have stock options in that company. Ana Oaknin has received support for travel or accommodation from Roche, AstraZeneca, and PharmaMar. Elena Helman is an employee of Guardant Health and may own stock or have stock options in that company. Gottfried E. Konczyk has received honorarium from Novartis. James D. Brenton has received nonfinancial support from Clovis Oncology and Aprea AB, and has a pending patent for a diagnostic method of relevance to the current work.
58 (PB-009) Poster
Finding determinants of PARP inhibitor resistance using genome-wide and focused CRISPR screens
S. Pettin1, D. Krastel2, F. Song1, J. Frankum1, R. Brough1, I. Brandsma1, J.M. Lee1, E. Swisher3, A. Ashworth4, C. Lord1. 1Institute of Cancer Research, Division of Breast Cancer, London, United Kingdom; 2National Cancer Institute, Center for Cancer Research, Bethesda, MD, USA; 3University of Washington, School of Medicine, Seattle, WA, USA; 4University of California San Francisco, Helen Diller Family Comprehensive Cancer Center, San Francisco, CA, USA

Background: We are using genomic approaches to study PARP inhibitor resistance mechanisms. Experimental study of potential resistance mechanisms can inform the ongoing clinical development of these drugs, as well as reveal new aspects of PARP1 function in normal and homologous recombination deficient cells.

Methods: To investigate mechanisms of PARP inhibitor resistance, we carried out genome wide CRISPR screens for mutants resistant to the potent PARP inhibitor talazoparib (BMN 673) in BRCA1 mutant human breast cancer cell lines. We also use a high density CRISPR tiling mutagenesis approach to specifically study mutations in PARP1 that confer PARP inhibitor resistance.

Results: Many PARP1 inhibitor resistant clones had loss of function PARP1 mutations, demonstrating that PARP trapping drives cytotoxicity of PARP inhibitors in HR-deficient cells. We showed that survival of PARP1 mutants in these cell lines depends on residual BRCA1 function, thus PARP1 could be tolerated despite the expected synthetic lethality relationship between these genes. PARP1 loss could not be tolerated in tumour cells with mutations in the BRCA1 BRCT domain, nor in cells with large engineered deletions of BRCA1. As well as PARP1 mutations we identified TP53BP1 mutants, and novel components of the 53BP1 pathway that confer resistance when mutated via a lack of protection from end resection at DNA double strand breaks and consequent restoration of homologous recombination activity.

To further investigate the role of PARP1 mutations in BRCA1 mutant cells, we used a high-density focused sgRNA library targeting only PARP1. We developed a reporter cell line that allows us to selectively isolate in-frame mutations that preserve PARP1 protein expression. By deep sequencing mutagenised and appropriately selected cells we identified a series of subtle mutations in PARP1 that result in PARP inhibitor resistance in BRCA1 mutant cells, giving us a detailed insight into structure-function relationships in PARP1. Among these, we found mutants that display trapping despite conferring PARP inhibitor resistance, suggesting that PARP trapping is not sufficient for cytotoxicity. Mutations that confer resistance are clustered in DNA binding domains and a network of residues in the WGR and helical domains of PARP1 that may be involved in intramolecular activation of PARP1 upon DNA binding and thus affect trapping. We found a missense mutation in the WGR domain cluster in a patient that showed de novo resistance to PARP inhibitors and confirmed that this mutation also caused loss of normal PARP1 DNA binding.

Conclusions: PARP1 mutation is a potential cause of drug resistance in patients with BRCA1 exon 11 mutations. Other potential resistance genes in the 53BP1 pathway have also been identified. We are now looking to see whether these are mutated in patients that progress after PARP inhibitor treatment.

No conflict of interest

61 (PB-012) Poster
DNA repair and its guardian angel protein: computational/experimental study of RAD51/ss-DNA interaction
D. Marson1, M. Ferrenglia1, E. Laurni1, S. Prici1. 1University of Trieste, MolBiNL @ UnitS – DEA, Trieste, Italy

Background: NA replication, repair, and recombination proteins form complex and agile networks. These networks organize the participating proteins into molecular machines that act on different substrates and channel them to different outcomes. Some of these machines display the capacity to accurately repair DNA damage or reestablish damage DNA replication forks without the loss of genetic information. Under other circumstances, action of the same molecular machines destabilizes the genome, leading to cancer or to the accumulation of toxic repair intermediates resulting in cell death. The central player in homologous recombination (HR) is the RAD51 DNA strand exchange protein (aka recombinase); its inactive conformation is tightly controlled by a double-heptameric doughnut-like assembly while its active form is a nucleoprotein filament assembled on the single-strand DNA generated at the site of DNA damage.

Here we will present the results of a study based on the combination of performance computing-based simulations, high resolution imaging techniques and biophysical methods targeted at getting a comprehensive structural and thermodynamic perspective of RAD51/ssDNA interaction.

Materials and Methods: Experimental structural studies of the RAD51/ssDNA were performed with a high resolution transmission electron microscopy (HRTEM) and by far-UV circular dichroism spectroscopy (far-UV CD). Massively parallel molecular simulations of the protein/nucleic acid interactions were performed using both in house and external European High Performance Computing facilities. RAD51/ssDNA interaction thermodynamics in solution was fully characterized by isothermal titration calorimetry (ITC).

Results: The combination of different experimental and HPC-based simulation techniques has allowed us for the first time to (i) dissect the initial mode of RAD51/ssDNA interaction, (ii) derive the energetics of the interface in the protein/nucleic acid interface, (iii) uncover the role of DNA and protein flexibility in their assembly formation, and (iv) determining the mechanism and binding thermodynamics of RAD51 to ssDNA.

Conclusions: Among other fundamental evidences, through the application of HRETM, HPC-based simulations, far-UV CD and ITC measurements this paper allowed us to evidence that, under important physiological conditions, RAD51 is able to bind ssRNA also in its double-heptameric doughnut-like assembly, corresponding to its inactive form, and that this interaction might have a specific biological role.

No conflict of interest

62 (PB-013) Poster
DNA-PK regulates the radiosensitivity of MET-addicted cancer cell lines via a novel MET phosphosite
J.P. Koch1, S.M. Roth1, A. Quintin1, J. Gavin2, E. Orlando1, M. Medolo1, R. Aebersold1, D.M. Stroka2, D.M. Aebersold3, Y. Zimmer1, M. Medolo1, 1Department for BioMedical Research, Radiation Oncology, Inselspital, Bern University Hospital, and University of Bern, Bern, Switzerland; 2Department for BioMedical Research, Visceral Surgery, Inselspital, Bern University Hospital, and University of Bern, Bern, Switzerland; 3Faculty of Science, University of Zurich, Department of Biology, Institute of Molecular Systems Biology, ETH Zurich, Zurich, Switzerland

Background: While the use of receptor tyrosine kinase (RTK) inhibitors is being investigated as a radio-sensitization strategy, a better understanding of the molecular crosstalk linking RTK signaling to the DNA damage response remains necessary to optimize treatment options. The present study focuses on the link between DNA-PK and the RTK MET, an oncogene mainly expressed in epithelial cells and involved in development and tissue regeneration but deregulated in numerous cancer and thus an attractive candidate for targeted therapies.

Methods: Using western blotting, DNA damage measurement, cell cycle and viability/toxicity assays, we tested the radiosensitivity of human cancer cell lines overexpressing constitutively active MET and transformed mouse fibroblasts ectopically expressing wild type or activating MET-mutated variants combined with a phosphodeficient Ser to Ala mutation. Assays include comet assay, γH2AX foci formation and γH2AX fluorescence intensity, DNA strand breakage; PI staining and CFSE dye dilution assay for cell cycle analysis; and crystal violet staining, resazurin fluorescence, EthDII/calcine staining and Annexin V/PI staining for cell proliferation, viability and toxicity. The radiosensitization effect of the Ser to Ala mutation was confirmed in vivo in a mouse xenograft setup.

Results: A study of post-translational changes in a MET-addicted cancer cell line upon MET inhibition and ionizing radiation revealed a yet unreported phosphorylation site on MET that is part of a consensus motif recognized by master DNA-damage response (DDR) kinases. Our results show that this site is phosphorylated by DNA-PK and that its phosphorylation fluctuations in response to MET inhibition and DNA-PK activity. MET-addicted transformed cells expressing the phospho-deficient (Ser to Ala mutation) form of active MET prematurely evade from irradiation-induced cell cycle arrest, leading to abnormal mitosis and lower proliferation, rendering them more radiosensitive than their nonmutated counterpart. A phosphoproteomics analysis revealed cell cycle proteins potentially involved in the downstream function of this phosphosite and we confirmed the radiosensitization effect of the Ser to Ala mutation in vivo by performing a mouse xenograft experiment.

Conclusions: In the present study we show that DNA-PK directly phosphorylates MET and that preventing this phosphorylation negatively affects viability after irradiation, providing new insights into the crosstalk linking MET and the DDR.

No conflict of interest
Development of tumor-targeted PARP inhibitors for the treatment of solid cancers

R. Aiello1, B. Ranjit2, D. Marshall3, J. Csengery3, P. Bourassa1, Q. Zhang1, B. Robinson1, L. Lopresti-Morrow1, J. Bechtold1, L. Tylaska1, R. Sundaram2, P. Hellsund2, P. Glazer2, V. Paralsk1. 1Cytexra Therapeutics, Biology, New Haven, USA; 2Yale School of Medicine, Therapeutic Radiology, New Haven, USA; 3Cytexra Therapeutics, Chemistry, New Haven, USA

Background: Poly (ADP-ribose) polymerase inhibitors (PARPis) are a new class of DNA repair inhibitors which have revolutionized the field of oncology. These agents are most effective against tumors with defects in key DNA repair pathways, such as homologous recombination deficient (HRD) cancers. Monotherapy PARP inhibitors have demonstrated efficacy against metastatic solid tumors, with the greatest activity observed in HRD cancers. However, resistance occurs rapidly, and there is limited activity against non-HRD cancers. Thus, these drugs are not curative for the vast majority of cancer patients. As such, there is great interest in combining PARPis with other systemic therapies, including chemotherapy. Pre-clinical studies indicate the potential for exquisite synergy between PARPis and DNA-damaging chemotherapies. Furthermore, PARPis combinations with TMZ have the potential to greatly enhance and amplify tumor activity against non-HRD cancers. However, bone marrow suppression is a major barrier to treatment efficacy when PARPis are combined with chemotherapy. As such, there is great unmet need to enable safer and more effective means to combine PARPis with conventional chemotherapy.

Materials and Methods: We sought to develop a new class of tumor-targeted PARPis which will (a) address the issues of off-target toxicity, and (b) increase treatment efficacy against both HRD and non-HRD cancers when used in combination with chemotherapy. These tumor-specific drugs (TSDs) thus will have a greatly enhanced therapeutic index. Tumor targeting is achieved by a novel peptide triggered by low pH to insert its C-terminus across the cell membrane into the cytosol. Cargoes attached to the C-terminus of pHLP1 can be targeted and delivered into cancer cells based on the acidity in the tumor microenvironment.

Results: Using our novel TSD platform, we have successfully conjugated a diverse range of structurally unique PARPis to pHLP1s and demonstrated the following: (1) pH-dependent delivery of functionally active drug into tumor cells in vitro; (2) sustained and selective in vivo tumor localization, without free drug detection in systemic circulation; (3) target engagement by the drug specifically in tumor tissue, at levels similar to that observed with free drug; (4) prevention of bone marrow toxicity when combined with chemotherapy, again compared with free drug; and (5) selective tumor cell killing in both HRD and non-HRD xenografts, including patient-derived xenografts (PDXs).

Conclusions: Our approach will greatly increase the safety and efficacy of PARPis in combination with chemotherapy and will expand their use into a wider range of HRD and non-HRD solid tumor types. Our TSD platform can be applied to other DNA repair inhibitors in the future, and it will allow safer and effective combinations with other systemic therapies, including immunotherapy.

No conflict of interest

Poster Session (Tuesday, 13 November 2018)
suggest a significant proportion of melanoma and lung cancer patients could benefit from treatment with this drug combination.

**No conflict of interest**

66 (PB-017) **Poster**

Measurement of SLFN11 protein in circulating tumor cells (CTCs) as a proposed liquid biopsy biomarker to predict response to DNA repair therapies

L. Fernandez, L. Chu, R. Richardson, R. Graf, Y. Wang, M. Landers, R. Dittamore. Epic Sciences, Translational Research, San Diego, USA

**Background:** SLFN11 regulates response to DNA damage and replication stress, and is being investigated as a potential predictive biomarker for response to platinum agents and PARP inhibitors (PARPi). In a Phase II trial in recurrent small cell lung cancer (SCLC), patients with high SLFN11 expression in tissue biopsies had better survival when PARPi was used. In clinical practice, recurrent lung biopsies are not common. It has been hypothesized that SLFN11 could also identify patients who might respond to an additional round of inexpensive platinum agents. A CTC-based test could aid these indications. The Epic Sciences platform plates all nucleated cells from a blood sample onto microscope slides and stains for DNA (DAPI), blood lineage marker (CD45) and epithelial marker (CK, cytokeratins), with a fourth channel for additional biomarkers. CTCs are characterized via automated microscopy. The technology was utilized to create the first commercially available predictive test in prostate cancer to be considered for CMS reimbursement. AR-V7. We applied the technology to develop an assay for SLFN11 and tested clinical feasibility.

**Material and Methods:** Contrived samples consisting of healthy donor blood spiked with cell line cells were used: three PARPi sensitive (DU145, MD-MBA-436 and PC3) and two PARPi resistant (MCF-7 and MD-MBA-231). Rabbit polyclonal anti-SLFN11 antibody was used to assess SLFN11 expression in the 4th channel. Contrived samples, along with SCLC, triple negative breast cancer (TNBC) and mCRPC patient samples were processed as patient samples in a CLA-like environment.

**Results:** DU145, MD-MBA-436 and PC3 PARPi sensitive cell lines express nuclear-specific localized SLFN11 signal, at times visualized in sub nuclear focal bodies. PARPi resistant MD-MBA-231 cell line showed low non-nuclear expression of SLFN11. BRAC1-wt PARPi resistant MCF-7 does not express SLFN11. The median difference in single-cell SLFN11 expression observed between the PARPi resistant and PARPi sensitive cell lines as contrived patient samples was approximately 12 fold. In patient samples, a diversity of SLFN11 expression was seen between and within samples in multiple indications.

**Conclusions:** The expression levels and localization of SLFN11 observed in individual cell line cells spiked into healthy donor blood and processed as patient samples is consistent with the established biology and emerging clinical biomarker associations of SLFN11. The assay is able to qualitatively assess localization, and semi-quantitatively assess the expression level of SLFN11. Use of this assay is underway in research studies with SCLC patient samples to assess correlation with PARPi and platinum agent response.

**Conflict of interest:** Other Substantive Relationships: We are employee of Epic Sciences

67 (PB-018) **Poster**

Preclinical evaluation of the ATR inhibitor VE-821 alone and in combination with the PARP inhibitor olaparib in neuroblastoma

H. Southgate, L. Chen, N.J. Curtin, D.A. Tweddle. Newcastle University, Wofson Childhood Cancer Research Centre, Northern Institute for Cancer Research, Newcastle Upon Tyne, United Kingdom; Newcastle University, Northern Institute for Cancer Research, Newcastle Upon Tyne, United Kingdom

**Background:** Neuroblastoma (NB) is the commonest extra-cranial malignant solid tumour of childhood and one of the most difficult to cure. Around 50% of high-risk NBs have amplification of the MYCN oncogene that promotes rapid DNA replication, leading to errors and replication stress (RS). Defects in the p53 pathway are frequently observed at NB relapse. Cancer cells with defective p53 signalling and/or inactivated p53 are acutely dependent on ataxia telangiectasia and Rad3-related (ATR) protein kinase signalling. Poly-ADP ribose polymerase (PARP) inhibition increases RS; therefore ATR inhibition can synergize with the cytotoxic effect of PARP inhibitors. This study aimed to determine which molecular features lead to sensitivity to the ATR inhibitor VE-821 alone and in combination with PARP inhibitors in NB cell lines.

**Materials and Methods:**

- **XTT (Roche) and clonogenic assays were used to evaluate cell proliferation and survival respectively in response to 72 hours treatment with VE-821 in a panel of 14 NB cell lines of varying MYCN amplification and TP53 mutation status.** The XTT cell proliferation assay was also used to determine the effect of ATR inhibition on olaparib cytotoxicity.

- **Results:** VE-821 caused significantly more growth inhibition in MYCN amplified cell line by XTT assay (p = 0.04 Mann-Whitney U test). A similar trend has been observed by clonogenic assay. No significant difference was found in sensitivity between p53 mutant and p53 wild type cell lines in either assay. Combinatorial index analysis (CalcuSyn) showed that ATR inhibition by VE-821 is synergistic with olaparib at sub lethal concentrations (<1 μM), although this effect is lost at higher concentrations.

- **Conclusion:** MYCN-amplification but not p53 mutation is a determinant of ATRi sensitivity in NB cell lines. In addition, ATR inhibition by VE-821 is synergistic with olaparib at sub lethal concentrations (<1 μM), suggesting the degree of replication stress may predict ATR inhibitor sensitivity in NB.

**Conflict of interest:** Corporate-sponsored Research: NJC is the grant holder of the MRC-Merck Industrial CASE studentship.

69 (PB-020) **Poster**

Multiple deletions as a prognostic factor in metastatic colorectal cancer with chromothripsis

E. Skuž, D. Kalniete, M. Nakazawa-Miklasevica, Z. Danebergas, G. Purkalne, E. Miklasevics, P. Stradiņ Clinical University Hospital, Clinic of Oncology, Riga, Latvia; Riga Stradins University, Riga, Latvia

**Background:** Chromothripsis is a massive chromosome shattering occurred as a single catastrophic event leading to random repair of chromosomes. Multiple deletions and amplifications are very common in cancer cells affected by chromothripsis. In our previously reported study, we found a correlation between DNA massive fragmentation and increased progression free survival (PFS) in metastatic colorectal cancer (mCRC), but not overall survival (OS). The aim of this study is to find overlapping deleted genome regions in selected mCRC patients with chromothripsis and detect possible cause of increased PFS, and find new genes or combinations, involved in colorectal cancer oncogenesis.

**Material and Methods:** 10 mCRC patients with chromothripsis receiving FOLFOX first-line palliative chemotherapy between August, 2011 and October, 2012 were selected for this study. Genotyping. Microarray analysis was performed using the Infinium HumanOmniExpress-12 v1.0 FFPE BeadChip kit (Illumina). BeadChip was scanned on HiScan (Illumina). Analysis was performed by GenomeStudio software (Illumina) and R. version 3.1.2. (https://www.r-project.org/). Copy number variation and breakpoints on the chromosomes were analyzed using the DNA copy package (http://bioconductor.org/pack-ages/release/bio/c/html/DNAcopy. html).

**Results:** Eight deleted tumor suppressor genes (ROBO2, CADM2, FAT4, PCDH10, PCDH18, CDH18, TSG1, CTNNA3) and four deleted oncogenes (CDH12, GPM6A, ADAM29, COL11A1) were identified in more than half of patients. In 70% patients’ deletion in COL11A1 was detected. Deletion of MIR1269, MIR4465, MIR1261 and MIR4490 in patients with longer time to progression was observed. Four patients (40%) with PFS over 14 months, presented with NRG3 deletion (oncogene, EGFR ligand) what could possibly decrease proliferation of cancer cells via decreasing EGFR activation.

**Conclusions:** Multiple chromosomal deletions (MIR1269, NRG3, ADK) in mCRC patients with chromothripsis are associated with better response to first line palliative FOLFOTX-type chemotherapy and increased PFS.

**No conflict of interest**

70 (PB-021) **Poster**

Characterization of small molecule inhibitors of ubiquitin specific peptidase 1 (USP1) as anti-cancer agents


**Background:** The deubiquitinase USP1, which plays an important role in the deubiquitination of various proteins, is overexpressed in various cancer types. This suggests that USP1 inhibitors may have the potential to be used as anti-cancer agents in combination with DNA damaging drugs. The previously published USP1 inhibitor ML323 is characterized by high selectivity, it has a rather low biophysical, cell biology and drug metabolism/pharmacokinetic assays.
were used to profile USP1 inhibitors generated from the internal chemistry program. For primary screening, USP1/USP1 associated factor 1 (UAF1) activity was monitored using an enzymatic assay with diubiquitin as substrate. For validation, surface plasmon resonance (SPR) and a cellular ubiquitin-proliferating cell nuclear antigen (Up-PCNA) deubiquitination assay were used. In vivo pharmacokinetic studies were performed in mice.

Results: We report the characterization of orally bioavailable USP1 inhibitors exhibiting highly potent activities against USP1/UAF1, excellent selectivity against other USPs, biophysical validation by SPR, and promising in vivo pharmacokinetics. The potencies against USP1/UAF1 were <10 nM in the enzymatic assay with a selectivity of >1000× against all other tested USPs. Compound binding was confirmed by SPR with Kd values in the sub-μM range. Inhibition of endogenous USP1 resulted in accumulation of ubiquitinated PCNA with IC50 <200 nM in multiple cell lines and there was a strong correlation between the values in this assay and the enzymatic assay data. The high-dose of USP1 had only a minor cytotoxic effect in the μM range, combination of USP1 inhibitors with DNA crosslinking chemotherapeutics such as cisplatin or mitomycin C resulted in strong synergy confirming the importance of USP1 in the DNA damage response. In in vivo pharmacokinetic studies, our compounds were characterized by a good oral bioavailability and dose linearity between the tested doses.

Conclusions: We have developed highly potent and selective USP1 inhibitors that show strong synergy with DNA damaging agents in vitro and have promising pharmacokinetic profiles, justifying advancement to in vivo efficacy studies.


72 (PB-023) Poster
CRISPR-mediated base editing screens to identify PARP inhibitor resistant mutations
D. Krastev1, S. Pettit1, C. Lord1, Institute of Cancer Research, Division of Breast Cancer, London, United Kingdom

Background: Three PARP inhibitors are currently in the clinic for the treatment of advanced ovarian and breast cancers with underlying defects in the homologous recombination DNA repair pathways. Acquired and de novo resistances have been observed, necessitating the mechanistic understanding of the mode of action of this class of drugs. We have investigated the mechanism of PARP inhibitor resistance via CRISPR-based mutagenesis of PARP1 and other candidate genes.

Material and Methods: We used tiling sgRNA libraries to direct various base editing enzymes (BE2, BE3, BE4-Gam, AID and AB7.10) to target genes in order to induce missense mutations in BRCA deficient cellular models. The pools of thus mutagenized cells were subsequently exposed to lethal doses of PARP inhibitors and resistant clones were selected. We devised a pipeline to identify and annotate these mutations through a deep sequencing approach. A selected set of missense mutations in PARP1 were functionally characterized by biochemical and biosensor approaches.

Results: We carried out the mutagenesis screen in BRCA1 mutant breast cancer cell line. This led to the identification of a number of missense mutations in PARP1 that confer PARP inhibitor resistance in this setting. Mutations were found in both the DNA binding Zn-finger domains, and the regulatory WGR and HD domains of PARP1. Interestingly, different base editor enzymes generated different sets of mutations around certain hotspots, showing the merit of using as many and as diverse as possible orthogonal reagents in order to saturate the mutational space. We have selected a set of mutations and validated that they impact PARP1 DNA binding and drug-induced "trapping" ability. However, other mutations showed a complex relationship between DNA interaction and enzymatic activity.

Conclusions: We have developed an experimental system, where CRISPR-mediated mutagenesis is employed for the isolation of drug resistance-inducing mutations in target proteins. In the case of PARP1, we have defined mutations, which functionally uncouple DNA binding and enzymatic activity. These observations support the notion of reverse allosteries in PARP1, and suggest a mechanism of the inhibitor-induced DNA trapping.

No conflict of interest

73 (PB-024) Poster
Investigating the effect of replication stress and other phenotypic factors as determinants of sensitivity to single agent ATR inhibitor, VE-821 in ovarian cancer cell lines
A. Bradbury1, D. King2, H. Bryant3, F. Zenke3, A. Drew1, N. Curtin1
1Newcastle University, Northern Institute for Cancer Research, Newcastle upon Tyne, United Kingdom; 2University of Sheffield, Academic Unit of Molecular Oncology, Sheffield Institute for Nucleic Acids StiFoNIA, Department of Oncology and Metabolism, Sheffield, United Kingdom; 3Merck KGaA, Biopharma R&D, Translational Innovation Platform Oncology, Darmstadt, Germany

Background: ATR is a key component of the DNA damage response (DDR), responsible for signalling to S and G2/M checkpoints and facilitating repair. Cancer cells are often defective in their G1 checkpoint control due to e.g. TP53 mutation, a common event in ovarian cancer. This, coupled with frequent activation of oncogenes that drive replication, such as cyclin E, which is reported to be highly amplified in 20% of high-grade serous ovarian cancers, make cancer cells much more likely to enter S phase with increased replication stress (RS). Therefore, cancer cells are more reliant on their G2/M checkpoint; making the S and G2/M checkpoint an attractive anti-cancer target.

Methods: The single agent activity of VE-821 has been assessed by western blot (CHK1 phosphorylation) and clonogenic survival assay in a panel of 14 ovarian cancer cell lines representing a spectrum of phenotypes. Expression of DDR proteins was assessed by western blot and levels of RS assessed by DNA fibre assay.

Results: Cells exhibited a range of sensitivities to VE-821 with LC50 values ranging from 0.61 ± 0.14 μM (BRCA1 mutated UWB1.289) to 11.84 ± 1.90 μM (NIH-OVCAR3). In IGROV1 cells, VE-821 significantly decreased replication fork speed, and increased the percentage of new origins and collapsed forks. However, there was no significant relationship between the expression of previously reported determinants of sensitivity to VE-821 (TP53, cyclin E, DNA-PKcs or ATM) and VE-821 cytotoxicity across the cell line panel.

Conclusions: VE-821 exhibits differential single agent activity in a panel of ovarian cancer cell lines; however, this does not appear to correlate with the expression of known determinants of sensitivity to ATR. Studies are ongoing to further to assess the impact of VE-821 on RS as well as baseline levels of RS as a determinant of sensitivity to VE-821 across the panel of cancer cell lines.

Conflict of interest: Corporate-sponsored Research: Alice Bradbury is jointly funded by MRC and Merck. Other Substantive Relationships: Frank Zenke is an employee of Merck, as stated in the author affiliations.

74 (PB-025) Poster
Deacetylase activity of Sirt1 is required to protect the genome by preventing excess replication origin initiation
A. Baris1, H. Fu1, B. Thakur1, S. Jang1, C. Redon1, P. Kiran1, A. Mint1
1National Cancer Institute, Developmental Therapeutics Branch, Bethesda, USA

Chromatin structure affects DNA replication patterns, but the roles that individual chromatin modifiers play in the regulation of DNA replication has yet to be elucidated. Sirtuins, homologs of the yeast gene silencing modifier (Silent Information modifier 2, or Sir2), are crucial in maintaining genomic stability. The largest homolog of Sir2 in metazoans, Sirt1, has been implicated in circadian signaling, epigenetic modifications, as well as cell cycle signaling and DNA damage repair. Using molecular combing, a method in which DNA is labelled with thymidine analogs and observed via microscopy, we can analyze replication patterns in cells with wild type and enzymatically inactive Sir1. We report that the deacetylation activity of Sirt1 is necessary for Sir1 to modulate DNA replication. We determined that enzymatically inactive Sir1 exhibits more active origins, as well as a decreased rate of replication and increased replication stalling. This combined data leads to the conclusion that deacetylation activity is required for Sir1 inhibition of dormant origins, which prevents excess DNA damage.

No conflict of interest
Ataxia-telangiectasia-mutated (ATM) plays an important role in cell cycle delay after double-strand breaks. Low expression, as well as loss-of-function mutation of ATM gene can contribute to mutagenesis and carcinogenesis. We investigated the relationship between mRNA and protein expression levels of ATM gene and the somatic mutation count using whole exome sequencing data derived from the Cancer Genome Atlas (TCGA) database in various cancer types.

**Materials and Methods:** mRNA expression, protein expression, and somatic mutation data of 22 cancer types were collected from the TCGA data portal, and their association with overall survival (OS) was analyzed. To validate the survival outcome in an independent cohort, MTCI Breast Cancer Survival Analysis Tool (http://gliodos.ucl.ac/I/BreastMark) was used for survival analysis in breast cancer.

**Results:** Out of 22 cancer types from the TCGA database, mRNA expression levels of ATM were inversely correlated with somatic mutation counts in 4 cancer types (adenoid cystic carcinoma, breast cancer, stomach cancer, and thyroid carcinoma). Tumors with higher somatic mutation counts above the median value had shorter OS compared to those with lower somatic mutation count (p = 0.001) in these cancer types. mRNA expression and protein level of ATM showed positive correlation in breast and stomach cancer (p < 0.001 and p = 0.001, respectively).

Regarding the subgroups (luminal A, luminal B, HER2, and triple-negative) of breast cancer, low ATM mRNA expression (the bottom 25% expression above the median value) was associated with high somatic mutation count (p = 0.023), and trends to shorter OS (median 114 months vs. 123 months, p = 0.072) in luminal A subtype. Likewise, OS was significantly shorter in tumors with low ATM mRNA compared to high in the luminal A tumors in MTCI validation cohort (n = 609, hazard ratio 0.54, 95% Confidence Interval 0.33–0.86, p = 0.009). In contrast, ATM mRNA expression was not associated with somatic mutation counts in TNBC.

Somatic mutation count in TNBC was higher than in the other subtypes. In TNBC, somatic mutation count-high tumors (above the median) was associated with longer OS (median not reached vs. 114 months, p = 0.037). In stomach cancer, MSI-high tumors had higher somatic mutation counts compared to microsatellite stable (MSS) or MSI-low tumors. ATM mRNA was lower in MSI-high tumors, but low ATM mRNA expression was not associated with high somatic mutation count, regardless of MSI status. In MSI-high tumors, low ATM mRNA level was associated with trend to better survival (median OS 16 months vs. 31 months, p = 0.129).

**Conclusions:** ATM mRNA expression is associated with somatic mutation count and prognosis in subtypes of breast and stomach cancers. These associations need to be assessed in the contexts of molecular subtypes of breast cancer and MSI status of stomach cancer.

**No conflict of interest**
Background: Ovarian cancer (OC) is the most aggressive and lethal gynaecological malignancy. Up to 70% of patients are diagnosed when the disease has already spread contributing to only 25% 5-year survival rates. The standard of care for OC patients consists of debulking surgery followed by systemic treatment with platinum compounds -- regimen that remains unchanged for almost two decades.

Interestingly, with the advent of Poly (ADP-ribose) polymerase inhibitors (PARPi), a new class of anticancer drugs, an important change in standard of care is being observed. PARPi have been thoroughly studied in tumours with defects in Homologous Recombination-based DNA repair -- a feature caused by mutations of BRCA1, BRCA2 and a number of other genes. Molecular profiling of BRCA genes has become inseparable part of the standard of care in OC patients' management. Next Generation Sequencing (NGS) is a robust tool to assess mutation in BRCA genes in -- germline and recently also in the somatic setting. Although with the appropriate diagnostic workflow, NGS of tumour derived DNA allows timely and accurate testing, a number of important areas still require further evidence generation.

Material and Methods: 201 consecutive ovarian carcinomas of the following histopathological subtypes: 155 (77.1%) serous, 21 (10.4%) endometrioid, 9 (4.5%) clear cell, 6 (3%) mucinous, 6 (3%) undifferentiated

Results: In the studied population pathogenic mutations were detected in 27% (54) of cases. 8% of cases (16) were assigned as variant of uncertain significance (VUS). Out of the 27% of identified BRCA mutations, 19% were cases with BRCA1 mutations and 8% affecting BRCA2 gene. Mutations were only found in the following histopathological subtypes: 31.6% of serous, 14.3% of endometrioid and 22.2% of clear cell. Interestingly in cases with over 50% AF of tumour-detected pathogenic variant 96% of mutations were detected by qMSP in plasma samples and surgical tissues, respectively. An antibody panel was used to quantify (modified H-score) expression change of JAM3 in CRC cells and mRNA level in plasma samples was detected by qPCR, and the methylation status of JAM3 was assessed there was variable expression of each of the DDR antigens ranging from an H score of 0–24. There was no correlation between individual antigen expression by IHC and HRR function (p = 0.13). When antigens were combined using Vectra multiplexed immunofluorescence assessment there was no pattern clustering within HRR vs HRC patient samples. The median progression free survival (PFS) for HRR tumours was 12 months compared to 11 months in the HRC group, (p = 0.075). Median PFS of cultures homogenous for HRR function was 11 months in comparison to 10 months for patients with heterogeneous subcultures.

Conclusions: Protein quantification of key proteins in DDR pathways are not predictive of HRR function when assessed in isolation or combination. FFPE-based protein biomarkers cannot replace functional assessment in live culture material and future work should focus on making culture and functional HRR assessment more reproducible in a diagnostic laboratory setting.

No conflict of interest

No conflict of interest
normal controls (mean 19.03%). Hypermethylation was also found to be frequent in 18 of 24 primary CRC tissues analysed, and there were statistically significant differences in the methylation status of JAM3 between CRC tissues (mean 82.62%) and normal controls (mean 38.84%), suggest that methylation modification may be involved in the silence of JAM3. And the methylation status of JAM3 was significantly associate with tumor stage in CRC tissue samples.

Conclusions: These results demonstrated that DNA methylation involved in the tumorigenesis of CRC was responsible for loss of JAM3, which suggest that detection of JAM3 gene methylation in plasma can be used as a potential noninvasive biomarker for CRC diagnosis and prognosis.

No conflict of interest
including germline MUTations in hereditary and sporadic papillary RCC, somatic mutations in head and neck carcinomas, and METamplification in lung, gastric, and multiple other cancers. Previous studies have demonstrated high Met protein abundance in papillary and clear cell RCC tissues compared to normal correlating with poor prognosis and over-all survival. The Cancer Genome Atlas (TCGA) provisional ccRCC data indicates that mutation, copy number alteration, and amplification/over-expression occur in ~12% of 446 total cases. Interestingly, we found that androgen receptor (AR) over-expression occurring in 6% of S34 ccRCC TCGA cases was associated with significantly improved over-all (OS) and progression-free survival (PFS). This potential benefit of AR overexpression was not seen in papillary RCC TCGA data (280 cases). Furthermore, previous studies reported AR loss in RCC compared to normal kidney tissue. Our initial investigation of the potentially beneficial role of AR signaling in ccRCC was designed to identify AR effects on the HGF/Met pathway. We found that AR and MET expression appear to be mutually exclusive in TCGA data for ccRCC and in kidney, bladder and prostate cancer cell lines, consistent with several reports that androgen deprivation leads to increased MET expression in prostate cancer. We found that reconstitution of AR in ccRCC cell lines with robust MET expression (766-0 and Caki-2) did not significantly reduce MET mRNA levels. However, it did reduce Met protein content, implying that an additional negative regulatory mechanism by AR may exist. To gain a more global view of how AR expression might potentially benefit ccRCC patients and identify potentially novel therapeutic strategies, we performed RNA-Seq on ccRCC cell lines over-expressing wild-type AR or the constitutively active variant Avn7 in the presence or absence of the AR ligand dihydrotestosterone (DHT). Data analysis using Partek Flow identified significant alterations in stemness pathways (e.g. PPAR, thyroid hormone, oxygen and estrogen receptor signaling) and RCC relevant pathways (e.g. Hippo, TGF-beta, Wnt, FOXO, oxidative phosphorylation pathways), including those of therapeutically targetable proteins. Androgen/AR suppression of Met has been demonstrated in many solid tumors. Androgen/AR signaling in prostate cancer and we found a similar relationship in ccRCC cell lines between AR and Met. AR over-expression could be beneficial for ccRCC patients since TCGA data showed significantly improved OS and PFS for that cohort.

No conflict of interest

85 (PB-035)  Poster
Discovery of a potent androgen receptor degrader for castration resistant prostate cancer
C. Wang1,2, X. Han1, E. Fernandez-Salas1, C. Qin1, W. Xiang1, T. Xu1, D. Mceachern1, S. Przybranowski1, C. Foster1, L. Bai1, J. Lv1, L. Huang1, S. Wang1
1University of Michigan, Internal Medicine, Pharmacology, 2University of Michigan, Internal Medicine, Pharmacology, Medicinal Chemistry, Ann Arbor, USA; 3Peking University, School of Pharmaceutical Sciences, Beijing, China

Background: Androgen receptor (AR) is a central therapeutic target in metastatic castration resistant prostate cancer (mCRPC). Previous successful strategies targeting AR signaling have focused on blocking the synthesis of androgen or abiraterone and inhibition of AR with AR-antagonists. These agents ultimately become ineffective against advanced prostate cancer through AR gene amplification, mutations and alternative splicing, despite the fact that continued AR signaling is retained in tumors.

Methods: Degradation of AR may achieve much better clinical efficacy than inhibition of the protein. Here we report an AR degrader, ARD-61, containing an AR inhibitor and a ligand for an E3 ligase utilizing PR0Teolysis TArgeting Chimeric (PROTAC) technology as a new therapeutic strategy for the treatment of CRPC.

Results: ARD-61 can effectively induce AR degradation in a dose- and time-dependent manner in all the cell lines tested, with observed low nanomolar 50% degradation concentrations (DC50). AR degradation resulted in over 40 times more potent anti-proliferation effects compared to the inhibitor. Degradation of AR in the cytoplasm is faster than that in the nucleus. Proteomics results confirmed AR degradation by ARD-61 and revealed no obvious off-target effects. Degradation of AR protein by ARD-61 leads to increased expression of mRNA encoding PSA and TRAPSS2 but not that of AR, indicating effective inhibition of AR signaling. ARD-61 showed potent AR degradation effects and anti-tumor activities in multiple murine xenograft models of prostate cancer at well-tolerated dosing schedules.

Conclusions: Our data provides clear evidence that targeting AR for degradation by the PROTAC methodology represents a very promising therapeutic approach for the treatment of CRPC retaining AR signaling. ARD-61 is a highly potent AR degrader with optimal drug-like properties for advanced preclinical development and future clinical trials for the treatment of CRPC.

No conflict of interest

86 (PB-037)  Poster
Combination effect of thymoquinone and extracts of Iksan526 callus in A375 human melanoma cell line
S.J. Kim1, Kongju National University, Biological Sciences, Gongju, Korea

Background: Iksan526 is resveratrol-enriched transgenic rice that over-expresses the stilbene synthase gene isolated from the peanut (Arachis hypogaea var. Palitwang). Experimental data show that resveratrol-enriched transgenic rice might down-regulates melanin synthesis in UVB-induced Guinea Pigs epidermal skin tissue. The aim of the present study was to investigate the effect of thymoquinone (TQ) and extracts of Iksan526 callus (IS526) treatment on A375 cells.

Materials and Methods: A375 were treated with TQ or IS526, and the effect of TQ or IS526 on cell proliferation, tyrosinase activity, reactive oxygen species (ROS) generation and Cyclooxygenase (COX)-2, pp38, pERK expression levels were respectively tested by the MTT assay, tyrosinase activity assay, ROS assay and western blot analysis.

Results: In this study, TQ has been shown to inhibit cell proliferation, ROS production and tyrosinase activity. TQ also induced COX-2, pERK, pp38 expression, IS526 reduced cell proliferation, tyrosinase activity and COX-2 expression. IS526 increase ROS production, pERK, pp38 expression. We found that compared with treatment alone, co-exposure of cells to TQ and IS526 resulted in a significant decrease in cell proliferation and tyrosinase activity, pERK expression in the cells that expressed TQ, which was inhibited by IS526 and these effects were enhanced by ERK1/2 and p38 inhibitors. TQ inhibits IS526-induced ROS, which reversed by PD98059 (PD) and SB203580 (SB). SB enhanced the decrease in tyrosinase activity by TQ combined with IS526 while, PD restored tyrosinase activity. The blockage of p38 kinase or ERK1/2 with SB and PD did not have any effect in cell proliferation by both drugs.

Conclusions: These results suggest that IS526 inhibits TQ-induced COX-2 expression and TQ suppresses IS526-induced ROS via the ERK1-2 and p38 kinase pathways. The ERK1-2 and p38 kinase pathways regulate tyrosinase activity by TQ combined with IS526.

No conflict of interest

87 (PB-038)  Poster
HDMD001-10D1, a novel humanized anti-HER3 antibody with a unique mechanism of action, demonstrates superior tumor inhibition in multiple tumor models compared to other EGFR family therapies
D. Thakkar1, M. Taguiam1, V. Sancenon1, S. Guan1, K. Paszkiewicz1, P. Ingram1, J. Boyd-Kirkup1, Hummingbird Bioscience, Discovery, Singapore, Singapore

HER3 is increasingly implicated as a critical node in oncogenic EGFR family signaling. HER3 is a potent heterodimer partner for EGFR and HER2, which signal through the MAPK pathway and, additionally, HER3 heterodimerization triggers signaling through the PI3K/AKT/mTOR pathway. Activation has thus been implicated in acquired resistance to EGFR/HER2 therapies and other MAPK pathway therapies such as BRAF inhibitors, where activation of the PI3K pathway represents a possible escape route for tumor signaling. Further, emerging clinical evidence has shown that solid tumors refractory to anti-PD1 therapy have higher HER3 expression compared to anti-PD1 responders. Despite this, existing anti-HER3 therapies such as MM-121 that inhibits the ligand-binding to HER3, and LM-J716 that blocks HER3 activation have shown only limited efficacy in clinical trials, likely due to high levels of ligand independent dimerization in many tumors.

HMHD-001-10D1 is a novel therapeutic antibody designed to directly block the heterodimerization surface of HER3. This precise targeting of a specific functional epitope at the dimerization interface was enabled using Hummingbird Bioscience’s proprietary Rational Antibody Discovery Platform. HMHD001-10D1 inhibits phosphorylation of HER3, demonstrating superior tumor inhibition in multiple tumor models compared to other EGFR family therapies. HMBD001-10D1 shows over 90% efficacy in pre-clinical and significantly decreases downstream signaling through the PI3K/AKT/ mTOR and MAPK pathways. As a result of its distinct molecular mechanism of action, HMHD-001-10D1 blocks both ligand-dependent and independent activation. Notably, HMHD-001-10D1 shows over 90% efficacy in pre-clinical
B. Umbayev1, T. Davis2, S. Askarova1, D. Kipling 2.

Freiburg, Germany;3Charles River, Discovery Services, Cell Biology & predictive biomarkers.

antitumor activity in various models of tumors. Here we studied Sabutoclax, a pan-Bcl-2 inhibitor targeting Bcl-xL, Bcl-2, Mcl-1 and Bfl-1 which has shown Venetoclax was approved as second line therapy in CLL while several BCl-2 family proteins is an emerging therapeutic option, the BCL2 inhibitor role in regulating programmed cell death and is involved in tumors apoptosis. Several broad spectrum activity of sabutoclax in haematological and solid cancer cell lines is associated with defined biomarkers.


13HFBiotec GmbH, Freiburg, Germany;24HFBiotec GmbH, Bioinformatics, Freiburg, Germany;3Charles River, Discovery Services, Cell Biology & Composite Screening, Freiburg, Germany;4Hendrix Pharmaceutical Consulting, Pumperneck, Netherlands

Background: The B cell lymphoma 2 gene family (BCL-2) plays a central role in regulating programmed cell death and is involved in tumors apoptosis evasion and resistance toward cytostatic agents. The selective inhibition of BCL-2 family proteins is an emerging therapeutic option, the BCI2 inhibitor Venetoclax was approved as second line therapy in CLL while several inhibitors are currently in clinical trials for other indications. Sabutoclax is a pan-BCL-2 family Bcl-xL, Bcl-2, Mcl-1 and Bfl-1 which has been shown to act as an antitumor activity in various models of tumors. Here we studied Sabutoclax efficacy in a broad range of tumor models to define possible indications and predictive biomarkers.

Material and Methods: The compound was tested in vitro in a monolayer assay in well-characterized human tumor cell lines from hematological cancers (n = 51) and solid tumors (n = 222). The cell lines molecular data generated by whole exome sequencing. Affymetrix snp 6.0 and Affymetrix u133 plus 2.0 arrays were used to elucidate determinants of drug response.

Results: In vitro assays revealed high anti-tumor efficacy (IC50 < 0.2 μM) in 42 out of 273 cell lines (15%). Similar IC50 geometric means (0.7 μM) were observed in both hematological cancers and solid tumors. Overall, Sabutoclax was active in a broad spectrum of tumors including lymphomas, leukemias (AML and ALL), sarcomas (soft tissues and osteosarcoma), pleuropneumothorax, adeno carcinomas of the lung, breast, head and neck, prostate, and bladder tumors. The compound was however active in a limited number of cell lines per tumor type, making the identification of predictive biomarker essential. The biomarker screening revealed BCL2L1 (Bcl-xl, probe 212132_at) to be among the most significant transcript that correlated with Sabutoclax IC50s (Spearman r = 0.35, p adjusted = 4.5E-05) while BCL2, Mcl-1 and Bf-1 inhibitors were not. Based on cell line gene expression profiles, we derived a signature classifying the cell lines into clusters with response rates ranging from 0 to 56%. The analysis also revealed 133 mutated genes which were associated with sensitivity to Sabutoclax (Fishier and Wilcoxon tests p < 0.05). Notably, at 5 out of 6 models mutated for BCL2 were found to be highly sensitive to Sabutoclax (IC50 < 0.2 μM. Fisher test p = 0.004). On the contrary, 48 genes including AXL, KMT2B, ADAMS17, and MUC17 were associated with tumor resistance when mutated (fisher p values <0.05).

Conclusions: Sabutoclax showed efficacy in lymphoma, leukemia as well as in wide range of solid tumors without however clear indication regarding the histological type. Molecular analysis revealed multiple genomic and transcriptomic predictors of tumor response which will need to be validated. They may serve to develop a predictive model helping in tumor selection for the next phases of the drug development.

No conflict of interest

98 (PB-039) Poster

Broad spectrum activity of sabutoclax in haematological and solid cancer cell lines is associated with defined biomarkers.


13HFBiotec GmbH, Freiburg, Germany;24HFBiotec GmbH, Bioinformatics, Freiburg, Germany;3Charles River, Discovery Services, Cell Biology & Composite Screening, Freiburg, Germany;4Hendrix Pharmaceutical Consulting, Pumperneck, Netherlands

Background: The B cell lymphoma 2 gene family (BCL-2) plays a central role in regulating programmed cell death and is involved in tumors apoptosis evasion and resistance toward cytostatic agents. The selective inhibition of BCL-2 family proteins is an emerging therapeutic option, the BCI2 inhibitor Venetoclax was approved as second line therapy in CLL while several inhibitors are currently in clinical trials for other indications. Sabutoclax is a pan-BCL-2 family Bcl-xL, Bcl-2, Mcl-1 and Bfl-1 which has been shown to act as an antitumor activity in various models of tumors. Here we studied Sabutoclax efficacy in a broad range of tumor models to define possible indications and predictive biomarkers.

Material and Methods: The compound was tested in vitro in a monolayer assay in well-characterized human tumor cell lines from hematological cancers (n = 51) and solid tumors (n = 222). The cell lines molecular data generated by whole exome sequencing. Affymetrix snp 6.0 and Affymetrix u133 plus 2.0 arrays were used to elucidate determinants of drug response.

Results: In vitro assays revealed high anti-tumor efficacy (IC50 < 0.2 μM) in 42 out of 273 cell lines (15%). Similar IC50 geometric means (0.7 μM) were observed in both hematological cancers and solid tumors. Overall, Sabutoclax was active in a broad spectrum of tumors including lymphomas, leukemias (AML and ALL), sarcomas (soft tissues and osteosarcoma), pleuropneumothorax, adeno carcinomas of the lung, breast, head and neck, prostate, and bladder tumors. The compound was however active in a limited number of cell lines per tumor type, making the identification of predictive biomarker essential. The biomarker screening revealed BCL2L1 (Bcl-xl, probe 212132_at) to be among the most significant transcript that correlated with Sabutoclax IC50s (Spearman r = 0.35, p adjusted = 4.5E-05) while BCL2, Mcl-1 and Bf-1 inhibitors were not. Based on cell line gene expression profiles, we derived a signature classifying the cell lines into clusters with response rates ranging from 0 to 56%. The analysis also revealed 133 mutated genes which were associated with sensitivity to Sabutoclax (Fishier and Wilcoxon tests p < 0.05). Notably, at 5 out of 6 models mutated for BCL2 were found to be highly sensitive to Sabutoclax (IC50 < 0.2 μM. Fisher test p = 0.004). On the contrary, 48 genes including AXL, KMT2B, ADAMS17, and MUC17 were associated with tumor resistance when mutated (fisher p values <0.05).

Conclusions: Sabutoclax showed efficacy in lymphoma, leukemia as well as in wide range of solid tumors without however clear indication regarding the histological type. Molecular analysis revealed multiple genomic and transcriptomic predictors of tumor response which will need to be validated. They may serve to develop a predictive model helping in tumor selection for the next phases of the drug development.

No conflict of interest

90 (PB-041) Poster

Tumor immune modulation by the PI3-kinase (PI3K) inhibitor MEN1611 via tumor-associated macrophages polarization.

S. Capano1, A. Fiacarelli1, A.M. Tomirotti1, A. Paoli1, M. Bigioni1, A. Bressan1, D. Bellarosa1, M. Salerno1, M. Binaschi1, Menarini Ricerche S.p.A., Experimental and Translational Oncology Department, Pomezia RM, Italy

MEN1611 (previously PA799) is a phosphatidylinositol 3-kinase (PI3K) inhibitor, that selectively targets PI3Kγ (both wild type and mutated forms) and PI3Kδ isoforms. Its inhibitory and anti-tumor activity has been mainly characterized against PI3Kα, known to be frequently mutated in various human cancers. On the contrary, its activity against PI3Kγ, that is highly expressed in leukocytes but not in cancer cells and is reported to be involved in inflammatory cell recruitment to tumors, is not well described yet. Previous studies have shown that selective targeting of PI3Kγ by IPI-549 can reshape the tumor immune microenvironment towards a less immunosuppressive phenotype and promote cytotoxic T cell-mediated tumor regression. These evidences suggest that MEN1611 might lead to anti-tumor immune response via PI3Kγ inhibition.

To determine whether PI3Kγ inhibition mediated by MEN1611 directly affects macrophage polarization we analyzed in vitro cell morphology, mRNA expression and cytokine release both in murine and human macrophages. Murine macrophages have been differentiated from bone marrow-derived monocytes by M-CSF stimulation, followed by M1 (lipopolysaccharides and interferon γ) or M2 (IL-4, M1 and M2 macrophages were obtained from buffy coats in the same way, iPI-549 treatment was used as positive control, while BYL-719, which selectively inhibits PI3Kγ, was used as negative control. In order to evaluate whether MEN1611 tumor growth inhibition in vivo might be mediated also by an immune modulatory activity, a syngeneic tumor model (4T1 breast cancer) has been established.

Both IPI-549 and MEN1611 treatments were able to modulate macrophage polarization towards an immune-activating phenotype and as a consequence M2 cell morphology was reverted towards an M1-like round cell shape, gene expression analysis revealed a significant increase of immunostimulating factors mRNAs (such as IL-1β, IL-12α and INOS, both in M1 and M2 macrophages), and the secretion of pro-inflammatory cytokines was enhanced both in M1 and M2 macrophages. This shift towards a more inflammatory M1-like state was not clearly evident in BYL-719-treated macrophages. 4T1 syngeneic mouse model characterization by flow cytometry revealed M1 and M2 macrophage subpopulation with CD4+ and CD8+ T cells infiltrates. The impact of MEN1611 treatment on macrophages and T lymphocytes subsets infiltrating tumors is currently under investigation.

In conclusion, we demonstrated that targeting PI3K with MEN1611 at clinically relevant doses can switch the activation of macrophages towards a less immunosuppressive state in vitro. Nevertheless further studies are needed.
Rational-based drug design of novel, highly potent MER inhibitors as potential treatment of cancers

A. Stanczak1, A. Yaman2, P. Olejkowska2, N. Piorowska2, M. Naitana3, P. Maliszewski4, K. Dubie5, J. Pieczylak6, M. Wieczorek7, A. Staniec8, Celon Pharma SA, Preclinical Research Development, Lomianki-Kiepiel, Poland; 2Celon Pharma SA, Medicinal Chemistry, Lomianki-Kiepiel, Poland; 3Celon Pharma SA, Research and Development Center, Lomianki-Kiepiel, Poland

Background: MER is a member of TAM (TYRO3, AXL, MER) family of receptor tyrosine kinases. MER is aberrantly expressed in several types of solid and hematological tumors, playing a substantial role in tumor growth and invasion. Inhibition of MER individually can be particularly prominent in solid tumors, whereas dual targeting of MER/AXL has greater potential in hematological tumors, playing a substantial role in tumor growth and in vivo treatment led to increased apoptosis as revealed with cleaved caspase-8, 9 and 3. In vivo treatment of nude mice bearing WSU-DLCL2 xenografts with TAS4464 (50 mg/kg intravenous injection twice a week) or ASTX660 (25 mg/kg orally for 7 consecutive days every other week) significantly reduced the tumour growth compared to the control and when combined, the tumours underwent regression (see table below). A putative mechanism for this enhanced activity was determined: NAE inhibition reduces the induction of non-canonical NF-kB-mediated resistance to IAP antagonism (e.g. cIAP2 and c-FLIP induction), and hence the combination of these two compounds effectively shuts down NF-kB signaling and leads to sustained apoptotic induction.

Materials and Methods: Combining novel STAT3 inhibitor YHO-1701 with multi-targeted tyrosine kinase inhibitor sorafenib improves anti-tumor response in solid tumor xenograft model

K. Taniguchi1, F. Nishisaka1, M. Tsugane1, G. Hirata1, A. Takagi2, S. Ishii3, H. Takahashi4, T. Iijima2, A. Asai5, T. Matsuoka6, Y. Shishido7, Y. Honshoa Co., Ltd., Yakult Central Institute, Tokyo, Japan; 2Yakult Honsha Co., Ltd., Pharmaceutical Department, Tokyo, Japan; 3Graduate School of Pharmaceutical Sciences University of Shizuoka, Center for Drug Discovery, Shizuoka, Japan

Background: Signal transducer and activator of transcription (STAT) 3 plays a key role in many cellular processes, including proliferation, invasion, and survival. Aberrant STAT3 signaling has been demonstrated in various malignant tumors. Using biochemical and virtual screening, we previously identified a novel orally active STAT3 inhibitor, YHO-1701. The aim of the present study was to investigate its anti-proliferative activity in a wide variety of cancer cell lines alone and in combination with the multi-targeted tyrosine kinase inhibitor sorafenib.

Material and Methods: First, the anti-proliferative potential of YHO-1701 was investigated in 20 human and murine cancer cell lines derived from various tumor types. Based on the results, human oral squamous cell carcinoma cell line SAS (known for IL-6 signaling) was mainly used for further analysis. The phosphorylation and dimerization levels of STAT3 and survivin were determined by immunoblotting. Anti-proliferative activity was measured by WST-8 dye-based assay after 48 hours of treatment with YHO-1701 and/or sorafenib. STAT3 nuclear translocation and DNA-binding were examined by immunocytochemistry and ELISA, respectively. The antitumor efficacy of YHO-1701 in combination with sorafenib was explored in an SAS
subcutaneous xenograft model. The test compounds were administered orally once a day for 5 consecutive weekdays followed by a 2-day rest at the weekend for 4 weeks.

**Results:** YHO-1701 showed cytotoxic activity against various cancer cell lines, particularly SAS, where the IC50 was <0.5 μM. Dimerization of STAT3 was blocked and pSTAT3 levels reduced in this cell line. In addition, YHO-1701 reduced cellular survival levels, a STAT3-regulated downstream target. Moreover, YHO-1701 treatment reduced STAT3 nuclear translocation and blocked STAT3 DNA-binding activity in a dose-dependent manner. In an in vitro cytotoxicity assay, YHO-1701 enhanced the anti-proliferative activity of sorafenib. In the SAS xenograft mouse model, the tumor growth inhibition rate increased to 74.6% when YHO-1701 was administered with sorafenib, whereas that for YHO-1701 and sorafenib alone was 26.5% and 56.9%, respectively. The results further revealed a close correlation between the additive effect and change in survivin levels, both in vitro and in vivo. It is also noteworthy that combination therapy with YHO-1701 and sorafenib was well tolerated in tumor-bearing mice.

**Conclusions:** The present study suggests that combination therapy with YHO-1701 and sorafenib is promising and worthy of further investigation in clinical trials.

**No conflict of interest**

94 (PB-045)  
Poster  
Distinct relationship of antitumor activity of lenvatinib (LEN) and sorafenib (SOR) to FGFR21 expression levels in preclinical hepatocellular carcinoma (HCC) models

S. Watanabe Miyano1, H. Taisuke1, J. Ito1, K. Kodama1, H. Watanabe1, K. Takase1, J. Matsui1, Y. Funahashi2, K. Nomoto2, Elsai Co., Ltd., Tsukuba Research Laboratories, Tsukuba, Ibaraki, Japan;2Eisai Inc., Oncology Business Group, Woodcliff Lake, NJ, USA

**Background:** LEN is a multitargeted tyrosine kinase inhibitor that selectively inhibits VEGFR1–3, FGFR1–4, PDGFRα, RET, and KIT. In a phase 3 clinical trial in unresectable HCC (REFLECT study), LEN showed statistical non-inferiority of overall survival compared to SOR and clinically meaningful outcome in objective response rate, progression-free survival, and time to progression. Preclinical data previously reported that LEN inhibited FGFR signaling pathway in preclinical HCC models in vitro and in vivo. Here, we investigated expression patterns of FGF ligands and their receptors in human HCC cell lines and patients, and the role of FGFR2 in tumor growth of HCC xenografts in nude mice.

**Methods:** Expression levels of genes in the FGFR signaling pathway were analyzed using TCGA (HCC patients) and CCLE (HCC cell lines) databases. Protein expression of FGFR2 and KLB, co-receptor of FGFRs, was examined by immunohistochemistry (IHC) using human HCC tissue arrays and historical tumor tissues from human HCC patient-derived xenograft (PDX) models. Antitumor and antiangiogenic activities were evaluated against human HCC cell lines and mouse xenograft models.

**Results:** The TCGA database analysis showed that elevated expression of FGFR1, FGFR3, and FGFR4 mRNA was found in human HCC cell lines. The CCLE database analysis showed that the expression of FGFR2 and KLB mRNA was lower in six HCC cell lines than in the control cell line. The expression of FGFR2 and KLB mRNA was lower in human HCC xenografts in nude mice than in the tumor cell lines. The results of the xenograft tumor growth experiments showed that the IC50 of LEN was lower in human HCC cell lines than in xenografts in nude mice.

**Conclusions:** The results of this study suggest that the expression of FGFR2 and KLB mRNA is related to the antitumor activity of LEN in preclinical HCC models. The results also suggest that the expression of FGFR2 and KLB mRNA is related to the antitumor activity of LEN in preclinical HCC models. The results also suggest that the expression of FGFR2 and KLB mRNA is related to the antitumor activity of LEN in preclinical HCC models. The results also suggest that the expression of FGFR2 and KLB mRNA is related to the antitumor activity of LEN in preclinical HCC models. The results also suggest that the expression of FGFR2 and KLB mRNA is related to the antitumor activity of LEN in preclinical HCC models. The results also suggest that the expression of FGFR2 and KLB mRNA is related to the antitumor activity of LEN in preclinical HCC models. The results also suggest that the expression of FGFR2 and KLB mRNA is related to the antitumor activity of LEN in preclinical HCC models.
assess apoptosis after 48 hrs of treatment. Cells were fixed and stained with FucciBlue violet stain and analyzed by flow cytometry to assess cell cycle following 48 hrs of treatment.

Mouse xenograft: bab/c mice were implanted subcutaneously with HCC70 cells or patient-derived breast cancer cells and randomized for treatment with test drug or vehicle when tumors reached 150–200 mm³. Mice were dosed BID through oral administration for 3 weeks.

Results: A series of CDK7 inhibitors were designed and profiled in biochemical assays and tumor cell lines. Analysis of 467 compounds revealed a correlation between CDK7 Kᵦ, CDK7 occupancy (EC₅₀) and cell growth inhibition (EC₅₀). A representative member of the class, compound A, exhibited selectivity over CDK12, CDK9, and CDK2 of 236-, 1174-, and 1202-fold, respectively. In breast cancer cell lines, compound A inhibited proliferation of triple negative breast cancer (TNBC) and ovarian (OVA) cells, with EC₅₀ in the low nanomolar range. Compound A induced apoptosis in a dose-dependent manner in multiple TNBC and OVA cell lines and also induced G2/M arrest. Strikingly, tumor growth inhibition in breast cancer CXD and PDX models was observed when Compound A was dosed orally at 4 mg/kg BID.

Conclusions: We designed and profiled orally available, CDK7 selective inhibitors with potent activity against TNBC and OVA cells and induced tumor growth inhibition in breast cancer cell and patient derived xenograft models. These data support the rationale for advancing one or more members of this class toward clinical development.

Conflict of interest: Ownership: Employees and stock holders of Syros Pharmaceuticals.

98 (PB-049) Poster

Involvement of Notch signaling pathway in a panel of human cancer cell lines

L. Astorgues-Xen1, M. Martinez1, E. Raymond2, S. Faire3

Background: Notch pathway has been involved in cell differentiation, cell proliferation, apoptosis, angiogenesis and drug resistance, as well as epithelial-to-mesenchymal transition. Our team has demonstrated the involvement of the PIGF/VEGFR1/Notch axis in the angiogenesis of hepatocellular carcinoma. What about the role of Notch in other cancer types? The aim of this work is to characterize the basal activation and the role of Notch1 and Notch4 in a panel of human cancer cell lines.

Materials and Methods: We characterized a panel of 8 pancreatic (PDAC), 5 cholangiocarcinoma (CK), 5 colorectal (CRC), and 8 head and neck (H&N) human cancer cell lines for Notch1 intracellular domain (NICD1), Notch4 intracellular domain (NICD4), Hes1 (a target gene of Notch activation), E-cadherin, and Vimentin expressions by Western Blot. In each tumor type, 2 cell lines were selected (one NICD4 high and one NICD4 low) to assess the role of Notch4 basal activation on cell proliferation and migration, using MTT and wound-healing assays, respectively. We also assessed the expression of NUMB, a protein involved in NICD proteasomal degradation, in the selected cell lines. Notch inhibition, using PF-03084014, was studied on cell signaling, proliferation, and migration.

Results: Notch4 is highly activated in 6 out of 8 (6/8) PDAC cell lines, 7/8 H&N cell lines, 3/5 CK cell lines and 2/5 CRC cell lines. In PDAC cell lines, Notch4 is activated in all CK lines and 3/8 H&N cell lines. In PDAC cell lines, Notch4 activation is correlated with high expression of Vimentin. In each tumor type, 2 cell lines were selected regarding NICD4 expression, to decipher why we observed differential Notch4 activation in our cell lines as well as to investigate the role of Notch4 basal activation in proliferation and migration. NUMB is overexpressed in all low NICD4 cell lines, suggesting a correlation between low NICD4 expression and degradation through the proteasome. Interestingly, Notch4 activation is associated with an increased proliferation rate in all tumor types and an increased basal migration of PDAC and CRC cell lines. Furthermore, all high NICD4 selected cell lines were more sensitive to PF-03084014 than the low NICD4 cell line. In addition, we explored the effect of Notch inhibition and Notch stimulation (using a specific ligand) on cancer cell signaling and migration. These results will be displayed at the conference.

Conclusions: In this study, we demonstrated the involvement of Notch4 basal activation on cell proliferation and migration, as well as in the sensitivity to Notch inhibition. This study could help to discriminate tumor types or tumor characteristics that are good candidate for Notch inhibition in the clinics.

No conflict of interest

99 (PB-050) Poster

Evaluation of drug–drug interaction of itraconazole and ivosidenib (AG-120), an oral, potent, targeted, small molecule inhibitor of mutant IDH1, in healthy subjects

B. Fan1, C. Prakash1, H. Liu2, G. Liu3, C. Korth4, H. Yang1, D. Dai1

Background: Mutant isocitrate dehydrogenase 1 (mIDH1) produces the oncometabolite D-2–hydroxyglutarate (2–HG), accumulation of which results in impaired myeloid differentiation. IDH1 mutations are found in myeloid malignancies, including acute myeloid leukemia (AML), myelodysplasia and acute myeloid leukemia (AML). Ivosidenib (IVO; AG–120) is an oral, potent, targeted, small molecule inhibitor of mIDH1 for the treatment of mIDH1–driven hematologic malignancies, including AML. Antimicrobial agents, including antifungal medications, are commonly prescribed in the AML population, and many are moderate to strong CYP3A4 inhibitors. As IVO is metabolized predominantly by CYP3A4, this study was conducted to evaluate the effect of itraconazole, a strong CYP3A4 inhibitor, on the pharmacokinetics (PK) of a single dose of IVO in healthy subjects, and to predict the effect of itraconazole on IVO exposure under steady-state conditions.

Materials and Methods: This was a two-period, single-sequence, crossover study (Clinicaltrials.gov NCT02831972). Twenty-two subjects received a single oral dose of IVO 250 mg on Day 1 of Period 1. In Period 2, subjects received itraconazole 200 mg once daily (OD) for 18 days and a single oral dose of IVO 250 mg on Day 5. PK sampling was carried out for 21 days in each period, and parameters were determined using
noncompartmental methods. The magnitude of drug–drug interaction (DDI) was assessed by analysis of variance of log-transformed AUC and Cmin. A physiologically based pharmacokinetic (PBPK) model was validated to further predict the DDI effects of strong CYP3A4 inhibitors under steady-state conditions. Trial status: complete; trial sponsor: Agios.

**Results:** Coadministration of a single dose of IVO 250 mg with iraconazole resulted in higher AUC, longer t1/2, and lower apparent oral clearance (CL/F) than for IVO alone (Table). Itraconazole increased IVO AUC by ∼169% (geometric mean ratio [GMR]: 269%) but had no effect on Cmax. PBPK modeling suggested a smaller effect of iraconazole on steady-state IVO AUC (GMR: 144%) than on single-dose IVO AUC because the CYP3A4 induction effect of IVO resulted in a reduced DDI. IVO administered alone or with iraconazole was generally well tolerated, with similar favorable safety profiles.

### Table. PK parameters following administration of IVO + iraconazole

<table>
<thead>
<tr>
<th>PK parameter</th>
<th>IVO 250 mg alone</th>
<th>IVO 250 mg + 200 mg QD iraconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (ng h/mL)**</td>
<td>107,500 (40.9)</td>
<td>282,000 (33.5)</td>
</tr>
<tr>
<td>AUC (ng h/mL)**</td>
<td>115,000 (38.6)</td>
<td>302,300 (31.4)</td>
</tr>
<tr>
<td>Cmax (ng/mL)**</td>
<td>2240 (25.2)</td>
<td>2750 (25.0)</td>
</tr>
<tr>
<td>t1/2 (hr)**</td>
<td>60.7 (22.5)</td>
<td>140 (65.2)</td>
</tr>
<tr>
<td>CL/F (L/hr)**</td>
<td>2.31 (0.778)</td>
<td>0.863 (0.247)</td>
</tr>
</tbody>
</table>

**Geometric mean (%CV).**

**Arithmetic mean (SD).**

**Conclusions:** Coadministration of single dose IVO with a strong CYP3A4 inhibitor, iraconazole, resulted in increased AUC but not Cmax. PBPK modeling predicted that the magnitude of DDI with strong CYP3A4 inhibitors at steady state would be ≤100% increase in AUC.

**Conflict of interest:** Corporate-sponsored Research: All authors are employees of and stockholders in Agios Pharmaceuticals Inc.

---

**100 (PB-051) Poster**

**Mutant p53 as a Therapeutic Target for the Treatment of Triple-Negative Breast Cancer**

N. Symnett 1, J. Crown 2, M.J. Duffy 2, 1University College Dublin, School of Medicine, Dublin, Ireland; 2Department of Medical Oncology, St Vincent’s University Hospital, Dublin, Ireland; 3School of Medicine, University College Dublin, Ireland

**Background:** p53 is the most frequently mutated gene in breast cancer, being mutated in over 80% of triple-negative (TN) cases. The aim of this study was therefore to investigate the potential value of the mutant p53 reactivating compound, APR-246 for the treatment of breast cancer with particular focus on TNs.

**Methods:** Cell viability was determined using the MTT assay. p53 protein levels were determined using Western blotting, ELISA and immunofluorescence. Knockdown of p53 protein was carried out in 3 different breast cancer cell lines using 3 independent predesigned Flextube sequences (Qiagen). Changes in the refolding of mutant p53 were investigated using the conformation-specific anti-p53 antibodies, PA81620 and PA8240.

**Results:** Using a panel of 23 breast cancer cell lines, significantly lower IC50 values were found for APR-246 in p53 mutant compared to p53 wild-type cells (p = 0.014). Of potential clinical value, we found a significant inverse correlation between IC50 values for APR-246 and endogenous p53 protein levels (p = 0.0001, r = −0.76), i.e., the higher the endogenous p53 protein, the greater the response. Knockdown of p53 expression reduced p53 protein levels by almost 90%. Reduction of p53 protein levels resulted in a significant decrease in the growth inhibitory effects of APR-246, in all 3 cell lines investigated, suggesting that p53 was the target for APR-246. To establish if APR-246 reversed the mutant unfolded state of p53, we treated the mutant p53 expressing cell line, SKBR3 with APR-246. This resulted in a dose-dependent increase in fluorescent staining with the WT associated p53 antibody, PA81620. Simultaneously, there was a dose-dependent decrease in fluorescence using the mutant specific p53 antibody, PA8240. In contrast, treatment of the WT-p53 cell lines, MCF7 with APR-246 failed to alter staining intensity using PA81620 or PA8240. This finding suggests that APR-246 alters the conformation of mutant unfolded p53 protein, converting it to a WT-like conformation. To confirm the changes in fluorescent staining were due to p53 refolding, we quantified the absolute p53 protein levels under the same conditions. To confirm the changes in fluorescent staining were due to p53 refolding, we quantified the absolute p53 protein levels under the same conditions. Using the PAb1620 or PAb240. This finding suggests that APR-246 treatment of the WT-p53 cell lines, MCF7 with APR-246 failed to alter staining intensity using PA81620 or PA8240. Simultaneously, there was a dose-dependent decrease in fluorescent staining with the WT associated p53 antibody, PAb1620. Simultaneously, there was a dose-dependent decrease in fluorescent staining with the WT associated p53 antibody, PA81620. This was found to be highly expressed in multiple different types of tumors. APR-246 triggers UPS cascade may demonstrate clinical benefits in solid tumors.

**Conclusions:** The proliferation of HCT-116 and PC-3 were potently inhibited (IC50 20 and 50 μM, respectively). Unfolded protein response (UPR) and apoptosis were induced after APR-246 treatment of 250 nM dose. CPL-410-005 exhibits higher metabolic stability on mice (4,7 μ/min*mg) and human (2,8 μ/min*mg) microsomes in comparison to MLN7243 (18,6 μ/min*mg and 3,8 μ/min*mg, respectively). The logD values are: pH 1.2 = 1.7; pH 4.5 = 3.06; pH 6.8 = 3.19; pH 7.4 = 3.40 which fall under the Lipinski rules. Moreover, no mutagenicity of CPL-410-005 was observed in Ames test. Finally, PK/PD analysis of CPL-410-005 on SCID mice bearing HCT-116 cells xenografts showed rapid elimination of CPL-410-005 from the plasma after single i.v. dosing of 5 mg/kg with long CPL-410-005 retention in tumor (up to 24 h).

**Conclusions:** We have designed a potent E1 inhibitor with promising efficacy in vitro and in vivo results. Preclinical findings may lead to the development of a novel anticancer therapy for solid tumors.

**No conflict of interest**
Overcoming resistance to AKT inhibition in Oesophageal Adenocarcinoma

L. Cairis 1, L. Stevenson 1, R. Douglas 1, N. Mccabe 1, E. Sutton 1, R. Kennedy 1, T. Harrison 1, R. Turkington 1. 1Queen’s University Belfast, Centre for Cancer Research and Cell Biology, Belfast, United Kingdom

Background: The incidence of Oesophageal adenocarcinoma (OAC) in the UK has risen by 6-fold in the last 40 years and is now the highest in Europe. The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB) signalling pathway is implicated in resistance to chemotherapy in OAC and has been investigated as a potential target to enhance therapy with limited success. We aimed to identify resistance mechanisms to AKT inhibition and develop novel combination therapeutic strategies.

Material and Methods: Parental OAC cell lines were exposed to repeated, increasing concentrations of a novel AKT inhibitor (AKTi) over a period of six months and 6 lines were selected to establish AKTi-resistant cell lines (AKTi-R). Sensitivity was assessed by MTT and clonogenic cell survival assays and transcriptional profiling was performed using the ALMAC Diagnostics Xcell Array. Migration and invasion were measured using the xCELLigence platform and receptor tyrosine kinase (RTK) activation was assessed using a phospho-RTK array. Induction of cell death was evaluated using Annexin V/PI flow cytometry coupled with western blot analysis for PARP cleavage.

Results: AKTi-resistant (AKTi-R) cell lines were confirmed to be 6-fold more resistant compared to their parental counterpart. AKTi-R cells displayed a mesenchymal morphology together with an increased migratory and invasive capacity (P = <0.0001) and elevated expression of mesenchymal markers. Functional analysis of gene expression data indicated upregulation of pathways governing epithelial-mesenchymal transitions (EMT). The RTK AXL showed increased activation in the AKTi-R cell lines and this was validated by western blot and RT-PCR. AXL knockdown reversed the mesenchymal phenotype of the AKTi-resistant cell lines but did not restore sensitivity to AKT inhibition. Further investigation identified the RTK MET as a potential mediator of resistance. MET knockdown restored sensitivity to AKT inhibition and targeting MET in combination with AKTi demonstrated enhanced cell death (P = <0.0001).

Conclusions: Inhibition of AKT in OAC cell lines increases migratory and invasive capacity together with an upregulation of mesenchymal markers and AKT activation. We identified the role of AXL in driving this EMT phenotype and we established that targeting MET restored sensitivity to AKT inhibition and caused enhanced cell death in combination with AKTi. Our work elucidates novel molecular mechanisms of resistance to AKT inhibition and indicates potential, innovative therapeutic combinations in the treatment of OAC.

No conflict of interest

Cyclin dependent kinase inhibition: a novel treatment strategy for glioblastoma

B. Murphy 1, J. Noonan 1, M. Jarzabek 1, F. Lincoln 1, B. Kavanagh 1, P. Arhona 1, L. Young 2, K. Ligon 3, H. Jahns 4, A. Ashkenazi 5, D. Zheleva 6, 1Royal College of Surgeons in Ireland, Neurosurgery, Dublin, Ireland; 2University College Dublin, Pathobiology, Dublin, Ireland; 3Royal College of Surgeons in Ireland, Surgery, Dublin, Ireland; 4Harvard Medical School, Pathology, Boston, USA; 5University College Dublin, Pathobiology, Dublin, Ireland; 6Genentech, Cancer Immunology, San Francisco, USA; 1Cyclacel, Research and Development, Dundee, United Kingdom; 2University of Stuttgart, Cell Biology and Immunology, Stuttgart, Germany

Background: Glioblastoma (GBM) is the most common primary brain tumour and no cure presently exists. The average survival rate for patients is only 15 months. The reasons for treatment failure are multi-faceted including, the tumour’s extreme resistance to apoptotic death which the treatment strategies of radiation and chemotherapy attempt to induce and the challenge of successfully delivering drugs across the blood brain barrier. Our group has previously described seliciclib, as a first generation cyclin-dependent kinase (CDK) inhibitor, that down-regulates the anti-apoptotic protein, Mcl-1, in GBM via its selective targeting by CDK9. Additionally, we have demonstrated that combining seliciclib with the death receptor ligand, tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), re-establishes apoptotic sensitivity in GBM cells grown as monolayers.

Materials and Methods: We have now assessed the effectiveness of first- (seliciclib) and second- (CYC065) generation CDK inhibitors, both alone and in combination with TRAIL receptor ligation, in clinically relevant preclinical models of GBM – patient-derived neurospheres and orthotopic patient-derived xenograft (PDX) model.

Cyclin-dependent kinase inhibition: a novel treatment strategy for glioblastoma

B. Murphy 1, J. Noonan 1, M. Jarzabek 1, F. Lincoln 1, B. Kavanagh 1, P. Arhona 1, L. Young 2, K. Ligon 3, H. Jahns 4, A. Ashkenazi 5, D. Zheleva 6, 1Royal College of Surgeons in Ireland, Neurosurgery, Dublin, Ireland; 2University College Dublin, Pathobiology, Dublin, Ireland; 3Royal College of Surgeons in Ireland, Surgery, Dublin, Ireland; 4Harvard Medical School, Pathology, Boston, USA; 5University College Dublin, Pathobiology, Dublin, Ireland; 6Genentech, Cancer Immunology, San Francisco, USA; 1Cyclacel, Research and Development, Dundee, United Kingdom; 2University of Stuttgart, Cell Biology and Immunology, Stuttgart, Germany
Results: Significant levels of apoptosis were observed in patient-derived neurosphere cultures upon treatment with TRAIL, highlighting that OATP1B1 substrates are highly vulnerable to phosphorylation-mediated drug interactions. No conflict of interest

107 (PB-058) Poster
The novel oral Cdc7 inhibitor, SRA141, demonstrates robust efficacy in preclinical cancer models

R. Hansen1, S. Mitulovic1, B. Strouse1, M. Hedrick1, G. Smith1, C. Hassig1. 1Sierra Oncology, Inc., Research, Vancouver, Canada; 2Sierra Oncology, Inc., Preclinical, Vancouver, Canada

Cell division cycle 7 (Cdc7), a serine-threonine kinase, is a novel therapeutic target with therapeutic potential. Preclinical validation of Cdc7 in vitro and in vivo demonstrates evidence of anti-tumor activity in both hematological and solid cancers in preclinical models. Cdc7 plays important roles in regulating DNA replication and is essential for progression through cell cycle phases. Cdc7 is an attractive target for cancer therapy due to its central role in DNA replication and mitosis. Cdc7 inhibitors have shown promising preclinical activity against various cancer cell types.

Conclusions: Cdc7 exerted dose-dependent activity against human colorectal (Caco-2, HT-29, and HEK293), breast (MDA-MB-231), and cancer xenograft models. Cdc7 inhibition led to DNA replication arrest and cell cycle arrest in colorectal cancer cells. Cdc7 inhibition led to cell cycle arrest in colorectal cancer cells and induced apoptosis in breast cancer cells. Cdc7 inhibition led to cell cycle arrest and apoptosis in cancer xenograft models.

No conflict of interest

108 (PB-059) Poster
Modulation of OATP1B1 function by LYN-kinase inhibitors

S. Hu1, M. Chen1, A. Gibson1, A. Sparreboom1, J. Sprovoll1. 1The Ohio State University, Pharmaceutics and Pharmaceutical Chemistry, Columbus, USA; 2D’Youville College, Pharmaceutical, Social and Administrative Sciences, Buffalo, USA

Background: Functional modulation of various cellular proteins is regulated by tyrosine phosphorylation, which is a key regulatory mechanism of intra- and intercellular signaling in metazoans. We previously reported the existence of a conserved regulatory mechanism in the major drug transporters, OCT2, and OCT3, and mass-spectrometric data from global phospho-proteome studies suggest that a substantial fraction of clinically-relevant transporters expressed in humans have conserved tyrosine-phosphorylated sites. We hypothesized that OATP1B1, a hepatic uptake transporter of various cancer drugs, including paclitaxel, may also be regulated by tyrosine-phosphorylation and is vulnerable to modulation by tyrosine kinase inhibitors (TKIs).

Materials and Methods: Putative tyrosine phosphorylation sites in OATP1B1 were identified utilizing computational prediction tools and site-specific tyrosine-to-phenylalanine (Y-F) mutants of OATP1B1 were constructed by site-directed mutagenesis. Functional studies were performed in vitro in HEK293 cells engineered to express OATP1B1, mouse mOatp1b2, or zebrafish zOatp1b1, using 5-fluorosocin-cAMP or extradiol-17β-o-glycuronide as prototypical test substrates, as well as in vivo in wild-type and mOatp1b2-deficient Oatp1b2(-/-) mice. RNAi kinase-library screens for all known kinase genes (Dharmacon) were performed using reverse transfection of transporter-overexpressing 293T cells.

Results: Mutation at one particular site (Y234F) in OATP1B1, conserved in human mOatp1b3, mOatp1b2, and zOatp1b1, was associated with >90% reduced OATP1B1 function. Based on this observation, we hypothesized that the second-generation CDK inhibitor, CYC065, even in the absence of prior kinase expression, could inhibit OATP1B1 activity in vivo. To test this hypothesis, we performed a screen with 32 FDA-approved TKIs and confirmed that several of these agents inhibited OATP1B1 function by at least 75%. Nilotinib was found to inhibit OATP1B1 function most potently at concentrations that can be achieved clinically (IC50 ~ 1 μM), and was also able to inhibit OATP1B3, mOatp1b2, and zOatp1b1. Consistent with a non-competing inhibitory mechanism, we found that nilotinib is itself a poor substrate of OATP1B1 and OATP1B3, and that the interaction with OATP1B1 is reversible and time-dependent. Nilotinib inhibited phosphorylation of known kinase targets in the mouse liver, including ABL, and potently inhibited mOatp1b2 in vivo. In line with LYN, identified as a lead hit from the RNAi screen, the putative kinase inhibitor, INNO-406, was itself a potent and selective orally-bioavailable inhibitor of Cdc7, with an IC50 of 1.4–4.0 nM and slow dissociation kinetics (koff = 0.003 at 5 nM). In cells, SRA141 (EC50 ~ 33 nM), an essential component of the replicative helicase complex involved in DNA replication, while having minimal effects against potential off-target kinases. Coincident with the inhibition of phospho-MCM2, SRA141 inhibited the phosphorylation of the Cdc7 substrate MCM2 (EC50 ~ 33 nM), which is essential for DNA replication and is a known target of SRA141.

Conclusions: The present studies have uncovered a previously unrecognized role of kinases in the post-translational regulation of OATP1B1. The widespread use of TKIs affecting LYN function in diverse therapeutic areas make OATP1B1 substrates highly vulnerable to phosphorylation-mediated drug-drug interactions.

No conflict of interest

109 (PB-060) Poster
Targeting the inhibitor of apoptosis proteins (IAPs) sensitises oesophageal adenocarcinoma to Akt inhibition

L. Stevenson1, L. Cairns1, R. Douglas1, N. Mccabe2, G. Gavory3, T. Harrison1, R.D. Kennedy4, R.C. Turckington1. 1Queen’s University Belfast, Centre for Cancer Research and Cell Biology, Belfast, Northern Ireland; 2Almac Diagnostics, Centre for Cancer Research and Cell Biology, Belfast, United Kingdom; 3Almac Discovery, Centre for Cancer Research and Cell Biology, Belfast, United Kingdom

Background: Oesophageal adenocarcinoma (OAC) is the predominant histological type of oesophageal cancer in North America and Europe and incidence rates have progressively increased 6-fold since 1975. Whole genome sequencing has identified P13K/AKT as the most frequently mutated oncogenic pathway in OAC with significant amplifications in upstream gene targets in the majority of OAC tumours. Akt is therefore an attractive therapeutic target in OAC. Inhibitor of apoptosis proteins (IAP) are overexpressed in a variety of human cancers and small molecule IAP antagonists that mimic the second mitochondria-derived activator of caspase (Smac) are promising cancer therapeutics. This preclinical study uses a novel allosteric Akt1/2 inhibitor, ALM301, to target Akt in OAC cell lines in combination with chemotherapy or IAP antagonist, BV6. We hypothesised that targeting IAPs could enhance the efficacy of Akt inhibition in OAC.

Materials and Methods: The effect of ALM301 in combination with 5-fluorouracil (5FU), cisplatin (DDP) or BV6 was examined in a comprehensive panel of 9 OAC cell lines, OE33 and FLO1 cell lines were continuously treated with increasing concentrations of ALM301 or DDP over ~3 months to generate ALM301- and DDP-resistant sub-lines respectively. Cell viability was measured using MTT, Cell Titre GLo® and clonogenic assays. Cell growth was determined by Ki67 expression, RNA seq transcriptionally profiled using the XcelTM array (Almac Diagnostics) and detection of cleaved PARP by Western blot. Cell lines were transcriptionally profiled using the XcelTM array (Almac Diagnostics) and expression of IAPs was examined by Western blot.

Results: Akt inhibition synergistically sensitised the OAC cell lines to chemotherapy (combination index < 1.0) but this sensitisation was context-dependent. Examination of IAPs following chemotherapy treatment revealed down-regulation of cIAP1 and XIP in the settings which synergised with ALM301 and high intrinsic XIAP expression correlated with resistance to ALM301. In addition, IAP gene and protein expression was up-regulated in the ALM301-resistant cell lines. BV6 was found to deplete cIAP1 and XIAP in the OE33, OE19 and FLO1 cell lines and sensitised to ALM301. Further examination in the broader OAC cell line panel showed BV6 inhibition (~IC50) doses sensitised to ALM301. BV6 also sensitised to ALM301 in the ALM301- and DDP-resistant sublines.
Conclusions: Sensitivity to Akt inhibition is correlated with IAP expression in sensitive and resistant in vitro models. Our preclinical findings support a potential clinical role for Akt inhibition in combination with the IAP antagonist BV6 in the treatment of OAC.

Conflict of interest: Other Substantive Relationships: Nuala McCabe, Richard Kennedy, Gerald Gavory and Timothy Harrison are employees of the ALMAC group.

110 (PB-061) Poster Development of limited proteolysis, a novel drug target deconvolution strategy

N. Beaton1, R. Bruderer1, K. Beeler1, I. Piazza2, L. Reiter1. 1Biognosys AG, Research and Development, Schlieren, Switzerland; 2Biognosys AG, Scientific Business Development, Schlieren, Switzerland; 3ETH Zurich, Institut für Biochemie, Zurich, Switzerland

Background: High attrition rates in target-centric drug development approaches, as well as a limited number of targets, have shifted the focus of drug development back towards phenotypic screening. In parallel, novel proteomics-based target deconvolution approaches to drug target identification have gained popularity. Limited proteolysis (LiP) is a new target deconvolution technique that exploits protein conformational changes driven by drug binding. A major advantage of LiP is its unique focus on novel “conformotyic” peptides that are generated by a limited, unspecific digestion and subsequently identified by proteomic analysis. Here we demonstrate the reproducibility and performance of LiP using the mTOR inhibitor, rapamycin, as well as the protein phosphatase inhibitor Calyculin A, in a human cell lysate.

Materials and Methods: Mechanically sheared HeLa cell lysate was incubated with compound at multiple concentrations. Next, a limited digest was performed using the unspecific protease, proteinase K. Finally, the limited digests were processed to peptides with trypsin for mass spectrometry analysis. A project-specific spectral library was generated using data-dependent acquisition (DDA) mass spectrometry and for quantitative analysis data-independent acquisition (DIA) data was recorded and analyzed using Spectronaut Pulsar X and the project-specific spectral library.

Results: Herein, we demonstrate in HeLa lysate that our modified LiP approach reproducibly identified several conformotyic FKBP1A peptides, the target of the potent immunosuppressant rapamycin, with high confidence (p-value < 0.01), a finding not yet shown in a mammalian context. Notably, these same peptides displayed a strong dose-response correlation with increasing rapamycin concentrations (IC50 = 144 nM). A similar dose-response relationship was observed, and several conformotyic peptides could be shown, for the protein targets of the phosphatase inhibitor Calyculin A, with IC50 values of 57 and 23 nM for PP1A and PP2A respectively. Importantly, known concentration differences in target affinity were confirmed via extrapolation of IC50 values. Additionally, a previously unknown target of Calyculin A (PP1B) was identified among the same phosphatase family with a higher IC50 than previously known targets at approximately 74 nM.

Conclusions: Collectively, our data demonstrates that LiP can be used to effectively identify protein targets, both known and novel, and characterize their binding properties (e.g. IC50). These capabilities make LiP a powerful target deconvolution strategy with the potential to become an essential part of the drug development pipeline.

Conflict of interest: Ownership: Nigel Beaton, Roland Bruderer, Kristina Beeler, and Lukas Reiter own stock options in Biognosys.

112 (PB-063) Poster Relationship between c-kit mRNA expression and prognosis in postoperative patients with rectal carcinoma

Y. Liu1, Y. Huang2, M. Ying3. 1Fujian Medical University Cancer Hospital, Department of Abdominal Surgery, Fuzhou, China; 2Fujian Medical University Cancer Hospital, Department of Radiation Oncology, Fuzhou, China

Background: There was no consensus on the relationship between c-kit and the prognosis of rectal carcinoma patients. Our study aimed to investigate the correlation between c-kit mRNA expression and prognosis in patients with rectal carcinoma.

Methods: The expression of c-kit mRNA in rectal carcinoma tissues (n = 66) was detected by multiplex branched-DNA liquid chip method. The patients were classified into the high expression group and the low one, according to the outcome of its expression. Chi-square test was utilized to analyze the relationship between expression and clinic pathological features. Kaplan-Meier estimates and multivariate analysis (Cox) were employed to assess the correlation between the expression of c-kit and prognosis.

Results: The high expression rate of c-kit was 27% (18/66) in tumor tissues. No significant correlation was found between the c-kit expression and gender, age, preoperative carcinoembryonic antigen, preoperative hemoglobin, distance to verge, lymph node metastasis, tumor thrombus, T stage, TNM stage, tumor differentiation (P > 0.05). Kaplan-Meier indicated that 1-, 3-, 5-year survival rate of the high c-kit expression group were respectively 100.0%, 77.8%, 77.8%, and those of the low c-kit expression group were respectively 93.8%, 56.3%, 45.8%. The difference were statistically significant (c2 = 5.056, P = 0.025). Multivariate analysis (Cox) showed that the expression of c-kit was a significant factor (HR) = 0.354, 95% CI (0.133–0.943), P = 0.038 and TNM stage (HR) = 6.781, 95% CI (1.100–41.799, P = 0.039) were independent prognostic factor of postoperative rectal cancer patients.

Conclusions: Low expression of c-kit was associated with poor prognosis of rectal carcinoma. It is worth further studying whether it could become a potential target or not.

No conflict of interest

113 (PB-064) Poster Cell-penetrating Alphabodies targeting the Wnt/β-catenin pathway


Background: BCL9 is a constitutive member of the WNT pathway, where it serves as a docking port for nuclear β-catenin. Aberrant WNT activation is frequently observed in multiple cancers, and leads to increased proliferation, stemness and metastatic potential of tumor cells. WNT pathway inhibition at the level of the enhancement by blockade of BCL9 - β-catenin interaction represents an attractive therapeutic approach to tackle WNT-dependent cancers. Complix has developed Cell Penetrating Alphabodies (CPAB’S), a novel and unique class of therapeutic proteins engineered to efficiently enter cells and inhibit intracellular protein-protein interactions, and applied its CPAB platform to identify and functionally characterize binders of BCL9.

Material and Methods: Alphabodies targeting the HD2 domain of BCL9 and its paralog BCL9L were identified via selection of an Alphabody library displayed on phage. Ranking of the best candidates was performed via dual reporter, cell proliferation and clonogenic assays, cancer stem cell (CSC) self-renewal and wound healing assays.

Results: A biopanning campaign on BCL9 and its paralog BCL9L were identified via selection of an Alphabody library displayed on phage. Ranking of the best candidates was performed via dual reporter, cell proliferation and clonogenic assays, cancer stem cell (CSC) self-renewal and wound healing assays.

Conclusions: Complix has developed an anti-BCL9/9L CPAB able to specifically inhibit the BCL9 - β-catenin interaction in the WNT enhancement, with low-nM KD. Treatment of cancer cells with anti-BCL9/9L CPAB leads to inhibition of WNT transcriptional activity and gene expression, and to suppression of CSC self-renewal, proliferation and migration in WNT-pathway activated cancer cells.
114 (PB-065) Poster
Anti-tumor activity of tarloxotinib, a hypoxia-activated EGFR/HER2 TKI, in HER2 driven cell lines
A. Estrada-Bernal 1, A. T. Le 1, A. E. Doak 1, R. C. Doebele 1. 1School of Medicine, University of Colorado-Anschutz Medical Campus, Department of Medicine, Div of Medical Oncology, Aurora, USA

Background: Lung adenocarcinoma is characterized by distinct subsets that can be classified by oncogene status. ERBB2 (HER2) gene amplification is present in ~3%, and ERBB2 activating mutations in ~3% of the patients. Although HER2-directed therapies are available for breast and gastric cancer, the use of HER2-directed monoclonal antibodies and tyrosine kinase inhibitors (TKIs) have been disappointing in lung cancer. Tarloxotinib (TRLX) is a prodrug, which is converted to the active metabolite TRLX-TKI, an irreversible EGFR/HER2/HER4 TKI, under hypoxic conditions. We have previously shown that tarloxotinib can overcome the intrinsic resistance of EGFR/Flexon 20 insertion mutations to existing TKIs in vitro and in vivo.

Methods: Using MTS, we evaluated the proliferation of three HER2-driven cell lines (Calu-3 and H2071 with ERBB2 amplification; H1781 with ERBB2p, G776VC insertion mutation) treated with TRLX-TKI or TRXL or 1st, 2nd, or 3rd generation EGFR/HER2 TKIs. We analyzed the on-target and signaling effects elicited by TRLX-TKI via immunoblots of pHER2, pHER3, pE3K, and pAKT. Using a nude mice xenograft model, we compared the effect of the TRLX produg with other TKIs on tumor growth.

Results: Our results demonstrate that HER2-driven lung cancer cell lines show the highest sensitivity to TRLX-TKI whereas the TRXL produg is ≤50-fold less potent under normoxic conditions, consistent with the required metabolism for activation by hypoxia. Malatin, which has previously been shown to have poor activity in HER2 mutation positive NSCLC, was the next most potent drug in vitro, followed by osimertinib and gefitinib. In immunoblot analysis, TRLX-TKI inhibits HER2 phosphorylation between 10 and 100 nM whereas afatinib inhibits pHER2 at 100 nM, consistent with the cell proliferation data. Notably, we also observed a concurrent reduction in pHER2 phosphorylation with TKI treatment. Analysis of downstream signaling pathways demonstrated that pERK was not inhibited by any of the TKIs, whereas AKT signaling was inhibited at similar doses to that of upstream HER2. Xenograft data will be presented at the meeting.

Conclusions: TRXL-TKI is a potent HER2 inhibitor in vitro that can inhibit HER2 (and HER3) phosphorylation at low nanomolar doses and with greater potency than currently approved TKIs for NSCLC. This activity was observed in cell lines harboring both amplified or mutant ERBB2. HER2-driven cells depend on the AKT pathway for survival whereas MAPK inhibition was not necessary to inhibit cell proliferation. Tarloxotinib represents a potential new therapeutic approach for NSCLC patients harboring ERBB2-gene alterations.


115 (PB-066) Poster
In-vitro characterization of the mechanism of action of abemaciclib in human bone marrow progenitors
M.P. Ganado 1, R. Torres-Guzman 1, G.E. Perez 1, C. Baquero 1, S. Barriga 1, B. San Antonio 1, J. Du 1, L. Prieto 1, A. De Dios 1, M.J. Lallena 1. 1Lilly and Company, Lilly Research Laboratories, Alcobendas, Spain; 2Eli Lilly and Company, Lilly Research Laboratories, Indianapolis, USA

Background: CDK4/6 are essential regulators of cell proliferation, permitting cell cycle arrest upon inhibition of Rb phosphorylation. Furthermore, altered CDK4/6 activity are showing clinical activity. The role of CDK4/6 inhibitors as it was required after chemotherapy treatment with CDK4/6 inhibitors as it was required after chemotherapy treatment. CDK4/6 inhibitors on the maturation of CD34+ human progenitors and mature neutrophils in vitro. Washout studies with GCSF were carried out as potential adjuvant for neutrophil counting recovery.

Materials and Methods: We used flow cytometry to study the effect of CDK4/6 inhibitors on the maturation of CD34+ human progenitors and mature neutrophils in vitro. Washout studies with GCSF were carried out as potential adjuvant for neutrophil counting recovery.

Results: 1. Different CDK4/6 inhibitors share a common mechanism of action to induce neutropenia, which is distinct from that of chemotherapy agents; it affects directly the maturation of human precursors and not mature cells being rapidly reversible. This reflects a cytostatic effect on neutrophil precursors from bone marrow supporting reversibility in vivo. 2. Addition of GCSF is not essential for the full recovery of the CD34+ maturation after treatment with CDK4/6 inhibitors as it was required after chemotherapy agent. 3. There is a robust correlation between the degree of inhibition of CDK6 kinase activity and prevention of CD34+ maturation in vitro.

Conclusion: Results described herein contribute to understand abemaciclib safety profile and confirm a clear difference from chemotherapy-induced neutropenia providing clarification on the role of CDK6 inhibition on maturation of myeloid line.

No conflict of interest

117 (PB-068) Poster
Molecular dissection of CDK4/CyclinD1 regulation: Prevention of pathway hyperactivation by continuous CDK4/6 inhibition
S. Gharbi 1, J. Gutierrez 2, A. Espada 1, M.S. Gutierrez 1, R. Torres-Guzman 1, M.F. Debets 1, J. Prieto 1, J. Du 1, A. De Dios 1, M.J. Lallena 1. 1Lilly and Company, Lilly Research Laboratories, Alcobendas, Spain; 2Eli Lilly and Company, Lilly Research Laboratories, Indianapolis, USA

Background: CDK4/6 are essential regulators of cell proliferation, permitting the stepwise progression through the cell cycle. Alteration in CDK4/6 pathway occurs frequently in numerous types of cancer and drugs targeting CDK4/6 activity are showing clinical activity. In vitro reports suggest however, that CDK4/6 inhibitors (CDK4i/6i) can stabilize an active complex and this could induce pathway hyperactivation upon compound removal. In some clinical trials with CDK4/6 inhibitors, a rebound in proliferation activity has been observed in patients during the treatment rest interval.

Methods: We developed an MS-based workflow including affinity purification-MS (AP-MS) and used hydrogen to deuterium exchange (HDX) to understand the dynamic behaviour of CDK4/6 during inhibition with abemaciclib and other CDK4/6 inhibitory agents. AP-MS was applied to quantify cellular CDK4 phosphorylation (p-HER3) and signaling pathway hyperactivation by continuous CDK4/6 inhibition recently approved by the FDA for HR-positive advanced breast cancer both as monotherapy and in combination with fulvestrant or letrozole. Abemaciclib is an ATP-competitive inhibitor, although similar to other CDK4i/6i, its enzyme kinetics suggests a mixed mode of action behavior. We sought to investigate how CDK4i/6i regulate CDK4/CyclinD complexes at the molecular level and in clinically relevant cellular models of breast cancer.

Results: CDK4-CyclinD1 complex is controlled by a coordinated interplay between innate negative regulators such as INK and CIP/KIP family of i conulatory and mitogenic signals, key events that control CDK4 activity. We show that compounds have affinity for both the active (phosphorylated) and the inactive (non-phosphorylated) form of CDK4. In addition, we confirm that they stabilize the activated complex in a breast cancer model sensitive to CDK4/6i; CDK4 inhibition stabilized the phosphorylated form of CDK4 and reduced levels of p21-bound to CyclinD1/CDK4 complex. This correlated with increased Rb phosphorylation and cell proliferation following compound removal.

Conclusions: Together, our data indicate that stabilization of a pre-activated complex lacking the native negative regulator p21 could lead to rapid pathway activation when compound levels decrease. This highlights the need for constant inhibition of the complex for efficient blockade of the pathway. Importantly, to overcome this rebound effect, continuous dosing schedule may be critical to reach best clinical results.

No conflict of interest

118 (PB-069) Poster
EGFR-mediated alterations to trastuzumab-mediated antibody-dependent cell-mediated cytotoxicity (T-ADCC) in TKI-resistant HER2+ breast cancer cell lines
N. Gavron 1, A. Canonic 2, M. McDermott 1, J. Crow 2, D. Collins 2. 1Dublin City University, National Institute for Cellular Biotechnology, Dublin, Ireland; 2St. Vincent’s University Hospital, Elm Park, Dublin 4, Ireland

Background: Trastuzumab is a monoclonal antibody therapy used in the treatment of HER2+ breast cancer. Trastuzumab inhibits HER2-related
intracellular signalling pathways and is capable of engaging the immune system through TADC. Lapatinib is a reversible, small molecule tyrosine kinase inhibitor (TKI) targeting HER-family members EGFR and HER2. Arafatinib is an irreversible small molecule pan-HER family TKI. Previous work has shown decreased T-ADCC and increased EGFR protein levels in a lapatinib-resistant HER2+ breast cancer cell line (SKBR3-L). This study examines the impact of EGFR activation on T-ADCC in parental and lapatinib- and a Rafatinib-resistant cell model lines (HCC1954L, SKBR3-A) of HER2+ breast cancer.

Methods: The HER2+ breast cancer cell lines SKBR3 and HCC1954 were exposed to afatinib (150 nM) and lapatinib (1 µM) for 6 months to generate the TKI-resistant SKBR3-A and HCC1954-L cell lines. Proliferation assays were used to determine resistance to TKI treatment. Expression of EGFR and pEGFR y1068 in parental and resistant cell lines was determined by Western blot and analysed by densitometry. For immune cell-mediated cytotoxicity assays, PBMCs were isolated from healthy volunteer blood using a Ficoll-Paque-based method. The target parental and resistant cell lines were treated with and without EGF (10 ng/ml) for 24 hours. The PBMCs and the target cancer cells were incubated at a ratio of 5:1 (PBMC:TC) for 12 hours with cytotoxicity assessed using a flow cytometry-based method. Direct cytotoxicity and T-ADCC were determined against each cell line. Comparative statistical analysis was performed using Student’s T test.

Results: Protein levels of EGFR were increased in SKBR3-A (p = 0.02) and decreased in HCC1954-L (p = 0.02) compared to parental cell lines. pEGFR (y1068) levels in the SKBR3-A were reduced relative to parental cell lines (p = 0.01). pEGFR (y1068) levels were unchanged between HCC1954-L and the parental cell line. TKI resistance resulted in increased direct cytotoxicity and T-ADCC compared to the parental cell lines in both SKBR3-A and HCC1954-L. EGFR pre-treatment of target cells increased T-ADCC by 15.1 ± 0.4% in the SKBR3 cell line but reduced direct T-ADCC by 12.4 ± 1.2% in the SKBR3-A cell line. T-ADCC levels were not effected in EGF pre-treated HCC1954 or HCC1954-L cap cell lines.

Conclusions: A TKI resistant phenotype in the cell line models examined resulted in increased susceptibility to T-ADCC. Activation of the EGFR pathway alters susceptibility of SKBR3 parental and afatinib resistant cancer cells to T-ADCC. Further work is warranted to examine the mechanisms and potential mediators of this effect in HER2+ breast cancer models.

Conflict of interest: Corporate-sponsored Research: Dr. Alexandra Canonici has received research funding from Boehringer Ingelheim. Dr. Deniz Collins has received research funding from Boehringer Ingelheim. Other Substantive Relationships: Prof. John Crown has had a consulting or advisory role with Boehringer Ingelheim.

119 (PB-070) Poster Chromosomal Aberrations in Chronic Myeloid Leukemia: Response to conventional tyrosine kinase inhibitors and risk of blast transformation

S. Maqsood1, N. Siddiqui1, F. Ali1, S. Shaikut Khanum Memorial Cancer Hospital and Research Center, Medical Oncology, Lahore, Pakistan

Background: Chronic Myeloid Leukemia (CML) is a common hematological malignancy with tyrosine kinase inhibitors (TKI) forming the main stay of treatment.

Methods: This is a retrospective chart review of all patients who were diagnosed with CML in chronic phase (CP) with additional chromosomal abnormalities (ACAs) over a period of 5 years from 2010 to 2015 at our institution.

Results: Response to initial TKI. A total of 283 patients were diagnosed with CML from January 2010 to January 2015. Thirty one patients were found to have ACAs at the time of diagnosis in addition to BCR-ABL fusion gene. 82 patients (28.6%) were males. 11 (35.5%) of patients had variant Philadelphia chromosome following by 7 patients (22.6%) with trisomy 8. 8 patients (16.1%) had multiple chromosomal abnormalities including trisomy 8, deletion 1 and isochromosome 17q. Risk of blast transformation.

Eight patients (28.6%) transformed to acute myeloid leukemia(AML) whereas 3 (9.7%) patients had a transformation to acute lymphoblastic leukemia(ALL). Therapy and response in transformed patients. Out of the 11 patients who transformed to AML and ALL, 6 patients underwent conventional chemotherapy while 5 were not considered fit for it on account of poor performance score and lack of resources. Out of these 6 patients, none responded to induction chemo.

Conclusion: In summary, this purpose of this project was to study the clinical presentation of patients with CML harboring additional chromosomal abnormalities at diagnosis, cytogenetic analysis, risk of blast transformation and response to treatment in transformed patients. Despite an early hematological response, more than half of the patients failed to achieve complete molecular and cytogenetic response which clearly shows suboptimal response to the tyrosine kinase inhibitors in this particular set of CML patients. There was transformation to acute leukemia in 35.5% of patients. We were able to give induction chemotherapy to only 6 out of 11 patients and none of them responded to it. This study showed that presence of ACA at the time of diagnosis is a high risk feature for patients with CML and confers poor prognosis when treated with conventional TKI. Further studies are required in our population regarding alternative therapy for such patient population.

No conflict of interest

120 (PB-071) Poster DCC-2618, a broad-spectrum inhibitor of KIT and PDGFR mutants, synergizes with inhibitors of the MAPK pathway

A. Gupta1, C. Leany1, A. Garcia-Valverde2, J. Arribas2, C. Serrano2, D. Flynn1, B. Smith1, 1Deciphera Pharmaceuticals Inc., Biochemistry, Waltham, MA, USA, 2Vall d’Hebron University, Preclinical Research Program, Vall d’Hebron Institute of Oncology, Barcelona, Spain

Background: Treatment of gastrointestinal stromal tumors (GIST) with the KIT-inhibitor imatinib leads to objective responses or stable disease, but complete responses are rare. Residual signaling through the MAPK pathway via complete suppression of KIT or activation of other pathways that feed into MAPK signaling are possible mechanisms allowing GIST cells to escape death. The stasis of GIST tumors in response to treatment with KIT inhibitors can lead to rebound tumor growth in the absence of drug or allow for drug-resistance mutations to arise. Inhibitors of the MAPK signaling pathway, such as MEK inhibitors, have been shown to synergize with imatinib to inhibit GIST cell growth in vitro and in vivo. DCC-2618 is a kinase switch control inhibitor that potently inhibits the MAPK signaling pathway in GIST and mastocytosis models.

Methods: DCC-2618 and control compounds, alone or in combination with several MEK inhibitors, were tested using a variety of cell-based assays, including apoptosis and clonogenic assays. Levels of phosphorylated KIT and other proteins were determined by Western blot or ELISA. Proliferation was measured using the fluorescent dye resazurin. Xenograft models were performed at MI Bioresearch (Ann Arbor, MI).

Results: KIT inhibitors block proliferation of the imatinib-sensitive cell line, GIST T1, however single-agent treatment does not commit most cells to apoptosis, and quiescent cells grow back when drug is removed. The combination of DCC-2618 with a MEK inhibitor leads to substantially higher apoptosis and cell death. Importantly, DCC-2618 also synergizes with MEK inhibitors in drug-resistant GIST and mastocytosis cell lines, leading to a greater blockade of signaling pathways and increases in apoptosis and cell death.

In vivo, treatment with DCC-2618 in combination with a MEK inhibitor led to complete regression of tumor growth during the dosing period, and long-term reduction in tumor growth after the dosing period in a GIST xenograft model. DCC-2618 also showed additive efficacy with a MEK inhibitor in mastocytosis models.

Conclusions: Combinations of DCC-2618 and inhibitors of the MAPK pathway lead to greater cell death in GIST and mastocytosis cell lines, and may have utility in the treatment of patients with GIST or mastocytosis. DCC-2618 is currently in a Phase 3 clinical trial in advanced GIST patients who have been treated with prior therapies (ClinicalTrials.gov Identifier: NCT03353753).

Conflict of interest: Ownership: A.G., C.L. D.F. and B.S. are full-time employees of Deciphera Pharmaceuticals and have stock/options in the company. Advisory Board: C.S. has served on advisory boards for Deciphera Pharmaceuticals. Corporate-sponsored Research: C.S. has received research funding from Deciphera Pharmaceuticals. Other Substantive Relationships: C.S. is an investigator on a clinical trial sponsored by Deciphera Pharmaceuticals.
Background: RX-5902 (Supinoxin) is a novel, oral, anti-cancer compound that targets phosphorylated p68 (DDX5) to attenuate nuclear shuffling of b-catenin. At elevated levels of b-catenin signaling have recently been implicated in resistance to immunotherapies, we sought to determine if RX-5902 could enhance or reverse these responses. In the current study, we utilized a novel humanized mouse model bearing MDA-MB-231 triple-negative breast cancer (TNBC) xenografts to determine if RX-5902 could enhance the anti-tumor activity of the PD-1 inhibitor nivolumab. Additionally, end-of-study immunologic analyses were performed to assess the potential mechanisms by which RX-5902 augments the therapeutic potential of immunotherapies in models of TNBC.

Methods: Humanized Sirpα mice from the BALB/cRag2 -/- IL2Rγc -/- (BRG) strain were used. Newborn pups were humanized through transplantation of 1 x 10^7 CD34+ cells purified from umbilical cord blood. Mice were evaluated for chimerism at 6 and 12 weeks. At 16 weeks, 3 x 10^6 MDA-MB231 cells were implanted on the right and left flanks mice. When average tumor size reached a volume of ~150–300 mm^3, mice were randomized into treatment groups according to % chimerism. At sacrifice, blood, bone marrow, lymph nodes, spleen and tumors were harvested for flow cytometry analysis of human immune cells.

Results: We observed significant tumor growth inhibition when RX-5902 was combined with nivolumab compared to either agent alone with an inhibition index of approximately 50% (p < 0.01). Immunologic analyses indicated that mice treated with RX-5902 demonstrated a significant increase in the number of activated T cells in tumor infiltrating lymphocytes (TILs) and a significant decrease in immunosuppressive myeloid-derived precursor cells (MDSCs) compared to vehicle (p < 0.05). In the RX-5902/nivolumab combination group, there was a significant increase in the percentage of CD4+ T cells in TILs and increased, systemic granzyme B production (p < 0.01).

Conclusions: RX-5902 enhanced the efficacy of nivolumab in a humanized, preclinical model of TNBC. Several changes in immunologic profiles were noted in mice treated with RX-5902 and the combination, including an increase in activated TILs and a decrease in MDSCs in the tumors. These finding indicate that RX-5902 may have important clinical immunomodulatory as well as anti-tumoral activity in the treatment of TNBC with a checkpoint inhibitor. A single-agent phase 2a clinical study in metastatic TNBC is ongoing.

No conflict of interest
CDK4/6-dependent cancers, we studied the effects of tria on chemo efficacy in CDK4/6-dependent tumors.

**Material and Methods:** A panel of patient-derived (PDX) and cell-based breast cancer xenograft models of varying sensitivities to tria (i.e., CDK4/6-dependence) were treated with different chemotherapies for up to six weeks, and tumor volumes evaluated to determine the impact of tria on chemo efficacy. The cell cycle kinetics of tumor cells and bone marrow cells, including hematopoietic stem cells (HSCs), were also evaluated before and during tria treatment. In the model most sensitive to CDK4/6 inhibition (MCFT), experiments varying the scheduling of tria around chemotherapies are ongoing to further expand the results described below.

**Results:** Single doses of tria administered prior to chemotherapies, different AVB-S6 dosing regimens were tested in animal models, and tria enhanced efficacy. Cell cycle analyses revealed a significantly higher frequency of proliferating tumor cells compared to HSCs, with ~40% of tumor cells in the SG2M phases vs. ~8% of HSCs.

**Conclusions:** Data reported in this abstract using CDK4/6-dependent tumor models demonstrate that intermittent dosing of tria with chemotherapies, does not antagonize tumor efficacy and can enhance efficacy in some models. A potential mechanism for these findings includes the differential cell cycle kinetics of tumor cells vs. HSCs with a larger proportion of proliferating tumor cells present at the time of chemo exposure, thereby rendering the tumor more sensitive to the chemo cytotoxic effects. Taken together with previous studies in immune competent models that demonstrate tria increases anti-tumor activity through myelosuppression and direct immune activation, these data support clinical testing of tria + chemotherapies in patients with CDK4/6-dependent tumors.

**Conflict of interest:** Ownership: We are all employees of G1 Therapeutics, Inc.

---

125 (PB-076) Poster

**Evolved protein of AVB-S6 through the use of a proprietary biomarker in healthy volunteers to guide dosing in oncology studies**

G. McIntyre1, L. Bonifacio1, D. Prohaska1, J. Shang2, Y. Yokota3, A. Moss4, M. Dodds1, R. Tabibiazar1, A. Giaccia5, M. Kowalewska1,2,7. 1Holycross Cancer Centre, Molecular Diagnostics, Kielce, Poland; 2Maria Sklodowska-Curie Institute, Oncology Center, Department of Molecular and Translational Oncology, Warsaw, Poland; 3Warsaw Medical University, Chair and Department of Obstetrics, Gynecology and Oncology, 2nd Faculty of Medicine, Warsaw, Poland; 4Holycross Cancer Centre, Gynecologic Oncology, Kielce, Poland; 5Maria Sklodowska-Curie Institute, Oncology Center, Department of Pathology, Warsaw, Poland; 6Holycross Cancer Centre, Surgical Pathology, Kielce, Poland; 7Warsaw Medical University, Immunology, Biochemistry and Nutrition, Warsaw, Poland

**Background:** There is a marked malignancy of gynecological tract and thus the molecular mechanisms of its carcinogenesis remain obscure. It originates from the progression of HPV-associated high-grade squamous intraepithelial lesions (HSIL), accounted for the usual type of neoplasia (dVIN) and differentiated-type type intraepithelial neoplasia (dVIN). As a consequence, currently targeted therapies for vulvar cancer are lacking. The aim of the study was to analyze and compare the profiles of somatic mutations in precancerous vulvar conditions (HSIL and dVIN) in vulvar squamous cell carcinoma (VSCC) using next generation sequencing technology.

**Materials and Methods:** 50 HSIL, 16 dVIN and 81 VSCC tumour samples, were screened for hotspot mutations in 50 genes covered by the Ion AmpliSeq Cancer Hotspot Panel V2 Kit (Thermo Fisher Scientific).

**Results:** Among the 50 analysed genes, TP53 (44%, 26%, 44%), CDKN2A (28%, 9%, 22%), PIK3CA (0%, 13%, 9%), FBXW7 (5%, 7%, 6%), FGFR3 (5%, 11%, 1%) were identified as the most often mutated in dVIN, HSIL and VSCC, respectively. The remaining genes – BRF1, ERBB4, KIT, HNFA1, KRAS, HRAS, GFR, STK11, AKT1, SMAD4, FLI3, JAK3, GNAQ and P1EN – were mutated at much lower frequencies.

**Conclusions:** Unexpectedly, premalignant vulvar lesions (dVIN and HSIL) were found to harbour mutational profile very similar to that of VSCC. The most common genetic alterations identified in VSCC tumours, mutations of TP53 and CDKN2A, are already present in premalignant lesions. Therefore, these mutations may be considered as an early events during VSCC carcinogenesis.

**Acknowledgement:** This work was supported by the Polish National Science Centre grant no. 2013/10/E/NSZ/00863.

**No conflict of interest**

---

127 (PB-078) Poster

**Targeting co-regulators of the androgen receptor as a novel therapeutic approach for prostate and breast cancer**

M. Preciop1, A. Fabe2,3,4, E. Corey5, W. Gallagher1, W. Watson6, J. Crown6. 1UCD Conway Institute, UCD School of Biomolecular and Biomedical Science, Dublin 4, Ireland; 2St Vincent’s University Hospital, Department of Pathology, Dublin, Ireland; 3UCD Conway Institute of Biomolecular and Biomedical Research, Research Pathology Core, Dublin, Ireland; 4Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, D4, Dublin, Ireland, UCD School of Medicine, Dublin, Ireland; 5University of Washington, Department of Urology, Seattle, USA; 6St Vincent’s University Hospital, Department of Medical Oncology, Dublin, Ireland

**Background:** Targeted treatments for prostate cancer mainly target the Androgen Receptor (AR); however, despite initial response, these treatments commonly fail. AR expression has also been associated with poor prognosis in a subset of advanced breast cancers; therefore, targeting AR in this setting is attracting increasing interest. Among the multiple mechanisms of resistance, aberrations in AR co-factors and co-regulators represent a promising therapeutic approach since co-factors and co-regulators are not
EGFR treatments for targetable genetic alterations. These include multiple Background: Clinical and preclinical data demonstrate poor cellular growth and DM-CHOC-PEN lacked cytotoxic activities (IC_{50} >5 mcg/mL). Both the poor growth and the lack of cytotoxicity could be reversed by simply replacing the GLM-free RPMI medium with RPMI plus GLM medium. The SCLC cell lines grew well with or without GLM, and were poorly sensitive to DM-CHOC-PEN (IC_{50} >5 mcg/mM/mL), irrespective of the presence or absence of GLM. Acivicin (3 mM) when added to NSCLC cells in cultures containing GLM, inhibited the activity of DM-CHOC-PEN (IC_{50} >5 mcg/mL) however, in GLM-free medium no changes in NSCLC cell growth were noted.

Conclusion: DM-CHOC-PEN requires GLM for transport into NSCLC cells. Complete support data will be presented. GLM can provide superior control to block the emergence of BRAF inhibitor-resistant melanoma.

C. Feddersen1, A. Varzavand2, J. Schillo3, M. Milhem4, Y. Zakharia5, M. Henry6, A. Dupuy7, C. Stipp7, 1University of Iowa, Anatomy and Cell Biology, Iowa City, USA; 2University of Iowa, Biology, Iowa City, USA; 3University of Iowa, Hematology/Oncology, Iowa City, USA; 4University of Iowa, Molecular Physiology and Biophysics, Iowa City, USA

Background: BRAF/MEK inhibition remains an important treatment option for patients with BRAF\textsuperscript{V600E} mutant melanoma who show disease progression on immunotherapy; however, the majority of patients treated with BRAF inhibitor (BRAFi) therapy develop BRAF-resistant disease within a mean of 6 months. Various genetic analyses of BRAF-resistant melanomas has identified somatic mutations associated with resistance, but has yet to identify novel drug combinations that can be used to prevent or reverse resistance.

Materials and Methods: To address this critical gap, we have recently completed a forward genetic screen using the Sleeping Beauty transposon mutagenesis approach to identify novel drivers of BRAFi resistance in A375

No conflict of interest
melanoma cells. Key results were independently confirmed in multiple melanoma cell lines.

Results: Validating our approach, our screen identified N-terminal truncation of BRAF – a known mechanism of vemurafenib resistance. In addition, we identified DBL family guanine exchange factors (GEFs) as novel drivers of BRAF resistance that we have functionally validated in multiple BRAF

**Poster Session (Tuesday, 13 November 2018)**

**131 (PB-082)**

**Intervenolin, a novel anti-tumor drug, suppresses cancer cell growth through modulation of tumor microenvironment**

M. Kawada1, J. Yoshida1, M. Amemiya1, H. Abe2, T. Watanabe2, M. Shibasaki1, B. Thomas2, S. Li1

**Background:** Tumor-stromal cell interactions are attractive targets for cancer chemotheraphy and we have been focusing on small molecules modulating the interactions. Intervenolin (ITV), a novel natural compound, inhibits the growth of tumor cells in the presence of stromal cells (fibroblasts) more strongly than that in the absence of stromal cells. ITV exerts efficient anti-tumor activity in animal models without adverse effects. In this study we examined the mechanism of action of ITV. Since conditioned medium (CM) of stromal cells pretreated with ITV showed strong growth inhibitory effect against cancer cells, we then analyzed what factors could be dysregulated by ITV. As a result, we found that ITV increased the secretion of organic acids such as lactic acid and malic acid from stromal cells and changed the medium to acidic. In that acidic condition we found that ITV suppressed the activity of p70 S6 kinase, a key enzyme in protein synthesis. Furthermore, concerning the secretion of lactic acid by ITV, we found that ITV inhibited mitochondrial complex I. These results suggest that ITV inhibits cancer progression and metastasis. The potential oncogenic roles that TAM RTKs may play in BCa are undefined.

**133 (PB-084)**

**BAT8001, a potent anti-HER2 antibody-drug conjugate with a novel linker for the treatment of HER2-positive gastric cancer**

J.C. Yu1, W. Tang1, X. Deng1, Z. Ou1, J. Gan1, Q. Dong1, B. Tan1, L. Lu1, B. Chen1, C. Bao1, B. Thomas2, S. Li1

**Background:** HER2 is overexpressed at meaningful levels in a number of tumors, including breast, gastric, salivary duct carcinomas, non-small cell lung cancer and colon cancer. Gastric cancer is the second leading cause of cancer death in the world and represents a significant unmet medical need. Trastuzumab emtansine (T-DM1), an anti-HER2 ADC, has shown efficacy in HER2-positive breast cancer metastatic patients and was approved by the FDA and EMA for advanced HER2-positive breast cancer. However T-DM1 causes grade 3 and 4 thrombocytopenia in up to 14.5% of patients as its major toxicity. The thrombocytopenia is likely caused by one of T-DM1’s catabolites and payload, DM1, indicating T-DM1’s linker can be cleaved. Here we adopted a novel noncleavable linker and created an anti-HER2 ADC, BAT8001, which is expected to be efficacious in HER2-positive cancers and have a better side effect profile relative to T-DM1 due to the stability of BAT8001’s noncleavable linker.

**Material and Methods:** The payload of BAT8001, a maytansine derivative, is a fermentation product from a bacteria strain Actinosynnema pretiosum. The maytansine derivative is then connected to the linker through chemical reactions, followed by conjugation with the antibody. Annexin V-staining apoptosis assay, 72-hr cell proliferation assay, mouse tumor chemical reactions, followed by conjugation with the antibody. Annexin V-staining apoptosis assay, 72-hr cell proliferation assay, mouse tumor models, pharmacokinetic and toxicology studies were performed using standard methods.

**Results:** BAT8001 is internalized in HER2-positive cells. It inhibits proliferation of HER2-positive tumor cells with IC50s of ~0.1 nM, similar to the potency of T-DM1. BAT8001 also induces apoptosis in HER2-positive cells. In both cell-line and patient-derived mouse xenograft (PDX) models, BAT8001 demonstrates strong inhibition activity on tumor growth. For example, in a PDX mouse model of gastric cancer (GA0060), BAT8001 demonstrates a dose response curve with complete responses in all animals tested at the 15 mg/kg dose level. Pharmacokinetics studies in mouse models reveals BAT8001 has similar Cmax, AUC, and t1/2 as T-DM1. The major catabolite of BAT8001 is the Cys-linker-payload containing product. No free payload is observed. This compares favorably with T-DM1 where free DM1, T-DM1’s payload, is one of the major catabolites. In a multiple dose toxicity study, BAT8001 had a NOAEL of 15 mg/kg versus 10 mg/kg for T-DM1.

**Conclusions:** BAT8001 exhibits similar potency to T-DM1 on inhibiting HER2-positive cell proliferation and tumor growth, yet demonstrates better multiple dose toxicity than T-DM1. The improved toxicity profile of BAT8001 suggests that the novel noncleavable linker utilized in BAT8001 is more stable than the linker utilized in T-DM1. BAT8001 is very efficacious in PDX and patient-derived xenograft models of gastric cancer. The preclinical profile of BAT8001 warrants further development for the treatment of gastric cancer and other HER2-positive cancers.

**Conflict of interest:** Ownership: All authors are employees of Bio-Thera Solutions, Ltd and are eligible for incentive stock options. Board of Directors:
Dr. Shengfeng Li is on the Bio-Thera Solutions, Ltd. Board of Directors. Corporate-sponsored Research: Bio-Thera Solutions, Ltd. is a private biopharmaceutical company based in Guangzhou, China. The research presented in this poster is funded entirely by Bio-Thera Solutions, Ltd.

134 (PB-065) Poster
Chemotherapy-induced metastasis: mechanisms and translational opportunities

G. Karagiannis1, L. Rivera Sanchez2, Y. Wang1, V. Sharma1, J. Burt1, D. Entenberg1, M. Oktay1, J. Condeelis1.
1Albert Einstein College of Medicine, Anatomy and Structural Biology, Bronx, USA

Background: We have previously discovered that chemotherapy induces prometastatic changes in the breast cancer microenvironment by promoting the assembly and function of cancer cell intravasation sites called tumor microenvironment of metastasis (TMEM), and by increasing the proportion of the highly-invasive MenaINV-HI tumor cells that utilize TMEM sites for hematogenous dissemination. Consequently, chemotherapy-treated animals have increased circulating-tumor cell and micrometastasis counts. Since the formation of metastases depends on both the presence of functional doorways for dissemination (TMEM) and cancer cells capable of using these doorways (MenaINV-HI tumor cells), we investigated the cellular molecular context required for chemotherapy-driven TMEM function and MenaINV expression.

Materials and Methods: We used spontaneous models of mouse breast carcinoma (MMTV-PyMT) and patient-derived xenografts treated with chemotherapy and co-treated with Tie2 inhibitor (rebastinib), Cxcl12 inhibitors (AMD3100) ormacrophage lineage depletion agents (clodronate). Prometastatic endpoints were measured by intravital imaging, multichannel immunofluorescence and standard metastatic dissemination assays.

Results: Since TMEM function depends on Tie22 macrophages and MenaINV expression on macrophage contact, we focused on inhibiting Tie2 receptors, as well as macrophage influx and/or function to suppress chemotherapy-induced metastasis. We demonstrated that chemotherapy-mediated induction of MenaINV-HI tumor cells depends on the presence of macrophages via multiple methods of macrophage suppression. In particular, the depletion of either the entire macrophage lineage using clodronate liposomes, or the use of specific inhibitors of the Cxcl12/Cxcr4 chemotactic pathway, both resulted in a significant suppression of the prometastatic MenaINV-HI tumor cell subpopulation in all mammary tumors examined. Moreover, inhibition of Tie2 by rebastinib blocked TMEM function and decreased the number of circulating tumor cells and metastatic foci, despite the unaffected macrophage infiltration and the chemotherapy-mediated induction of MenaINV-HI tumor cells. Distance analysis algorithms further demonstrated that direct tumor cell-macrophage touching is required for chemotherapy-mediated MenaINV induction. Accordingly, the acute inhibition of Notch pathway, using gamma-secretase inhibitors along with chemotherapy eliminated the MenaINV-HI prometastatic phenotype.

Conclusions: Our data indicate that both the MenaINV-HI disseminating cancer cell population and the TMEM doorways are necessary but not individually sufficient for metastasis. As such, suppression of either of the two, MenaINV-HI population or TMEM function, can suppress chemotherapy-induced metastasis, thus providing targets to improve clinical care and eliminate all the non-beneficial effects of chemotherapy.

No conflict of interest

136 (PB-087) Poster
Inhibiting multifunctional ERK-protein complexes for cancer therapy

T. Kacou1, W.H. Johnson1, N.D. Ebelt2, A. Piscierchio3, S.V. Ravenstein1, D. Zamora-Oliveras4, R. Edupuganti5, R. Sammons1, M. Cand6, M. Warthaka1, C.D. Tavares1, J. Park2, P. Ren3, R. Ghose5, K.Y. Tsai6, E.V. Anslay7, K.N. Dalby1, 1The University of Texas at Austin, College of Pharmacy, Chemistry, Biochemical, and medicinal Chemistry, Austin, USA; 2City of Hope National Medical Center, Department of Experimental Therapeutics, Duarte, USA; 3The City College of New York, Department of Chemistry, New York, USA; 4The University of Texas at Austin, Department of Chemistry, Austin, USA; 5The University of Texas at Austin, Department of Biomedical Engineering, Austin, USA; 6Dana-Farber Cancer Institute, Harvard Medical School, Department of Cancer Biology, Boston, USA; 7University of Texas MD Anderson Cancer Center, Department of Breast Medical Oncology, Houston, USA; 8H. Lee Moffitt Cancer Center and Research Institute, Departments of Anatomic Pathology & Tumor Biology, Tampa, USA

Background: Mutations in pathways that enhance the activity of ERK1 and ERK2 are frequently present in human cancers, reflecting their important roles in tumor initiation, and progression. This notion is reinforced by observations in BRAF V600E mutant melanoma where the majority of the mechanisms of resistance to FDA-approved combination therapies targeting BRAF and MEK involve reactivation of ERK1 and ERK2. Recently, the direct targeting of the ERK enzymes using ATP-competitive inhibitors has emerged as an attractive strategy to overcome acquired resistance to current combination therapies. The ERK enzymes employ unique mechanisms of molecular recognition to engage protein components of the MAPK pathway.

Material and Methods: Chemical, Biochemical, cell biology and in vivo studies have been employed to characterize the mechanism of action of the first-in-class covalent inhibitor of ERK docking interactions.

Results: Here we report the potent targeting of an ERK-protein docking interaction by a small molecule thiotriazole, which abrogates ERK signaling in vivo. The thiotriazole binds covalently to a highly conserved cysteine residue within the D-recruitment site of ERK1/2 with more than a 100-fold discrimination over other MAPKs (e.g. JNK1/2, p38MAPKs and ERK5). Treatment of various BRAF-inhibitor naive or inhibitor-resistant melanoma cell lines expressing BRAF V600E with the thiotriazole for 2 hours induces dose-dependent inhibition of ERK activation and downstream signaling. Inhibition is maintained for up to 5 hours following thiotriazole washout and induces apoptosis and growth inhibition. Treatment of mice carrying a BRAF V600E A375 melanoma xenograft with the thiotriazole blocked tumor growth. Transient expression of a mutant form of ERK2, which is resistant to the thiotriazole, promotes survival of A375 and HEK 293 cells treated with thiotriazole.

Conclusions: This study lays the foundation for developing a new modality for the treatment of solid tumors driven by excessive ERK signaling.

No conflict of interest
Cell-surface tyrosine kinase receptors (RTKs) represent a prime target family for drug discovery. Historically, drug identification programs have been dominated by efforts to develop inhibitors that compete for binding with endogenous ligands at orthosteric sites. Despite their advantage over orthosteric antagonists, including greater safety and/or selectivity, allosteric modulators are more difficult to identify.

Here, we report the identification of the next generation of extracellularly-acting allosteric modulator of FGFRs (Fibroblast Growth Factor Receptor) leading to an unprecedented irreversible mechanism of action. Using molecular modeling studies, biochemical and cellular assays, we demonstrated that EVT601 interacts with the D3 domain of FGFRs, then inducing a conformational change of the ectodomain allowing the release of a protease-dependent cleavage site in the juxta-membrane domain of the receptor. Consequently, proteases belonging to the MMP-ADAMS family cleaves FGFRs inducing the release of its ectodomain. This biological process is dependent cleavage site in the juxta-membrane domain of the receptor.

FGFR allosteric antagonism offers a new generation of anti-FGF therapies with an unique mode of action. These results open the way for safer anti-FGF therapies in the treatment of cancers and FGFR-dependent diseases.

No conflict of interest
Background: Kinase inhibitors are used as precision medicine in specific patient populations that are defined by the presence of predictive drug response biomarkers. The application of these inhibitors could be further extended and refined by the identification of new response biomarkers. One of the best ways to do this, is by in vitro profiling in cancer cell line panels.

Materials and Methods: Oncolines is a panel of 102 genetically characterized cell lines from diverse tumour origins, on which proliferation assays are run in parallel. Earlier work has shown that the Oncolines workflow generates highly reproducible data, as necessary for biomarker discovery. Previously, we profiled all kinase inhibitors that were approved before November 2013 in the panel. Here we supplement this study by profiling the seventeen inhibitors that have been approved for oncology indications between November 2013 and June 2018. This includes the ALK inhibitors ceritinib, brigatinib, and alectinib, the CDK4/6 inhibitors palbociclib, abemaciclib, and ribociclib, the BTK inhibitorsibrutinib and acalabrutinib, and nine other novel marketed inhibitors. Assays were based on ATP-lite readout, with a nine-point duplicate dilution series of the compounds. Drug response was quantified by calculation of IC50, G1/2 passage inhibition and maximum effect. Response was associated with the genomic status of the cell lines as retrieved from the COSMIC and Cancer Cell Line Encyclopedia (CCLE) databases. Mutations in patient hotspot locations and copy number changes in well-characterized cancer genes were used as input for ANOVA tests. Basal gene expression levels of 400 clinically actionable genes were used as input for correlation analysis.

Results: Profiling of CDK4/6 inhibitors confirms previously reported biomarkers, such as loss of CDKN2A and wild-type RB1. Some sensitive cell lines have wild-type CDKN2A but exhibit downregulation of CDKN2A on an mRNA level. Our analysis also suggests new biomarkers, such as TP53 mutation and CCNE1 overexpression. ALK-targeting drugs strongly inhibit cell lines with high expression levels of ALK and also of JAK3. The dual ALK/JAK3 inhibitory profile of brigatinib might therefore explain its high cellular selectivity for cell lines with the NPM-ALK translocation, despite its moderate biochemical selectivity.

Conclusions: The profiling of newly approved kinase inhibitors in the Oncolines cell line panel shows that nearly all clinically-used drug response biomarkers can be identified from tumor-agnostic profiling in a hundred cell lines. Clinically useful biomarkers, such as NPM-ALK translocation or mutant BAF(V600E), are characterized by high potency differences between cell lines. The combination of these inhibitors could be further extended and refined by the identification of new response biomarkers. The application of these inhibitors could be further extended and refined by the identification of new response biomarkers.

Conflict of interest: Ownership: Guido Zaman and Rogier Buijsman are founders and shareholders of Netherlands Translational Research Center B.V.

140 (PB-091) Poster Cell panel profiling yields additional drug response biomarkers for kinase inhibitors approved for clinical use

J.C.M. Uitdehaag1, J.J. Kooijman1, J.A.D.M. De Roos1, M.B.W. Prinsen1, J. Dyusen1, N. Wijn-Seegers1, D.M. Jos1, S.J.C. van Gerwen1, R.C. Buijsman1, G.J.R. Zaman1. 1Netherlands Translational Research Center B.V., Biology, Oss, Netherlands

Poster Session (Tuesday, 13 November 2018) Abstracts, 30th EORTC-NCI-AACR Symposium

Background: Oncogenic somatic mutations in IDH1 or IDH2 genes are prevalent in diverse solid tumors. Therapeutic responses to IDH inhibitors are infrequent, partly due to the presence of simultaneous alterations in compensatory molecular pathways.

Material and Methods: We used a panel of TP53 mutated HGSOC cell lines (BRCAwt (OVCAR5 and OVCAR8), BRCA2 mutant (PEO1) or BRCA2 gain-of-function (PEO4)) to validate the screening findings. We examined the cell viability and IC50 of Prex and LY302 by XTT assay. Combination index (CI) was calculated by CompuSyn software. Caspase 3/7 activity was examined for apoptosis. Immunofluorescence staining of γH2AX was performed for DNA damage. All experiments were performed at least three times. Data were analyzed using Student’s t-test and shown as means±SD. P < 0.05 was considered statistically significant.

Results: IC50 of Prex and LY302 in all cells ranged from 7 to 53 nM and 276 to 726 nM, respectively. Prex/LY302 combination indicated synergistic (CI < 1) in all cells. As shown in Table 1, the combination therapy using clinically attainable concentrations of Prex (5 nM) and LY302 (200 nM) attenuated cell viability at least additively compared to single agent alone in all cells. In addition, this combination induced greater caspase 3/7 activation compared to monotherapy alone in all cells. We also observed the combination treatment induced greater DNA damage measured by γH2AX foci formation in OVCAR5, OVCAR8 and PEO1 cells, and a trend in PEO4 cells.

Table 1. The effects of Prex (5nM) and LY302 (200nM) treatments on cytotoxicity, apoptosis and DNA damage

<table>
<thead>
<tr>
<th>Cell viability(%)</th>
<th>OVCAR5</th>
<th>OVCAR8</th>
<th>PEO1</th>
<th>PEO4</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prex</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LY302</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Combination 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Combination 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

#P < 0.05; *compared to Prex; #compared to LY302.

Conclusions: Our results suggest that inhibition of CHK1 and PI3K/mTOR pathways together results in greater cytotoxicity in both BRCAwt and BRCA mutant HGSOC cells by inducing DNA damage and apoptosis.

No conflict of interest

143 (PB-094) Poster Simultaneous molecular alterations and clinical outcomes in solid tumors with IDH1 or IDH2 mutations

A. Swilling1, J. de Groot2, M. Javie3, M. Penas-Prado4, R. Shroff5, R. Luthra6, K. Banks7, R. Lannan7, A. Conley7, R. Broadus8, M. Davies9, S. Kopetz9, W.A. Yung10, J. Heymach11, S. Fu12, K. Shaw13, F. Meric-Bernstam11, F. Janku1. 1The University of Texas MD Anderson Cancer Center. 2Investigational Cancer Therapeutics, Houston, USA. 3The University of Texas MD Anderson Cancer Center, Neuro-Oncology, Houston, USA. 4The University of Texas MD Anderson Cancer Center, Gastrointestinal Medical Oncology, Houston, USA. 5The University of Arizona College of Medicine, Medicine, Tucson, USA. 6The University of Texas MD Anderson Cancer Center, Hematopathology, Houston, USA. 7Guardant Health, Medical Affairs, Redwood City, USA. 8The University of Texas MD Anderson Cancer Center, Sarcoma Medical Oncology, Houston, USA. 9The University of Texas MD Anderson Cancer Center, Thoracic Head and Neck Medical Oncology, Houston, USA. 10The University of Texas MD Anderson Cancer Center. 11Institute of Personalized Cancer Therapy, Houston, USA

Background: Oncogenic somatic mutations in IDH1 or IDH2 genes are prevalent in diverse solid tumors. Therapeutic responses to IDH inhibitors are infrequent, partly due to the presence of simultaneous alterations in compensatory molecular pathways.

Conclusions: Our results suggest that inhibition of CHK1 and PI3K/mTOR pathways together results in greater cytotoxicity in both BRCAwt and BRCA mutant HGSOC cells by inducing DNA damage and apoptosis.

No conflict of interest
Autophagy induction was determined by the MTT viability assay and by colony formation. We here wish to establish the influence of autophagy on the antiproliferative activity of nintedanib and regorafenib was determined by the MTT viability assay and by colony formation. Autophagy induction was determined by immunocytochemistry and Western blot of LC3 and by the Autophagy Blue™ fluorescence assay. CRC cell lines with attenuated Beclin1 expression were obtained by stable Beclin1 knock-down (sh-Beclin1).

**Results:** Nintedanib and regorafenib showed comparable activity toward a panel consisting of 12 well-characterized CRC cells, with average IC50 values between 2 and 2.8 μM. However, the activity profile between the two agents was different. Immunochemistry and Western blot of the autophagic marker LC3 as well as the Autophagy Blue™ fluorescence assay showed that nintedanib triggers an autophagic response, which was much less prominent for regorafenib. Interestingly, addition of the autophagy inhibitor 3-methyladenine decreased the cytotoxic activity of nintedanib up to 3-fold, but had no influence on the activity of regorafenib. In agreement, genetic models with attenuated expression of the autophagy regulator Beclin1 showed up to 3-fold decreased sensitivity to nintedanib, but the same sensitivity to regorafenib.

**Conclusions:** Autophagy contributes to the cytotoxic activity of nintedanib. Tumor cells with high autophagic flux may be selectively sensitive to nintedanib.

**Conflict of interest:** Corporate-sponsored Research: This project was financed in part by Boehringinger-Ingelheim.
were subjected to size exclusion chromatography and dimerization was purpose, soluble variants of WT or oncogenic D5D5 mutants were expressed in different variants of KIT D4D5 fragments to form dimers in solution. To the background, Gastrointestinal stromal tumors are mesenchymal tumors arising in the stomach and small bowel and more rarely in the rectum, esophagus, peritoneum and retroperitoneum. These tumors are characterized by KIT or PDGFRA mutations. KIT mutations are all in frame and lead to constitutive tyrosine kinase domain activation without ligand binding. Mutations concern four exons (9, 11, 13 and 17) but mainly exon 11. Here we report two new KIT exon 9 mutations, belonging to the D5 domain in the extracellular portion of the receptor – namely the deletion of two residues ΔA502-Y503 and the missense substitution K509N – found in a GIST patient at diagnosis. The patient was treated with the tyrosine kinase inhibitor (TKI) Imatinib, and responded well.

Materials and Methods: Immunoprecipitation and immunoblotting assays were performed to measure the tyrosine kinase activity for wild type and the oncogenic KIT mutants. The relationship between the receptor’s half-life and the responsiveness to ligand stimulation was studied by treating KIT-expressing cells with cycloheximide, which prevent protein synthesis, and monitoring the degradation of the KIT receptor. To explore the effect on oncogenic KIT activation, IP and IB assays were performed after cellular incubation with imatinib (and sunitinib for comparison). The efficacy of Imatinib against oncogenic KIT mutations were further examined by following the effect of Imatinib on colony formation in soft agar of NIH 3T3 cells harboring oncogenic KIT mutants. To understand the mechanism of ligand independent activation of the oncogenic ΔA502-Y503 and K509I D5 KIT mutants, we performed experiments aimed at comparing the capacity of different variants of KIT ΔD45 fragments to form dimers in solution. To the purpose, soluble variants of WT or oncogenic ΔD55 mutants were expressed in Sf9 insect cells and purified. Different concentrations of these variants were subjected to size exclusion chromatography and dimerization was monitored using multilayer light scattering and refractometer index detectors to obtain their weight average molecular mass (Mw). The corresponding dimerization constants Kd were determined by fitting the Mw. Results: We found that full length KIT variants harboring these mutations exhibit elevated basal tyrosine kinase activity, which can be further stimulated by SCF binding. The dimerization constant of isolated D45 ΔKIT domains of both oncogenic ΔA502-Y503 and K509N are increased by up to 20 fold as compared to WT D5, resulting in elevated tyrosine autophosphorylation of unoccupied KIT.

Conclusions: Both ΔA502-Y503 and K509N are ligand-sensitized oncogenic KIT mutations, and both Imatinib and Sunitinib can suppress growth in cell culture, albeit to 20 fold as compared to WT D5. This is a novel T cell redirecting agent targeting the c-Kit receptor, and both Imatinib and Sunitinib can suppress growth in cell culture. ERY974, a novel bispecific T cell activating antibody, can selectively induce T cell-mediated tumor cell death by activating T cells against KIT expressing tumor cell line, ERY974-induced cellular cytotoxicity and the T cell activation status were evaluated using expanded Tregs or CD4+ T cells derived from the same donor as effector T cells (Teffs). Furthermore, we evaluated the suppressive activity of Tregs against ERY974-activated Teffs in various Teff/Treg ratios.

Results: In the GPC3-positive cancer cell line, CD4+ Teffs showed ERY974-dependent activation, proliferation, and cellular cytotoxic activity, while expanded Tregs did not. In addition, Tregs inhibited ERY974-dependent proliferation and granzyme B production of CD8+ Teffs in a manner dependent on the Teff/Treg ratio. As for the amount of Tregs, ERY974 treatment did not expand the suppressive Tregs.

Conclusions: These preclinical data suggest that ERY974 is unlikely to increase Tregs by CD3 stimulation, but Tregs potentially attenuate the antitumor efficacy of ERY974 against GPC3-expressing tumors. Now, the combinational effect of ERY974 and an anti-CTLA4 antibody is being further investigated in mouse syngeneic tumor models to explore the effect of a novel T cell-targeting agent on the antitumor activity of ERY974 against GPC3-positive cancer.

No conflict of interest
**Tuesday, 13 November 2018**

**Poster Session**

**Vaccination**

**149 (PB-100) Poster**

**Novel Apolipoprotein-A1 (ApoA-I) multimers, Cargomer®,** as new targeted delivery platform for therapeutic cancer vaccines with tumor neo- and shared-antigens


**Background:** Therapeutic vaccination is a highly promising tumor-specific approach in immuno-oncology but has shown insufficient clinical efficacy, so far, in part due to poor cross-presentation efficiency and weak cytolytic CD8 (CTL) response. The development of new technologies to target the delivery of Tumor Antigens (TA) and immunostimulants into antigen-presenting cells (APC) and induce a potent tumor-specific CTL response both alone and in combination with immune checkpoint inhibitors (ICIs) remains a major goal.

ApoA-I is the major protein of HDL that plays an essential role in lipid transport and delivery via receptors including the scavenger receptor SR-B1 that is expressed on hepatocytes, macrophages and dendritic cells. Cerenis® recombinant human (rh)ApoA-I pre-b discoïdal HDL mimetic, is currently evaluated in phase III clinical trials in patients with genetic HDL deficiencies. Cargomer®-treated groups and demonstrated synergistic efficacy up to 100% and 62.5% of tumor-free animals at d30 and d70, respectively.

**Results:** The mice immunized with Cargomer®/TAs/Chol-CpG showed a significant inhibition of tumor growth, which synergized when combined with the ICI. ICI alone, TAs/CpG-Chol alone (i.e., not formulated in Cargomer®) or rhApoA-I alone did not show efficacy. Survival was increased in all Cargomer®-treated groups and demonstrated synergistic efficacy up to 100% and 62.5% of tumor-free animals at d30 and d70, respectively.

**Conclusions:** This work demonstrates that rhApoA-I Cargomer® can be effectively loaded with neo and shared peptide antigens and TLR agonists to generate a robust immune and therapeutic responses.

**Conflict of interest:** Other Substantive Relationships: Consulting services paid by Cerenis Therapeutics SA.

**Wednesday, 14 November 2018**

**Poster Session**

**Adoptive Cell Transfer therapy**

**150 (PB-001) Poster**

**Biomarkers of Fc-gamma receptors (FcγRs) on mononuclear phagocyte system (MPS) cells in blood of patients with advanced gastric cancer are upregulated as compared to patients with metastatic breast cancer**

W. Kirschbrown1, A. Lucas2, C. Li1, S. Girish1, W. Zamboni2, A. Garg1.

**Background:** Nanoparticles (NPs), monoclonal antibodies (mAbs), and antibody-drug conjugates (ADCs) are cleared via the mononuclear phagocyte system (MPS). Variability in MPS function has been shown to predict variability in the pharmacokinetics (PK) and pharmacodynamics (PD) of NPs. The MPS serves as a non-target-mediated mechanism of antibody
clearance via their Fc-gamma-receptors (FcγRs). Due to differences in types and affinity of FcγRs, variation in surface expression can lead to significant differences in the ability of MPS cells to clear mAbs and ADCs, which affects the PK and PD of these agents. Studies have reported lower mAb and ADC trough concentrations/faster clearance in patients with advanced gastric cancer (aGC) compared to metastatic breast cancer (mBC) and other solid tumors, which were not explained fully by patient covariates (e.g. age, gender, anti-drug antibodies). Thus, the difference in biomarkers of MPS cells in blood from patients with aGC and mBC were evaluated.

Material and Methods: Biomarker studies were performed in 15 adult female patients diagnosed with mBC (n = 15) or aGC (n = 15). Patients were excluded if they received NP or biological therapy within the past month or were treatment naive at the time of sample collection. FcγR expression (CD64, CD32, and CD16) and function (phagocytosis and reactive oxygen species [ROS] generation) for MPS cells (monocytes) in whole blood were evaluated by flow cytometry.

Results: There was high inter-patient variability (2- to 5-fold) in expression of FcγRs. There was higher CD64 expression in patients with aGC compared to mBC (8.43 ± 2.512 vs. 6.762 ± 1.344 antibodies bound per cell, respectively; p = 0.037). Additionally, CD16 expression was higher in patients with aGC compared to mBC (10.954 ± 4.385 vs. 7.519 ± 3.302 ABC, respectively; p = 0.029). CD32 expression was similar between the two populations. Similar variability and mean phagocytic function were seen in the two patient populations. The innate ROS production (i.e. unstimulated) within MPS cells was higher in patients with mBC compared to aGC (4,043 ± 1,331 vs. 3,170 ± 617, respectively; p = 0.035), as well as after stimulation. In addition, there was an association between monocyte count and MPS function after stimulation in patients with aGC but not mBC.

Conclusions: Increased expression of CD64 and CD16 FcγRs on MPS cells was observed in patients with aGC compared to mBC. This is significant as CD64 is the only FcγR to bind monomeric IgG1, the isotype commonly used in therapeutic mAbs. FcγR expression was inversely related to ROS production in MPS cells indicating there may be a compensatory relationship between MPS FcγRs and function. In summary, variability in FcγR expression could be explored as a potential covariate for the faster and clinically relevant clearance difference of mAbs and ADCs observed in patients with aGC.

Conflict of interest: Ownership: (1) Glytics, LLC has licensed technology on biomarkers of MPS function. WCZ is CSO and holds equity in Glytics, LLC. Corporate-sponsored Research: Funding for technology and study: (1) Genentech & (2) Eshelman Institute for Innovation. Other Substantive Relationships: (1) WCZ and ATL filed ROI for provisional patent on MPS FcγR technology.

151 (PB-002) Poster
IL13Rα2 chimeric antigen receptor T cells combined with checkpoint blockade to treat glioblastoma in human and canine models
Y. Yin1, Z. Binder1, R. Thokala1, D. O’Rourke1
1University of Pennsylvania, Neurosurgery, Philadelphia, USA

Background: Glioblastoma (GBM) is an inherently invasive tumor with a median overall survival of approximately 15 months, following surgery, radiotherapy, and chemotherapy. T cells can be redirected to kill cancer cells using chimeric antigen receptors (CAR), a promising method to treat solid tumors. IL13Rα2 is expressed in many solid tumors but not normal tissues, providing a tumor-specific target for CAR T cells.

Methods: Human and canine tumor cell lines were screened for IL13Rα2 expression by flow cytometry and RT-PCR. Different IL13Rα2 single-chain variable fragments (scFv) were tested in CARs against human and canine recombinant IL13Rα2 protein to identify a cross-reactive clone. Humanized IL13Rα2 CARs expressed in human and canine T cells showed antigen-specific stimulation by cytokine secretion and target cell lysis. NSG mice bearing subcutaneous and orthotopic xenografts received a single treatment of 0.8–5 million CAR T cells with or without anti PD-1, CTLA-4 or TIM3 mAb checkpoint blockade.

Results: IL13Rα2 was detected on human GBM (D270, U251 and U87), canine osteosarcomas (BW-, CS-, MC- and SK-KOSA) and canine lung cancer cell lines (Caca3, Caca5). Two different humanized IL13Rα2 CARs were developed, one that recognized human IL13Rα2 but not IL13Rα1. Of these, one also recognized canine IL13Rα2. IL13R2 CAR T cells demonstrated tumor growth inhibition in human and canine GBM tumors grown both subcutaneously and orthotopically. Use of fewer CAR T cells initially controlled tumor growth, followed by tumor outgrowth correlating with expression of T cell exhaustion markers. Combined treatment of CAR T cells with checkpoint blockade mAbs, delivered either systemically or in situ, had synergistic effects. Anergic T-cell function was selectively restored by specific checkpoint blockades and improved tumor growth inhibition.

Conclusions: IL13Rα2 is highly expressed on human and canine tumors but not normal tissue. IL13Rα2 specific CAR T cells are successfully activated in response to human and canine tumors and inhibit GBM growth in a xenograft mouse model, although this effect was transient at lower treatment doses. Addition of specific checkpoint blockade mAbs to CAR therapy was beneficial for the treatment of solid tumors. We plan to utilize this treatment in a canine spontaneous cancer preclinical model and translate into clinical trials for patients with GBM.

Conflict of interest: Corporate-sponsored Research: Dr. Donald O’Rourke receives research support from Novartis.

Wednesday, 14 November 2018
POSTER SESSION
Clinical Methodology

152 (PB-003) Poster
Detection of exfoliative cytology and VELscope fluorescence in oral cancer and oral leukplakia
Z. Sun1, Y. Liu2, X. Guan3
1Beijing Stomatological Hospital, Capital Medical University, Dept. Oral Mucosa, Beijing, China

Background: To access the feasibility of exfoliative cytology and VELscope fluorescence in the early detection of oral squamous cell carcinoma (OSCC) and prediction oral cancer risk of oral leukplakia (OLK).

Methods: We collected oral mucosa of patients with oral leukplakia (OLK, n = 57), OSCC (n = 70), and healthy subjects (n = 28). Exfoliative cells were collected and analyzed with traditional DNA quantitative analysis and oral cancer risk index (OCRI) analysis. Meanwhile, VELscope fluorescence was used in other patients. All the results were compared with histopathology to determine the sensitivity and specificity of these two methods.

Results: The sensitivity of DNA quantitative analysis, OCRI and VELscope was 92.68%, 100%, and 93.10% respectively. The specificity was 100% in all groups. Among 28 leukplakia patients with an OCRI less than 0.5, none of them developed cancer during follow-up.

Conclusions: Exfoliative cytology-based method for quantitative prediction of cancer risk (OCRI) and VELscope fluorescence detection (localization of the lesion) could be used in early detection of OSCC and assessment of cancer risk of OLK patients during clinical follow-up.

No conflict of interest

153 (PB-004) Poster
Circling tumor cell-free DNA correlates with the total tumor volume and survival in patients with advanced cancers
1University of Texas, MD Anderson Cancer Center, Cancer Systems Imaging, Houston, USA; 2University of Texas, MD Anderson Cancer Center, Diagnostic Radiology, Houston, USA; 3University of Texas, MD Anderson Cancer Center, Investigational Cancer Therapeutics, Houston, USA; 4University of Texas, MD Anderson Cancer Center, GI Medical Oncology, Houston, USA

Backgrounds: The association between the amount of tumor circulating cell-free DNA (cfDNA) and tumor burden is not well understood partially due to inadequate imaging evaluation tools such as RECIST. We hypothesize that, unlike RECIST, volumetric image analysis can accurately estimate total tumor burden and can correlate with the amount of tumor circulating cfDNA with the ultimate translational goal to use cfDNA assessment as a surrogate marker for response to cancer therapy.

Materials and Methods: We performed a retrospective volumetric assessment of pretreatment clinical imaging (CT and/or MRI) to estimate the Total Tumor Volume (TTV) using the 3D slicer 4.3.1 and MATLAB in patients with advanced cancers with KRAS12G13 mutations. KRAS12G13 mutations were detected in 16 ng of unamplified plasma cfDNA collected at the time of imaging and quantified as % variant allele frequency (VAF) using the multiplex droplet digital PCR screening kit. Correlations between VAF and tumor volumes were performed and results compared to overall survival (OS).

Abstracts, 30th EORTC-NCI-AACR Symposium
Results: Total of 76 patients (colorectal cancer [CRC]; 51; other cancers: 25) had a shorter median OS compared to CRC patients during a phase 1b trial (NCT 02398058) in patients with advanced and metastatic carcinoma. Hepatic Tumor Volumes (HTVs) positively correlated with the KRASG12D/G13VAF in cfDNA while LTVs (r = 0.48, p < 0.001), but not with Lung Tumor Volumes (LTVs; r = 0.02, p = 0.89) or Brain Tumor Volumes (BTVs; r = −0.15, p = 0.20). Patients with higher median KRASG12D/G13VAF in cfDNA had a shorter OS compared to patients with lower VAF (5.7 vs 7.3 months, P = 0.03). Patients with higher median TTVs showed a trend towards shorter OS compared to patients with lower TTVs (5.2 vs 6.6 months, P = 0.07). In the largest disease specific subgroup of 51 patients with CRC, HTVs, TTVs (r = 0.38, p = 0.006) and HTVs (r = 0.56, p < 0.001) also positively correlated with the KRASG12D/G13VAF in cfDNA while LTVs (r = −0.03, p = 0.81) or BTVs (r = 0.20, p = 0.90) did not. Patients with CRC and higher median KRASG12D/G13VAF in cfDNA also had a shorter median OS compared to CRC patients with lower VAF (6.0 vs 9.8 months, P = 0.04) while CRC patients with higher HTVs had similar OS to patients with lower HTVs (5.4 vs 7.5 months, P = 0.26).

Conclusions: The amount of mutated KRASG12D/G13 in plasma cfDNA correlates with HTVs on imaging and higher % VAF of KRASG12D/G13 in cfDNA, but not TTVs were associated with shorter OS.

No conflict of interest
Cell-free DNA testing allows for rapid patient screening, identification, and enrollment by cfDNA. Patients were accrued over a period of 19 months. Among the 68 enrolled. A total of 1559 patients were pre-screened: 53% by tissue and 47% by G360; 33 (5.4%) were eligible based on MET gene alterations. Patient samples were tested using the Guardant360® 73-gene cfDNA NGS assay (G360) with a turnaround time of 7 days from date of receipt. Data pertaining to the number of clinical trial matches, physicians contacted, and referrals provided. The ability to identify and enroll patients on clinical trials in a timely manner is critical for providing patients access to potentially life-saving therapies and the drug development process at large. Requirements for tissue biopsies for molecularly-guided clinical trials impose barriers to patient enrollment related to long test turnaround times, insufficient or inaccessible tumor material, test failure, and biopsy refusal. In a recent study, 30% of patients had clinical deterioration due to this delay or had insufficient tissue for analysis, both rendering them trial ineligible. In contrast, blood-based cell-free DNA testing, accounting for almost 30% of patients enrolled, allows for accelerated trial enrollment without patient risk of biopsy-driven complications.


159 (PB-010) Poster
The PROCLIPI Study: A prototype registry for rare cancers with global collaboration for establishment of a prognostic index in mycosis fungoides and Sezary syndrome

J. Scarisbrick1, A. Cozzio2, R. Willemsz3, Y. Kim4
Cutaneous Lymphoma International Consortium. 1EORTC, Cutaneous Lymphoma Taskforce, Brussels, Belgium; 2EORTC, Cutaneous Lymphoma Taskforce, Brussels, Belgium; 3Leiden University Medical Centre, Dermatology, Leiden, Netherlands; 4Stanford Medical Centre, Dermatology, Stanford, USA

Background: The PROCLIPI Study is a multi-national PROSpective Cutaneous Lymphoma International Prognostic Index study to develop a prognostic index in mycosis fungoides (MF) and Sezary syndrome (SS).

Materials and Methods: PROCLIPI was launched in 2015 collecting pre-defined clinical, haematological, radiological, immunohistochemical, genotypic, quality of life and treatment data using a secure web based database system. Additionally, central clinical and pathological review is performed by experts to confirm diagnosis and stage.

Results: 956 patients are registered from 46 international sites from 5 continents. 680 early stage (I-IIIa) patients; 1.75 males: 1 female and 276 advanced stage (III-IV); 1.65 males: 1 female. The median age at diagnosis of advanced disease was 65 years compared to 57 yrs at early stage (p < 0.0001). The median time of MF-like lesions prior to diagnosis was 36 months in both early and advanced disease confirming diagnostic delay. Raised serum lactate dehydrogenase (LDH) and large cell transformation (LCT) in skin are among the candidate prognostic factors. LCT is found in 20/ 680 (3%) early stage patients and in 69/276 (25%) advanced patients p < 0.01. 9% of early stage and 30% advanced patients have raised serum LDH at diagnosis (p < 0.01). Treatment data including responses and quality of life are collected and will be measured against survival and tumour phenotype (clinical, histological and genotypic) to determine the most efficacious therapies in improving outcomes.

Conclusions: PROCLIPI has confirmed a diagnostic delay in all stages of MF/SS. There is a need to develop improved diagnostic techniques to quicken the diagnosis. Identifying patients with a poorer prognosis should allow optimal treatments, better patient experience and improve survival. PROCLIPI is a a prototype registry for rare cancers and this study design may benefit other cancer groups.

No conflict of interest

160 (PB-011) Poster
Complete pathologic response is a strong predictor of event free survival and distant recurrence free survival, regardless of tumor subtype or investigational agent, in women with early breast cancer at high risk for recurrence in the I-SPY 2 TRIAL


1University of Pennsylvania, Hematology Oncology, Philadelphia, USA; 2University of Minnesota, Medicine, Minneapolis, USA; 3University of California, UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, USA; 4University of Texas, M.D. Anderson Cancer Center, Houston, USA; 5Georgetown University, Medicine and Oncology, Washington D.C., USA; 6Loyola University, Hematology and Oncology, Maywood, USA; 7University of California, Pathology, San Francisco, USA; 8University of Alabama Birmingham, Pathology, Birmingham, USA; 9University of California San Diego, Pathology, La Jolla, USA; 10University of Minnesota, Pathology, Minneapolis, USA; 11Loyola University, Surgical Oncology, Maywood, USA; 12Mayo Rochester, Pathology, Rochester, USA; 13Swedish Cancer Institute, Pathology, Seattle, USA; 14Gemini Group, Advocate, Ann Arbor, USA; 15University of California, San Francisco, Imaging, San Francisco, USA; 16Quantum Leap Healthcare Collaborative, I-SPY TRIALS, San Francisco, USA; 17Berry Consultants, LLC, Statistics, Houston, USA

Background: While pathologic complete response (pCR) is associated with an improved event-free (EFS) and distant recurrence-free survival (DRFS) with standard, approved treatment in the neoadjuvant therapy of
breast cancer, it is unknown whether this would hold for investigational agents. We tested this hypothesis in nine investigational arms and controls in the ongoing, adaptive, phase 2 platform trial, i-SPY2.

Methods: Women with breast tumors >2.5 cm were adaptively randomized to better performing agents compared with control therapy within molecular subtypes, using HER2, hormone-receptor and the 70-gene assay. Hormone receptor +/HER2-70-gene low-risk tumors were excluded. EFS and DRFS were evaluated by pCR status within subtypes. Hazard ratios (HR) were based on Cox proportional hazards regression. Associations between pCR and EFS were analyzed by therapeutic arm using Bayesian and hierarchical modeling with adjustments for molecular subtype.

Results: The analysis was restricted to patients who had at least 2 years of follow-up, with 741 patients (of over 1200 randomized) eligible for analysis; median follow-up was 2.7 years. Three-year EFS and DRFS for patients achieving pCRs were 94% and 95%, respectively. For pCR versus non-pCR, mean HR = 0.20 for both endpoints, regardless of modelling method, with the 95% probability interval ranges from 0.1–0.35. HRs were similarly low within subtypes and specific treatment arms. Even when assigned treatments were not superior to control, achieving a pCR on any treatment arm resulted in significantly lower HR compared to non-pCR.

Conclusions: Achieving pCR after neoadjuvant chemotherapy for women at high risk of early recurrence predicts an excellent outcome, regardless of subtype, even with the addition of investigational drugs, with 94% of patients remaining disease-free at 3 years. pCR is an early endpoint that can serve to support the development of personalized treatment strategies for drug development trials designed to optimize outcomes. Results by treatment arm will be shown.

No conflict of interest
antitumor agents with high cytotoxic activity against many experimental cell lines and tumor models (colon, lung, pancreatic, breast, prostate). Here, we investigated whether QDs conjugated with unsymmetrical bisacridines affect cytotoxicity of these compounds in human lung carcinoma cells as well as normal lung fibroblasts.

Materials and Methods: Two unsymmetrical bisacridine derivatives: C-2028 and C-2045 and two human cell lines: non-small cell lung carcinoma H460 and fetal lung fibroblast MRC-5 were selected for these studies. UAs were non-covalently attached to the Ag-In-Zn-S/MUA nanocrystals. The amount of UAs compounds immobilized at QD nanocrystal surface was determined at the potential ca. –0.6 V (cyclic voltammetry). The cytotoxicity against H460 and MRC-5 cells was assessed by the MTT assay following 72 h of incubation with QD$_{C-2028}$ and QD$_{C-2045}$ conjugates as well as QD$_{C-2028}$ and QD$_{C-2045}$ alone.

Results: Both compounds exhibited high cytotoxicity against lung cancer H460 cells (IC$_{50}$ 0.035 μM for C-2028 and 0.273 μM for C-2045), being less active against normal lung fibroblast MRC-5 (IC$_{50}$ 0.47 μM for C-2028, 0.45 μM for C-2045), C-2028 and C-2045 conjugated with QD$_{red}$ and QD$_{green}$ decreased IC$_{50}$ values of both compounds (QD$_{red}$ 1.5 and 1.9 fold, QD$_{green}$ 1.2 and 1.3 fold, respectively). Interestingly, UAs conjugated with both red and green QD were much less cytotoxic against normal MRC-5 cells. IC$_{50}$ value for QD$_{red}$-C-2028 increased 22 fold, QD$_{green}$-C-2045 28 fold, QD$_{green}$-C-2028 2.5 fold and QD$_{green}$-C-2045 11 fold. Moreover, QDs alone did not influence cancer and normal cells proliferation. Comparing the obtained values from voltammetric measurements with the maximum amount of UAs compounds immobilized at QD surface one can conclude that the efficiency of the nanocojugates synthesis was higher for QD$_{red}$ nanocrystals.

Conclusions: Our results indicate that conjugation of unsymmetrical bisacridines with QDs improves drugs cytotoxicity in lung cancer cells and protects normal lung fibroblast from drugs action. These effects were more pronounced in the case of QD$_{red}$ which were more effectively loaded by bisacridines.

These studies were supported by the National Science Center, Poland, Grant No. UMO-2016/23/B/NZ7/03324.

No conflict of interest

164 (PB-015) Poster BT5528, an EphA2-targeting Bicycle Toxicin Conjugate (BTC): profound efficacy without bleeding and coagulation abnormalities in animal models

G. Bennett1, A. Brown1, G. Mudd1, P. Park2, N. Keen2. 1Bicycle Therapeutics Limited, Therapeutics, Cambridge, United Kingdom; 2Bicycle Therapeutics Limited, Therapeutics, Lexington, USA

Background: Ephrin receptor A2 (EphA2) is a member of the Ephrin receptor family of cell-cell junction proteins[1] and is highly overexpressed in a range of cancer types. Bicycle binders for EphA2 were identified using a phage libraries of Bicycles, conjugated to cleavable linkers & toxins to form Bicycle Toxicin Conjugates (BTCs). The small size of BTCs offers a significant advantage over other targeted cytotoxic approaches such as antibody-drug conjugates due to rapid extravasation, renal clearance and improved tumour penetration. The efficacy of BTCs was evaluated using xenograft models (cell- and patient derived) in baliic nude mice, with treatments administered intravenously once weekly.

Toxicology studies were performed in Han Wistar rats and Cynomolgus monkeys, with treatments administered intravenously once weekly. Standard evaluations of weight, condition, haematology, clinical chemistry, coagulation parameters, and urine were undertaken as well as macro- and micro-scopc pathology and measurement of D-Dimer.

Results: We selected the candidate BTC BT5528 from a panel of >75 BTCs, based on in vivo efficacy, tolerability and drug-like properties. BT5528 is effective in EphA2-expressing xenograft models, with complete tumour regression seen from 1 mg/kg weekly.

Expression of EphA2 is high across a range of cancers of high unmet medical need and correlation with efficacy in several tumours including lung, breast, oesophageal, ovarian, prostate, gastric and sarcoma models were shown. No significant efficacy was seen in tumours without EphA2 expression. Efficacy was maintained even when treating very large (>1000 mm$^3$) tumours and heterogeneous xenograft models.

While ADC approaches to EphA2 have been hampered by profound bleeding & coagulation toxicity in the clinic and in preclinical species, BT5528 shows no sign of bleeding in exploratory toxicity studies in rat and NHP. No changes were seen in coagulation parameters or liver enzymes after treatment with BT5528, unlike previously reported data for MEDI-547 [MK2].

Conclusions: The EphA2 targeting BTC BT5528 shows potent anti-tumour activity in a range of solid tumour xenograft models without the limiting toxicity observed with previous Antibody Drug Conjugates approaches. IND-enabling studies for BT5528 are currently underway.

No conflict of interest

166 (PB-017) Poster EB1-dependent long survival of glioblastoma cancer stem-like cell tumor-bearing mice after daily oral treatment with the novel Tumor Checkpoint Controller BAL101553

R. Berges1, A. Tchoghandjian1, A. Serge2, D. Figarella-Branger3, F. Bachmann4, H. Lane5, D. Braguer5, 1Aix-Marseille Univ, Cnrs-inp, Marseille Cedex 05, France; 2Aix-Marseille Univ, CNRS, Inserm, Marseille, France; 3Aix-Marseille Univ, CNRS-INP, Marseille, France; 4 Basilea Pharmaceutica International Ltd, aa, Basel, Switzerland

Background: Glioblastoma (GBM) are the most aggressive brain tumors in adults, and treatment options are very limited. Cancer stem-like cells, which contribute to GBM invasiveness, represent promising target. We have previously shown that overexpression of microtubule + End-binding 1-protein (EB1) correlates with GBM progression and poor survival and that EB1 is a predictive biomarker of response to BAL101553. BAL101553 is the prodrug of BAL27862, a small molecule that binds microtubules and promotes cell death through activation of the spindle assembly checkpoint. BAL27862 inhibited proliferation and migration in vitro of glioblastoma stem-like cells (GBM6). In an EB1-dependent manner. Three i.e. administrations of BAL101553 over the course of one week were sufficient to provoke an EB1-dependent survival benefit in tumor-bearing mice. Moreover, BAL27862 inhibited stem-cell properties and triggered astrocytic differentiation of GBM.
stem-like cells in an EB1-dependent manner. Here, we evaluate a long-term oral schedule of treatment on this orthotopic mouse model.

**Material and Methods:** GBM6-GFP downregulating EB1 (GBM6 GFP shEB1) or not (GBM6 GFP sh0), were orthotopically grafted in mice. Thirsty-five days later, BAL101553 (30 mg/kg) or vehicle was orally administered 5 days a week for 100 days. Survival duration was measured to determine the final tumor size. At the end of treatment, tumors were excised and measured in a caliper for tumor volume estimation. The degree of tumor volumes and invasiveness were estimated by flow cytometry analysis and 3D tumor reconstruction and immunostaining of stem cell markers were performed. Phenotype of stem cell differentiation during BAL101553 treatment was determined in vitro.

**Results:** Survival was increased by 326 days and 155 days in GBM6 GFP sh0 and GBM6 GFP shEB1 respectively, as compared with respective controls. Patterns of invasion and quantification of tumor cells in brain demonstrated that the anti-proliferative and anti-invasive effects of BAL101553 were more prominent in mice bearing control tumors than in EB1-downregulated tumors. Moreover, BAL101553 decreased the proportion of stem-like cells (ASB5+) in the tumor in an EB1-expression level-dependent manner, as long as the treatment was administered. Furthermore, BAL27862/BAL101553 had a strong effect on tumor vessels; it inhibited GBM6 endothelial cell differentiation in vitro and in vivo and it inhibited GBM6 migration on preformed endothelial capillary-like tubes in an EB1-dependent manner.

**Conclusion:** Long-term oral treatment with BAL101553 provides a large EB1-dependent survival benefit. Moreover, the drug counteracts tumor angiogenesis by acting on cancer stem-like cells. These findings support the potential of BAL101553 as a novel therapeutic strategy for enhancing the efficacy of cisplatin chemotherapy.

**Conflict of Interest:** Ownership: Heidi Lane and Felix Bachmann are shareholders of Basilea Pharmaceuticals Ltd. Corporate-sponsored Research: Financial support for Research by Basilea Pharmaceuticals Ltd. Other Substantive Relationships: Heidi Lane and Felix Bachmann are full-time employee of Basilea.

168 (PB-019) Poster

**CP-506, a next-generation hypoxia-activated prodrug, as a promising novel anti-cancer therapeutic**

S. Deschoemaeker 1, S. Thilloy 1, J. Gilissen 1, A. Stampella 1, L. Dubois 2, A. Yaromina 1, T. Shyr 2, A. Ashoorzadeh 3, C. Guise 4, M. Abbattista 4, J. Heverin 5, A. Ashoorzadeh 3, C. Guise 4, M. Abbattista 4, J. Heverin 5.

**Materials and Methods:** In vitro potency assay: Cells are seeded under anoxic (ANX) (<0.01% oxygen) or normoxic (NRX) conditions. Post 24 hours, cells are incubated with a dose range of CP-506 for 4 h under ANX or NRX conditions. Next, the cells are washed and maintained under NRX conditions for 96 h when viability is evaluated using resazurin staining.

**Results:** In vivo studies: Immunocompromised mice were injected subcutaneously (NCl-H1650, NCI-H69) or orthotopically (MDA-MB-436, MDA-MB-436-3D) with human tumor cells. Treatment was initiated after randomization, once the tumor volume (TV) reached 250 mm³. TV and body weight were measured and the time to reach 4 times the TV at treatment start was used as surrogate endpoint for survival. To assess tumor hypoxia and DNA damage, tumors from vehicle and CP-506 treated groups were collected 24 h after the 2nd treatment. One hour before sacrifice, mice were injected ip with 60 mg/kg of pimonidazole. Tumors were processed for anti-pimonidazole (hypoxia) or anti-phH2AX (DNA damage) immunostaining and analysis.

**Results:** CP-506 significantly inhibits the viability of a tumor cell line panel under anoxic conditions in an in vitro potency assay. In vivo studies in different lines of treatment on this orthotopic mouse model confirmed that 5 consecutive administrations of CP-506 at 600 mg/kg results in a significant inhibition of tumor growth and in an increase in survival without significant body weight loss. Histological analysis showed that CP-506-treated tumors presented a significant increase in DNA damage compared to vehicle-treated tumors and as expected CP-506 treatment induces DNA damage in the hypoxic regions of the tumor. Additional dose regimen studies showed that a repeated dose dense treatment regimen can control tumor growth over time without additional body weight loss.

**Conclusions:** Our pre-clinical data show that CP-506 is a potent highly selective HAP with the highest in vivo efficacy when dosed with a repeated dose dense treatment schedule. Building on these results, we are currently identifying the key parameters determining the sensitivity to CP-506 (hypoxia, reductases and sensitivity to the alkylating activity of CP-506) which will be part of an integrative biomarker which aims at selecting potentially sensitive patients to CP-506 and pave the way to a successful clinical development.

169 (PB-020) Poster

**Augmentation of NAD+ by NQO1 activation attenuates cisplatin-mediated hearing impairment**

Y. Sei-hoon 1, K.B. Kwon 2, H.S. So 3, School of Medicine Wonkwang University, Internal Medicine, Iksan-city, Jeonbuk, South Korea. 4School of Medicine Wonkwang University, Center for Metabolic Function Regulation, Iksan-city, Jeonbuk, South Korea

**Introduction:** Cisplatin is an extensively used chemotherapeutic agent, and one of its most adverse effects is ototoxicity. However, the precise mechanism underlying cisplatin-associated ototoxicity is still unclear. The co-factor nicotinamide adenine dinucleotide (NAD+) has emerged as a key regulator of cellular energy metabolism and homeostasis. It remains unclear whether modulation of NAD+ levels has an impact on cisplatin-induced hearing impairment.

**Material and Methods:** To investigate whether augmentation of NAD+ by NQO1 activation using b-Lapachone (b-Lap) attenuates cisplatin-mediated hearing impairment, male C57BL/6 mice and NQO1 knockout mice on a C57BL/6 background were used. We measured the enzymatic activity of SIRT1, PARP1, ROS production, NAD+/-NADH ratio, mRNA levels of miR-34a and pro-inflammatory cytokines. Immunohistochemistry and western blot analysis were also performed.

**Results and Discussion:** We have demonstrated for the first time that both the protein expression level and the activity of SIRT1 were suppressed by the reduction of intracellular NAD+ levels in cisplatin-treated cochlear tissue. We also found that the decrease in SIRT1 protein expression and the activity of cisplatin exposure were mediated by the increase in transcriptional activity of p53 for miR-34a expression and PARP-1 activation causing NAD+ depletion, respectively.

**Conclusion:** Considering that b-Lap itself did not attenuate the tumoricidal effect of cisplatin, these results suggest that the direct modulation of the cellular NAD+ level by pharmacological agents could be a promising therapeutic strategy for enhancing the efficacy of cisplatin chemotherapy without its adverse effects.

**No conflict of interest**

170 (PB-021) Poster

**TAS1553, a novel class of RNR inhibitor, demonstrates antitumor activity in preclinical models**

H. Ueno 1, T. Hoshino 1, S. Tsukiuoka 1, W. Yano 1, T. Suzuki 1, A. Hashimoto 2, S. Miyahara 1, S. Hara 1, Y. Ogin 1, K. Ichikawa 1, K. Matsuo 1, K. Miyadera 1, T. Utsugi 1, Y. Iwasawa 1, Taiso Pharmaceutical Co., Ltd., Discovery and Preclinical Research Division, Tsukuba, Japan

**Background:** Ribonucleotide reductase (RNR) is a key metabolic enzyme in nucleic acid metabolism which has long been targeted by antimetabolites. RNR regulates a rate-limiting step for supply of dNTPs via converting
ribonucleotides to deoxyribonucleotides. Because dNTPs are the building blocks of DNA, inhibition of cancer cell growth and survival, RNR is considered to be a promising therapeutic target for cancer treatment. TAS1553 is a novel class of RNR inhibitor which has a potent inhibitory activity against RNR via abrogating the protein-protein interaction between RNR subunits. In vitro study suggests that TAS1553 shows wide-ranging antitumor activity by causing DNA replication stress and apoptosis. Here we describe the antitumor activity of TAS1553 in in vivo models.

**Material and Methods:** TAS1553 was designed and synthesized at Taiho Pharmaceutical Co., Ltd. The antitumor efficacy of TAS1553 was evaluated in athymic nude mice or nude rats bearing MV-4-11 (human acute myeloid leukemia) and HCC38 (human breast cancer). The amounts of nucleotide pools (dATP and dTTP) in tumors were measured by LC-MS/MS analysis in order to evaluate RNR inhibitory effect of TAS1553. The protein and phospho-protein expression level in tumors were determined by Western blot analysis to evaluate the induction of DNA replication stress and apoptosis.

**Results:** The antitumor effect and RNR inhibitory effect of TAS1553 were evaluated in MV-4-11 rat xenograft model. Once daily dosing of TAS1553 not only inhibited tumor growth at 150 mg/kg but also caused tumor shrinkage (and tumor disappearance in 3 of 5 rats) at 300 mg/kg without severe effect on body weight gain. Importantly, single oral administration of TAS1553 at 150 mg/kg caused a marked decrease of the intratumoral dATP pool (T/C < 20%) while the administration did not affect the intratumoral ATP pool. Furthermore, the administration of TAS1553 induced an increase of pChk1 level followed by the induction of pChk1 and pPARP and cleaved caspase-3 in tumors, suggesting that TAS1553 exerts antitumor effect by inhibiting RNR, resulting in DNA replication stress and apoptosis. In HCC38 mouse xenograft model, the antitumor activity of TAS1553 at 10mg/kg was also efficacious, showing a tumor growth inhibition compared to weekly administration of paclitaxel at 20 mg/kg.

**Conclusions:** These findings demonstrated that TAS1553, a novel class of RNR inhibitor, is orally available and had potent antitumor activity in preclinical models for both hematological and solid tumors. Therefore, TAS1553 could be a promising therapeutic option for cancer patients.

**Conflict of interest:** Corporate-sponsored Research: We are employees of Taiho Pharmaceutical Co., Ltd.

**171 (PB-022) Poster**

**Title:** TAS1553, a novel protein-protein interaction inhibitor against RNR, causes the inhibition of tumor cell proliferation via the induction of dATP pool reduction and DNA replication stress


**Background:** Ribonucleotide reduction (RNR) is a key metabolic enzyme that catalyzes a rate-limiting step for dNTP biosynthesis. It is composed of two non-identical subunits, R1 and R2, and the interaction between these subunits is necessary to its enzymatic activity. Based on the role in supplying dNTP for DNA synthesis, RNR is considered as a promising target for cancer therapy. The conventional RNR inhibitors have the shortcomings of low potency and selectivity. Here, we report a novel, selective and highly potent RNR inhibitor TAS1553, which disrupts the protein-protein interaction between RNR subunits.

**Material and Methods:** Inhibitory effect on enzymatic activity of human RNR was assessed by measuring the formation of dCDP from the substrate CDP. Material and Methods: Inhibitory effect on enzymatic activity of human RNR was assessed by measuring the formation of dCDP from the substrate CDP. RNR inhibitor TAS1553, which disrupts the protein-protein interaction caused the inhibition of tumor cell proliferation via the induction of DNA replication stress and apoptosis. The conventional RNR inhibitors have the shortcomings of low potency and selectivity. Here, we report a novel, selective and highly potent RNR inhibitor, was orally available and had potent antitumor activity in preclinical models for both hematological and solid tumors. Therefore, TAS1553 could be a promising therapeutic option for cancer patients.

**Conflict of interest:** Corporate-sponsored Research: We are employees of Taiho Pharmaceutical Co., Ltd.

**172 (PB-023) Poster**

**Title:** The expression of topoisomerase IIα(topo IIα) protein in young breast cancer patients (<35 years) and its relationship with prognosis

**Authors:** Y. Lin, H. Yunxia, Y. Mingang, Fujian Medical University Cancer Hospital, Department of Abdominal Surgery, Fuzhou, China; Fujian Medical University Cancer Hospital, Department of Radiation Oncology, Fuzhou, China

**Background:** There was no consensus on the relationship between topoisomerase IIα (Topo IIα) protein expression and the prognosis of young breast cancer patients. Our study aimed to assessed the long-term prognostic and predictive value of Topo IIα expression in young patients (<35 years) with breast cancer.

**Methods:** A total of 131 young breast cancer patients from 2001 to 2006 were retrospectively analyzed at our institute. We finally enrolled 50 patients for Topo IIα expression detection by immunohistochemistry technology. The association of Topo IIα expression and clinicopathological features were studied by Chi-square test. The 10-year disease free survival (DFS) and overall survival (OS) of these 50 patients were calculated by Kaplan-Meier analysis. The Cox regression model was employed for multivariate analysis.

**Results:** There were 42 patients (84%) with Topo IIα protein expression in the patients we analyzed. There was no correlation between Topo IIα protein expression and age, tumor size, lymph node metastasis, TMN stage, molecular typing, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER-2) (P > 0.05). The expression of Topo IIα was only positively correlated to Ki67 (r = 0.533, P = 0.002) as displayed by Spearman’s correlation test. The 1-, 3-, 5-, 10-year DFS in Topo IIα positive vs. negative group were 95.2% vs. 87.5%, 76.2% vs. 37.5%, 66.7% vs. 12.5% and 64.3% vs. 12.5%. The difference between two groups was statistically significant (C = 5.056, P = 0.025). There were 16 (32%) death in our follow-up, the median OS (mOS) of Topo IIα positive and negative group were 144 months and 105 months (C = 1.652, P = 0.199). In the multivariate Cox regression model, Topo IIα expression [RR = 0.341, 95% CI:0.126–0.919, P = 0.033] was an independent prognostic factor for DFS in young patients with breast cancer. Also, TMN staging [RR = 5.920, 95% CI: 1.89–19.738, P = 0.004] and lymph node metastasis [RR = 5.411, 95% CI: 1.16–26.222, P = 0.036] were both independent predictors for OS.

**Conclusions:** Topo IIα expression was more likely to have high expression in young breast cancer patients, and positively correlated to Ki67 expression. Patients with high expression of Topo IIα presented a favorable DFS.

**No conflict of interest**
Materials and Methods: A series of biaryl heterocyclic DNA binders were linked to an immunomodulator and the cytotoxic activity of the resulting molecules were evaluated in a panel of solid tumor cell lines. The most potent molecules were incorporated into linked payloads. The payloads were conjugated to monoclonal antibodies and the resulting ADCs [R1] that displayed desirable physicochemical properties and in vitro activity were evaluated in vivo xenograft models. Payload and ADC processing was assessed in tumor cells in culture as well as in the presence of lysosomal extract and in plasma. Bystander capability of the cytotoxic payload released from the ADC was also assessed in co-culture experiments using antigen-positive and antigen-negative cell lines. The lead ADCs were evaluated in vivo xenograft models, PK, and rat tolerability head-to-head against a known DNA mono-alkylator (IGN mono-alkylators).

Results: SAR studies led to the identification of a key indole unit in the DNA binder portion that significantly improved potency while providing a site for antibody conjugation. The lead I-BiP series exhibited low picomolar activity in a broad panel of solid tumor cell lines, including cell lines resistant to anti-tubulin agents. These in vitro cytotoxicities correlated with in vivo activity and the corresponding I-BiP ADCs were also highly active in vivo in aurin-resistant xenograft models. Co-culture experiments with I-BiP ADCs showed that the extent of bystander killing could be modulated via simple structural variations on the indole unit. Unlike typical PBD dimers and IGN mono-alkylators, I-BiP ADCs are more hydrophilic and therefore are not limited to DAR 2. DAR 4-5 I-BiP ADCs with high monomeric content were readily achieved without resorting to site-specific conjugation. Prior to this anti-tumor activity was observed for I-BiP ADCs at single doses of 1 or 3 mg/kg in a variety of solid tumor xenograft models. In toxicity studies in rats, the ADCs were well tolerated after single dose administration.

Conclusions: Given their potent antitumor efficacy in a variety of solid tumor models, favorable therapeutic index and hydrophilicity relative to PBD dimers and IGNs, I-BiPs are a promising new class of DNA damaging agents for ADC development [R1] defined in the title.

No conflict of interest

174 (PB-025) Poster Testing the COXEN method of predicting drug response with a prospective trial in dogs with osteosarcoma
D. Gustafson1, K. Collins2, J. Fowles1, E. Erhartz1, K. Weishaar1, D. Duval1, D. Thamm1
1Colorado State University, Flint Animal Cancer Center, Fort Collins, USA; 2Colorado State University, School of Biomedical Engineering, Fort Collins, USA

Background: COXEN is a method of predicting drug response of tumors based on gene expression and drug sensitivity of cell lines (Proc Natl Acad Sci USA 104:13086). Testing of predictive algorithms in clinical populations are difficult due to a number of factors including cost and the time associated with the implementation of the algorithm. In this study, dogs with cancer were treated for their disease by specialty trained veterinarians and here we report the use of this population to validate the ability of the COXEN algorithm to enhance drug response in canine osteosarcoma (cOSA).

Materials and Methods: Gene expression modeling (GEM) to predict the chemosensitivity of canine cancer cell lines and tumor drug response in cOSA using COXEN has been previously reported (BMC Bioinformatics 17:93). Following up on these findings, we designed a prospective clinical trial in dogs with cOSA that involved use of the previously derived COXEN algorithm to predict response to doxorubicin (DOX) and carboplatin (CARBO). Dogs with pathologically confirmed cOSA underwent amputation of the affected limb and tumor samples were collected from independent trephine biopsies. Biopsies underwent pathology review for viable tumor content followed by mRNA isolation and processing for gene expression analysis using the Affymetrix® canine 2.0 array. The previously described COXEN algorithm was then implemented to predict tumor sensitivity to DOX and CARBO. Following surgery and adjuvant chemotherapy with DOX, CARBO or the combination (DOX/CARBO), dogs are evaluated every two months for disease progression by chest x-ray to detect lung metastasis.

Results: Sixty-two canine patients have been evaluated for the trial with 19% of the dogs being disease free currently while 8/22 dogs predicted as drug resistant have a disease free interval (DFI) greater than one year whereas 19% of the dogs predicted as resistant have a DFI >1 year. 16/34 dogs predicted as drug sensitive are disease free currently while 8/22 dogs predicted as drug resistant. Ten dogs continued to be monitored for disease progression and the completion of this study allow for the prospective testing of the COXEN-based approach.

Conclusions: This is the first example of a GEM algorithm being used for drug selection in a canine cancer patient population and establishes their use for testing molecular-based therapeutic approaches in the adjuvant setting. Enrolled dogs will continue to be monitored for disease progression and the completion of this study allow for the prospective testing of the COXEN-based approach.

No conflict of interest

175 (PB-026) Poster Elevated serum substance P levels as a predictive marker for chemotherapy induced nausea and vomiting: prospective cohort study
H.S. Park1, H.S. Won1, H.J. An1, B.Y. Shim1. 1St. Vincent Hospital, College of Medicine, The Catholic University of Korea, Division of Medical Oncology, Department of Internal Medicine, Suwon, South Korea; 2Uijeongbu St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, Division of Medical Oncology, Department of Internal Medicine, Uijeongbu, South Korea

Background: Chemotherapy induced nausea and vomiting (CINV) is an unavoidable side effects. Moderate emetic risk chemotherapy induces CINV in a wide range of frequency (30 to 80%), but prevention strategy is the same to all these patients. Therefore, selection of high emetic risk patients and more active anti-emetics strategies are mandatory to improve tolerability of chemotherapy. The aim of this study is to find predictive clinico-biological marker for CINV.

Material and Methods: In this prospective cohort study, patients who received moderate to high emetic risk chemotherapy were enrolled. All patients were received granisetron patch at the day before start of 1st cycle chemotherapy. As predictive biomarkers, leptin, substance-P and ghrelin were measured at baseline, day 3 and day 14 after 1st cycle chemotherapy. Nausea and vomiting were measured each day during 4 days of 1st cycle chemotherapy, respectively. Logistic regression was used to evaluate the association between clinico-biological marker and CINV. Continuous variables were dichotomized according to the best cut-off value by receiver operating characteristic (ROC) curve analysis.

Results: Eighty-eight patients were enrolled and median age was 61. Male was predominant (70.5%), and colorectal cancer (56.8%) and gastric cancer (35.2%) were common cancer type. Most of patients (93.2%) received moderate emetic risk chemotherapy. During 4 days receiving chemotherapy, 50 patients (58.0%) had nausea and 20 patients (22.7%) experienced vomiting. Baseline median level of leptin, substance-P and ghrelin was 1.33 ng/mL (range 0.03–29.03), 302.2 pg/mL (range 54.7–1228.4) and 201.0 ng/mL (12.3–1700.0), respectively. There are no significant differences among biomarkers at different time point, but ghrelin level of day 14 was increased compared to baseline (P = 0.052). Patients with nausea had higher level of substance-P than patients without nausea at baseline and day 3. In univariate analysis, higher level of baseline substance P (≥22.6 ng/mL) is the only significant predictive marker for chemotherapy induced nausea (odds ratio (OR): 2.8, 95% confidence interval [CI]: 1.1–7.1, P = 0.027). Regarding chemotherapy induced vomiting, patients with high level of substance-P had more change of vomiting, but that was not statistically significant [OR: 2.4, 95% CI: 0.7–8.1, P = 0.148]. Regimen of chemotherapy and cancer type [Gastric cancer compared to colorectal cancer, OR: 29.0, 95% CI: 2.2–182.4, P = 0.029] is independent predictive marker for chemotherapy induced vomiting.

Conclusions: Baseline serum substance-P which is target of aprepitant is independent predictive marker for chemotherapy induced nausea. Active anti-emetics strategy should be considered to patients with high level of substance-P and further prospective study is needed to validate the predictive value of substance-P.

Conflict of interest: Corporate-sponsored Research: This study was supported by grant from LG Chem, Ltd (to B.Y. Shim).

176 (PB-027) Poster Influence of tether variations on the biological activity of tesirine analogues
A. Tiberghien1, N. Patel1, B. Vijayakrishnan1, L. Adams1, S. Corbett1, N. Aota2, J. Hartley1, P. Howard1. 1Medimmune, Spirogen, London, United Kingdom

Background: Pyrrolobenzodiazepine (PBD) dimers are DNA crosslinking agents, and exert potent cytotoxic activity across a wide spectrum of cancer cell lines. For these reasons, they were identified as ideal candidates for targeted delivery by antibody drug conjugates. Amongst many PBD drug-
Antibody-drug conjugates (ADCs) are an exciting new class of anticancer therapeutics. ADCs combine the potency of a cytotoxic agent with the selectivity of an antibody to enable targeted killing of tumour cells. BTC Bicycle Toxin Conjugates (BTC), which bind to cell-surface targets on solid tumour cells and deliver a cell-killing toxin, are a new generation of ADCs designed to achieve a DAR 1 homogeneous ADC. The unique flexibility of the PBD linker allows a variety of molecular motifs suitable for enhancing activity. Drug-linker analogue 3b was identified as a more potent analogue of SG3249.

**Conflict of interest:** Ownership: Authors and co-authors are MedImmune/ AstraZeneca employees (exception SC) with ownership interest.

177 (PB-029) Poster

**DAR 1 ADCs: Advances and opportunities**

B. Vijayakrishnan, R. Beard, L. Masterson, A. Tibergien, N. Dimasi, P. Howard.

1MedImmune, Conjugation, London, United Kingdom; 2MedImmune, Research and Development, Technology, Gaithersburg, USA; 3MedImmune-Immunomedica, Chief Scientific Officer, London, United Kingdom

**Background:** Antibody-drug conjugates (ADCs) are an exciting new class of anticancer therapeutics. ADCs combine the potency of a cytotoxic agent with the selectivity of an antibody to enable targeted killing of tumour cells. PBD dimers are emerging as a particularly attractive class of payload for the growth of ADC field due to the combination of their potency and different modes of action to antimitic warheads such as auristatin and maytansines. Tesirine (SG3249) is currently being used in multiple clinical trials by MedImmune, external licencing companies and others.

**Results and Discussion:** Expanding the therapeutic window is a key driver for successful ADCs and frequently narrow therapeutic indices (TI) have been reported for candidates under evaluation in the clinic. This is mainly due to off-target toxicity, which is linked to deconjugation, competition with unconjugated antibody, aggregation and faster clearance due to hydrophobicity. In our quest to improve the TI, we designed a PBD payload to achieve a DAR 1 homogeneous ADC. The unique flexibility of the PBD platform technology allowed us to add two functional maleimides to the potential PBD payload SG3199, which resulted in the creation of a symmetrical PBD payload SG3710. This double maleimide is capable of bridging site-specific cysteines in an engineered antibody. Conjugation conditions were optimized to produce homogeneous DAR 1 ADCs using anti-Her2, anti-EphA2 and non-targeting antibodies.

The resulting DAR 1 ADCs were tested in an in vitro cytotoxic assay along with DAR 2 ADCs using tesirine payload. It is worth noting that both SG3710 and tesirine releases the same potent PBD warhead, SG3199. In Her2 overexpressing gastric cancer cell line NCI-N87 both ADCs showed similar EC50 values (0.2–0.5 ng/mL). In a EphA2 overexpressing prostate cancer (PC3) cell line both ADCs exhibited comparable EC50 values (2.2–5.0 ng/mL). The DAR1 and DAR2 anti Her2 ADCs were evaluated in an in vivo NCI 87 human xenograft model. At dose of 0.3 mg/kg the DAR1 ADC was as active as the DAR2 despite delivering only half the amount of SG3199 warhead.

**Conclusion:** In summary, in this study we have developed a unique, site specific DAR 1 ADC by exploiting the flexible PBD platform and a cysteine engineered antibody. DAR 1 ADC shows similar potency in vitro and cell killing assays and in vivo mouse xenograft models. This work shows the potential of DAR 1 ADCs and we are currently exploring alternate payloads with varying potency.

**Conflict of interest:** Ownership: The authors declare the following competing financial interest(s): All authors are employees of MedImmune a division of AstraZeneca and own AstraZeneca stocks.

178 (PB-029) Poster

**Preliminary pharmacokinetic assessment of BT1718: A phase I/IIa trial of BT1718 (a first in class Bicycle Toxin Conjugate) in patients with advanced solid tumours**


**Background:** Bicycles® are novel therapeutics: bicyclic peptides constrained via a chemical scaffold, which confers structural stability leading to high affinity and selectivity. These Bicycles have been used to generate Bicycle Toxin Conjugates (BTC), which bind to cell-surface targets on solid tumour cells and deliver a cell-killing toxin. BTC’s low molecular weight results in rapid and extensive delivery of payloads into preclinical solid tumour models, associated with a short duration of systemic exposure and liver-sparing rapid renal elimination.

The BTC, BT1718, is a MT1-MMP binding peptide linked to the maytansinoid toxin DM1 via a cleavable disulphide linker that demonstrated significant anti-tumour activity in a range of preclinical experiments. Following intravenous administration to preclinical species, BT1718 exhibited a short terminal half-life (ca. 20 min in Balb c mice) and a volume of distribution similar to extracellular fluid volume (ca. 0.2 L/kg). Here we present the preliminary initial pharmacokinetic (PK) data from the ongoing phase I/IIa clinical study.

**Materials and Methods:** This is a first in human, multicentre, dose escalation study in patients with advanced solid tumours. The study aims to establish the recommended Phase II dose (RP2D) for once and bi-weekly dosing of BT1718, along with pharmacokinetics and exploratory pharmacodynamics. Expansions will further explore preliminary efficacy, tolerability and pharmacodynamics of BT1718 in approximately 60–70 patients with MT1-MMP expressing NSCLC, TNBC or other tumours (as assessed by IHC) (Clinical trial NCT03486730).

**Results:** Dose escalation in Phase I is ongoing and no DLTs have been reported to date (up to cohort 3). The preliminary PK data in patients are in line with the preclinical PK data and confirm dose dependent increases in exposure of BT1718 accompanied with a short terminal half-life.

**Conclusions:** BT1718 is a first in class BTC and is well tolerated at doses up to 2.4 mg/m². The preliminary PK data confirm the expected PK profile based on preclinical data. Updated clinical PK data from the ongoing study will be discussed along with further preclinical tumor and plasma data illustrating mechanism of action of this new treatment modality.

**Conflict of interest:** Other Substantive Relationships: BT1718 is being developed by Cancer Research UK (CRUK) and Bicycle Therapeutics. A number of the authors are employed by CRUK or Bicycle Therapeutics.

179 (PB-030) Poster

**Combinations containing the anti-CD205 antibody drug conjugate MEL1309/OBT076 show strong pre-clinical activity in diffuse large B cell lymphomas**

Purpose of this study is to investigate cancer-specific metabolic pathways and to find small-molecule inhibitors targeting those pathways. Biological Background: Still too many individuals affected by lymphoma succumb due to their disease: novel therapeutic approaches are needed. MEN1309 was combined with other targeted agents in three DLBCL cell lines (HBL1, OCI-LY-10, TMD8). MEN1309 plus the anti-CD20 monoclonal antibody rituximab was synergistic in 3/3. The combination was then evaluated in vivo in a DLBCLmodel (OCI-LY-10). Mice were divided into four groups and treated with MEN1309 (2.5 mg/kg IV, D1 and D12), rituximab (3 mg/kg IV on D1; 5 mg/kg IV on D12), MEN1309 plus rituximab (same schedule as single agents), or with vehicle only. The combination induced tumor eradication with significant differences at D17 (P < 0.05) versus both single agent arms. Benefit in all three cell lines was also observed with MEN1309 in vitro combined with the BCL2 inhibitor venetoclax (ABT-199) and with the BTK inhibitor ibrutinib. The combinations with the PI3K-delta inhibitor idelalisib and the immunomodulating champignon Andosan™ significantly increased Th1 type and pro-inflammatory cytokines in treated animals. Moreover, Andosan™ treated mice had improved cell-mediated cytotoxicity with Luminex multiplex analysis. Conclusions: The A/J Min/+ mouse model for human familial adenomatous polyposis (FAP) develops spontaneously multiple adenocarcinomas in colon and small intestine. Agaricus blazei Murill (ABM) mushroom is related to champignon and used in traditional medicine against cancer. It contains immunomodulating β-glucans and has anti-tumor effects in murine cancer models. Andosan™ is an ABM-based extract. Materials and Methods: A/J Min/+ and A/J wild-type mice were given tap water with 10% Andosan™ for 22 weeks, exanguinated, their intestines preserved and serum frozen. Intestines were microscoped blindly and stained for tumor-associated protease, legumain. Serum cytokines were measured by Luminex multiplex analysis. Results: Andosan™ treated A/J Min/+ mice had significantly fewer intestinal adenocarcinomas and 60% reduced tumor load compared to control. Legumain expression was also reduced in intestines from Andosan™ treated animals. Moreover, Andosan™ had significant cytotoxic/apoptotic effect on human cancer colon cell line, Caco-2. There were significantly increased Th1 type and pro-inflammatory cytokines in Andosan™ treated mice. Conclusions: The results from this mouse model for colorectal cancer shows significant protection of orally administered Andosan™ Agaricus blazei-based mushroom extract against intestinal cancer development. This is supported by finding of less legumain in intestines of Andosan™ treated mice and increased systemic Th1 cytokine response. The mechanism is probably both immunomodulatory and growth inhibition of tumor cells by induction of apoptosis.

182 (PB-033) Poster A translational platform using primary human immune cells in vitro, syngeneic and humanized models in vivo to support and advance immuno-oncology drug discovery

M. O’ourke1, O. Aziz1, 1Charles River Labs, Biology, Essex, United Kingdom

Charles River Laboratories (CRL) are establishing a powerful translational immuno-oncology platform with the capability of progressing biologics or small molecule modulators of immune response from in vitro to in vivo assays using human and mouse variants of current check-point inhibitors and small molecules. The platform is supported by an internal blood donor panel which ensures highly reproducible data and high quality immune cells which are prepared immediately once sampled.

Our in vitro platform includes primary human immune cell assays which profile T cell activation, T cell mediated cancer cell kill, expansion of T cell populations, mixed lymphocyte reactions (MLR), T cell invasion, and antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC).

The platform is currently being expanded to determine the effect of activated immune cell populations on tumour cell spheroid cultures. We are in the process of developing a range of GFP expressing cell lines which will be used to support co-culture experiments. The platform has been validated with a standard of care chemotherapeutics, including anti-CTLA4, anti-PD1, and a selection of small molecule inhibitors of targets known to modulate immune responses including IDO inhibitors.

Ex-vivo and in vivo activated mouse splenocytes response to check-point inhibitors measured as cytokine release and modulation of immune cell populations, as measured by flow cytometry supports the translation of important compounds from the bench to pre-clinical models.

Syngeneic mouse tumour models have frequently been used to profile immune responses in tumours. CRL have optimized and profiled existing check-point inhibitors to support immuno-oncology drug discovery using mouse and rat antibody variants of anti-CTLA4 and anti-PD1.

To confirm the translational development of our platform CRL have developed and optimized humanized mouse models using sub-cutaneous implanted patient derived xenografts (PDX) with human engraftment via CD34+ haematopoietic stem cells in NCr mice which were treated with anti-CTLA4 and anti-PD1. Infiltration of human immune cells and PDL-1 expression was detected by flow cytometry (FC) and immunohistochemistry (IHC) in hematopoietic organs and tumor tissue, supporting the initial in vitro response in primary immune cells.

We present a screening platform which will support translation of compounds from in vitro primary immune cell assays, to modulation of mouse immune cell population in spleen and tumours, resulting in efficacy and tumour immune cell activation in humanized mouse models.

No conflict of interest

183 (PB-034) Poster In silico screen for predictive ERBB mutations

M. Hakanen1, D. Chakroborty1, K. Kurpia1, K. Elenius1. 1University of Turku, Biomedicine, Turku, Finland

Background: Mutations or copy number variations in ERBB genes have been identified in several cancer types and aberrant ERBB signaling has been associated with the progression of e.g. breast, colorectal and lung cancer. Several ERBB-targeting drugs have been approved for clinical use. These selectively target either EGFR (ERBB1) or ERBB2 (HER2), or inhibit all kinase-competent ERBBs (EGFR, ERBB2 and ERBB4) simultaneously. While targeted therapies can be effective for a subgroup of patients, identification of individuals who benefit from treatments has remained a challenge. The predictive clinical significance for the vast majority of the thousands of mutations in the cancer tissues also remains unknown. Here, we describe an approach to identify predictive biomarkers for ERBB-targeted therapeutics that takes advantage of publicly available in silico cell line databases.

Materials and Methods: Three databases collectively including data from 272 different cancer cell lines were used: Cancer Cell Line Encyclopedia (CCLE), Genomics of Drug Sensitivity in Cancer (GDSC), and Cancer Therapeutics Response Portal (CTRP). An R script was written to collect data about cell lines with mutations in any of the four ERBB genes, the ERBB mutations and copy number variations in these cell lines, and drug response data for 10 ERBB-targeting drugs. Area under curve (AUC) value was used as an indicator of the drug response. Responsiveness of cells expressing clinically actionable "standard" predictive EGFR mutations (EGFR L858R or exon 19 deletions) to EGFR-selective tyrosine kinase inhibitors was used as a threshold to identify other ERBB mutant cell lines responsive to any of the 10 ERBB inhibitors. Candidate predictive mutations were validated by expressing individual mutants in Ba/F3 cells. The sensitivity of the Ba/F3 cells expressing ERBB mutants to ERBB inhibitor drugs was assessed using MTT assays.

Results: The analysis identified 41 cell lines harboring putatively predictive ERBB mutations. Included were 9 out of 17 (53%) ERBB mutations previously classified as "oncogenic" by cBioPortal, initially validating the screen. Nine candidate predictive mutations were selected for further functional validation in the Ba/F3 cells. Functional analyses are ongoing and will be followed by biochemical and structural analyses to understand the mechanisms by which the mutations lead to ERBB receptor activation and drug sensitivity.

Conclusions: The method can be used to identify activating and putatively predictive mutations in ERBB receptors, and possibly in other oncopgenes.

No conflict of interest

184 (PB-035) Poster Chemical genomic analysis on autophagic regulation mechanism in human lung cancer A549 cells

T. Kataura1, M. Imoto1. 1Keio University, Biosciences and Informatics, Yokohama, Japan

Background: Autophagy, an intercellular bulk degradation system is a key regulator of cellular homeostasis. Recently, autophagy has been reported to involve with various diseases such as cancer. Especially, it is indicated that cancer cells utilize autophagy to acquire drug resistance or adapt to undernutrition. Therefore, elucidation of the mechanism of autophagy regulation in cancer cells is becoming important. Additionally, diverse small molecules have been reported to induce autophagy. However, the predominant mechanism of autophagy induction by each small molecule is still unclear. Thus, we conducted comprehensive analysis on autophagy modulating pathways based on chemical genomic approach.

Material and Methods: GFP-LC3-RFP, a novel fluorescent autophagic probe was recently reported. This probe is divided into GFP-LC3 and RFP by ATG4 endoenzyme protease. GFP-LC3 is degraded in lysosomes along with autophagy progression while RFP continues to stay in cytosol. Therefore, induction or inhibition of autophagy can be estimated by GFP/RFP fluorescence intensity ratio. We produced A549 cells stably expressing this probe using retrovirus vector transduction and FACs cell sorting. Automated quantification of autophagic activity was performed by using OPERA phoenix high content imaging system. Clustering and heatmap analyses were conducted with R programming software.

Results: We first screened autophagy inducers from 400 small molecules. As a result, 30 compounds including several anti-cancer drugs were identified as autophagy inducers as judged from the decreased signal ratio of GFP/RFP. Next, we examined the effect of 300 different number of signal transduction inhibitors on autolysosome formation induced by autophagy-inducing compounds to produce fingerprints. Then, all fingerprints were normalized, and comprehensive analysis was conducted using hierarchical clustering to classify the regulation pattern of autophagy. The results of clustering, each autophagy-inducing compound was grouped depending on its fingerprint action, indicated that this chemical genomic analysis was validated. From the perspective of the signal transduction inhibitors, we confirmed MAPK and JAK-STAT signaling pathways broadly regulate autophagy as reported. Interestingly, we found that inhibitors of specific pathways such as CDKs selectively inhibited autophagy flux activated by the autophagy inducers including anti-cancer drugs, which categorized in same cluster. It is indicated that these specific pathways regulated the autophagy against cancer chemotherapeutic agents.

Conclusions: We proposed that several signaling pathways selectively regulated autophagy induced by anti-cancer drugs based on chemical genomic approach. Inhibition of these pathways might improve the efficacy of anti-cancer drugs by suppressing autophagy.

No conflict of interest

185 (PB-036) Poster Genetic background affects the drug efficacy in 3D organoid model

W.W. Chen1, J.M. Chang1. 1Development Center for Biotechnology, Department of Pharmacology, Institute for Drug Evaluation Platform, New Taipei city, Taiwan

Background: For decades, 2D cell culture is developed for drug screening and mechanism study in cancer research. It provides lots of information in...
preclinical drug development but few drugs can pass through the clinical trial because the 3D environment of tumor cells. Tumor cells grow in 3D environment which is more resistant to anti-cancer drug than growing in 2D cell culture. Here we developed a 3D organoid model for drug screening, which the size of tumor is larger than other traditional methods using U-bottom or cell sphere formation.

Materials and Methods: The human large adenocarcinoma cell lines HCC-B27 and NCI-H727 were cultured on the 96-well nanoculture plate, which the 3D organoid was formed at 3 days and subject to the treatment of a panel of chemo-drugs, target drugs, and immune-modulator drug. For improvement of 3D organoid model, adhesion molecules were added in the 3D organoid culture. A panel of anti-cancer drugs at a range between 0.01 and 10 μM was tested in 3D organoid model for 24 hours including taxol, CPT-11, cisplatin, doxorubicin, gemcitabine, aflatinb, gefitinib and erlotinib. The viability of cancer cells was measured by caspase3/7 activity by using cell imaging analysis.

Results: The size of 3D organoid was increased by adding adhesion molecule. In a panel screening, aflatinb appeared had a good anti-tumor activity on HCC-B27 lung adenocarcinoma cells with carrying the PI3K mutation in ES45K and EGFR mutation in T790M, or other TKI inhibitors and chemodrug. HCC-B27 cells with were sensitive to aflatinb about 100-fold than NCI-H727 cells with KRAS mutation in G12S and G12V.

Conclusions: In conclusion, aflatinb was responsible for wild type of kras but not in kras G12D mutation cells in a panel of drug screening with different genetic background. Within the candidate panels we found cancer cell lines in 3D organoid screening, the companion diagnostic for predicting the drug efficacy by genome test for mutation can be discovered for the selection of the responder patients.

No conflict of interest

186 (PB-037) Poster
Golgi morphology and drug resistance in cancer cells

J.J. Garcia Gomez1, M. Esteras1, J. Rodrigues Simoes da Costa2, J. Kristen1, S.P. Harley1, D. Hochhauser1.

1UCL Cancer Institute, Oncology, London, United Kingdom; 2MRC Laboratory for Molecular Cell Biology, Cell Signalling and Autophagy, London, United Kingdom

Background: Recent studies suggest a link between the DNA damage response and regulation of the Golgi apparatus. In cancer cells, DNA damage triggers DNA-dependent protein kinase (DNA-PK) phosphorylation and activation of GOLPH3 (Golgi-associated protein) resulting in fragmentation of the Golgi apparatus. Golgi fragmentation increases cell survival after DNA damage making this pathway a potentially useful therapeutic target. Our aim is to identify drugs which block Golgi fragmentation after DNA damage to improve efficacy of DNA-targeting anticancer therapies.

Results: In colorectal cancer cells, DNA damage following treatment with cisplatin, SN38, and ionizing radiation triggered GOLPH3 pathway-dependent Golgi fragmentation. We found that GOLPH3 knockdown abrogates Golgi fragmentation after DNA damage, blocks signalling through PI3K/Akt pathway, and increases sensitivity to SN38. We identified 16 drugs that impeded Golgi fragmentation after DNA-damaging chemotherapy treatment, and 6 additional drugs that increased chemotherapy-triggered Golgi fragmentation. We examined whether drugs with known activity as inhibitors of prostaglandin synthesis, and modulators of steroid hormone signalling.

Conclusions: Our results suggest that chemical inhibition of Golgi fragmentation after DNA damage has important therapeutic potential. Our goal is to improve the outcome of DNA-damaging anticancer treatments by combining them with drugs that inhibit Golgi fragmentation to sensitize cells to DNA damage.

No conflict of interest

187 (PB-038) Poster
A pharmacological screening to improve the anti-lymphoma activity of BET inhibitors

G. Golino1, C. Tarantelli1, F. Spriano1, L. Scalise1, A. Arribas1, L. Cascione1, E. Zucza2, A. Stathis3, F. Bertoni3, E. Gaudio1. 1Università della Svizzera italiana, Institute of Oncology Research, Lymphoma and Genomics, Bellinzona, Switzerland; 2Ente Ospedaliero Cantonale, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland

Introduction: Despite a widespread preclinical anti-proliferative activity of the bromo- and extraterminal domain (BET) inhibitors in lymphomas, the clinical activity in early trials has been limited. Here, we aimed to identify drugs that improve BET inhibition activity performing a pharmacological screening with the BET inhibitor birabresib (OTX015/MK-6267) in combination with a library of 348 compounds in two lymphoma cell lines.

Material and Methods: Two cell lines derived from germinal center B cell (GCB) diffuse large B cell lymphoma (DLBCL) (OCI-LY-19 and WSU-DLL2) were exposed to birabresib (single dose, 100 nM) in combination with two different doses (20 and 1,000 nM) of 348 compounds. Compounds giving a 1.5-fold decreased proliferation with the combination than with the individual compounds were further evaluated in additional cell lines (the GCB-DLBCL SU-DHL-8 and FAREG, the mantle cell lymphoma REC1 and the chronic lymphocytic leukemia MEC1) exposed (72 h) to increasing doses of birabresib alone and in combination with increasing doses of other compounds. Combinations were validated using another BET inhibitor (CPI-0610). Synergy was assessed with Chou-Talay combination index (CI): strong synergism (<0.3), synergism (<0.9), additive (0.9–1.1), antagonism/ no benefit (>1.1).

Results: The combinations of birabresib with a series of compounds achieved improved anti-tumor activity than single agents. Besides HDAC and mTOR inhibitors, in accordance with what previously reported by us and others, the ABL/SRC inhibitor dasatinib, the AKT1/2/3 inhibitor MK-2206, the JAK2 inhibitor TG101209 and the LRRK2 inhibitor LRRK2-IN appeared as active combination partners. The screening results were validated in additional four cell lines. The combination of LRRK2-IN with birabresib and with CPI-0610 was synergistic in 6/6 and 5/6 cell lines (no synergism in WSU-DLL2), respectively. Dasatinib in combination with birabresib or with CPI-0610 was synergistic/strong synergistic in 5/6 cell lines (no synergism in REC1). MK-2206 in combination with OTX015 or with CPI-0610 was synergistic/strong synergistic in 6/6 cell lines. The JAK2 inhibitor TG101209 in combination with OTX015 and with CPI-0610 was synergistic in 4/6 and 3/6 cell lines respectively.

Conclusion: A chemical screening has identified novel BET inhibitors – combinations containing with anti-tumor activity in lymphoma cell lines, to be further studied. Work supported by a San Salvatore Foundation grant.

No conflict of interest

188 (PB-039) Poster
Enzyme adsorption by drug aggregates: probing the molecular basis of inhibitor promiscuity using molecular dynamics simulations

M. Ghattas1, N. Atreth1, S. Arawashedi2, R. Bryce2. 1Al Ain University of Science and Technology, Pharmacy, Al Ain, U.A.E.; 2University of Manchester, Division of Pharmacy and Optometry, School of Health Sciences, Manchester, United Kingdom

Background: Promiscuous inhibitors are one of the PAINS (pan-assay interference compounds) that make scientists’ life difficult in the early drug discovery process. They cause false positives in inhibition assays, forming colloid aggregates in solution which have the ability to adsorb enzyme molecules. The molecular basis of this promiscuous inhibition is difficult to study due to the transient stability of aggregates.

Methods: Molecular dynamics (MD) simulations were employed to study the interaction between a drug aggregate and enzyme in aqueous solution. A large aggregate of drug was first obtained from a separate MD simulation and then solvated along with the enzyme molecule. These systems were then simulated multiple times from different initial configurations.

Results: In accord with experiment, MD simulations indicate direct interactions between the inhibitor aggregate and the enzyme molecule. The protein showed no major changes in its overall tertiary structure. Interestingly, however, there were changes in its active site conformation after being adsorbed to the drug aggregate, that made it unsuitable for substrate binding.
Conclusions: This study provides initial insights into the molecular mechanism of aggregation-based false inhibition and a basis for further investigations.

No conflict of interest

190 (PB-041) Poster

The NCI Program for natural product discovery: a new resource for cancer drug discovery

1. National Cancer Institute, National Institutes of Health, Bethesda, MD, USA; 2. Leidos Biomedical Research, Inc., Frederick, MD, USA; 3. University of Maryland, College Park, MD, USA; 4. Data Management Services, Inc., Natural Products Biology Laboratory, Center for Cancer Research, Frederick, USA

The US National Cancer Institute’s Natural Product Repository is one of the world’s largest, diverse collections of natural products containing over 230,000 unique extracts derived from plant, marine and microbial organisms that have been collected from biodiverse regions throughout the world. Importantly, this national resource is available to the research community for the screening of extracts and the isolation of bioactive natural products. However, despite the success of natural products in cancer drug discovery, compatibility issues that make crude natural product extracts challenging have reduced enthusiasm for the high-throughput screening (HTS) of crude natural product extract libraries in targeted assay systems. To address these limitations and make the NCI’s Natural Products Repository more amenable to HTS, we have initiated the prefractionation of extracts using an automated, high-throughput robotics platform capable of generating a library of 1,000,000 partially purified extracts. The talk will discuss this and other mechanisms to increase the utility of the NCI Natural Products Repository in cancer-related drug discovery.

No conflict of interest

191 (PB-042) Poster

3D growth system of MCF-7 is valuable model for anticancer drugs screening

T. Nikolaenko1, L. Garmanchuk1, V. Nikulina1, S. Taran Shvchenko National University of Kyiv, Educational and Scientific Centre ‘Institute of Biology and Medicine’, Kiev, Ukraine

Introduction: Gene expression profiles in spheroid cultivated cells are more similar to natural tumors, than profiles of the same cells in monolayer culture. Tumor spheroids are heterogeneous cellular aggregates that, when greater than 500 μm diameter, are frequently characterized by hypoxic regions and necrotic centers. Architecture of three-dimensionally (3D) propagated cells is very similar to avascular tumor areas. The gradient of diffusion in cell aggregates leads to reduced proliferation rates and increased drug resistance. The purpose of the work was to conduct a comparative study between 3D and monolayer growth systems of MCF-7 cells, and prove the value of spheroid model.

Materials and Methods: As experimental model was used adhesion line of breast adenocarcinoma MCF-7. Cells in 2D and 3D culture were incubated during 5 days under conditions of starvation. The number of live cells was evaluated using MITT-colorimetric assay. Apoptotic index was assessed by flow cytometry.

Results: MCF-7 cells growth parameters differ significantly in 2D and 3D growth systems. Cells in 2D system are more sensitive to serum starvation than 3D cultures. Cell viability increases dramatically in 3D system. The level of apoptotic and necrotic cells for 2D growth in serum starvation conditions (39.2 ± 7.3% and 33.5 ± 2.8% respectively) were twice increased in comparison with conditions of complete culture medium (19.0 ± 1.3% and 11.4 ± 1.7% respectively), whereas incomplete medium have no detectible effects on 3D cultured cells. However, the 3D cells percentage in G0/G1 phase of the cell cycle was increased in 1.6 times in serum free conditions, whereas it was not changed in complete medium that can indicate similarity to natural tumors.

Conclusions: Therefore, the 3D growth system has been proposed as an adequate and valuable model to study tumor growth and response to therapeutic substances.

No conflict of interest

192 (PB-043) Poster

Quantification of target occupancy in cells and tumor tissue using FCCS

F. Becker1, K. Hansen1, M. Croshiere2, L. Yosef2, S. Hannus1
1. Intana Bioscience GmbH, Research, Planegg, Germany; 2. Karyopharm Therapeutics, Research & Translational Development, Newton, MA, USA

Quantification of target occupancy in cells and tissues is an important parameter to correlate the biochemical signature of a drug with in vivo efficacy. Occupancy data can confirm the target hypothesis and support the identification of the best regimens for drug dosing. Fluorescence Cross Correlation Spectroscopy (FCCS) has been used successfully in the past to monitor and analyze drug-target interactions. The technology is sensitive, fast and specific and allows the detection of drug target interactions in small sample volumes. Here we adopted FCCS to quantify the occupancy of cells and tumors treated with lead molecules.

The application uses a fluorescently labeled tracer to sense and quantify the amount of unoccupied binding pocket of the target. Additionally, target specificity is confirmed using an antibody with a second fluorescent label. Target occupancy studies can be carried out in different relevant cell lines and xenograft tumors retrieved from treated animals. As such the approach can be applied to study target occupancy in vitro and in vivo.

Focus of the presentation will be the selection and characterization of necessary tools to perform occupancy studies using this technology.

The use of this method to support preclinical and clinical studies will be discussed.

Conflict of interest: Ownership: Founder and Shareholder of Intana Bioscience GmbH. Corporate-sponsored Research: Study was sponsored by Karyopharm.

194 (PB-045) Poster

Synergy between IAP inhibitors and a cytotoxic antibody-based chimeric protein

D. Antignani1, D.J. Urban2, J.S. Roth3, M.D. Hsi2, D.J. Fitzgerald3
1. NCI, Laboratory of Molecular Biology, Bethesda, USA; 2. NCATS, Division of Preclinical Innovation, Rockville, USA

Background: Screening of drug libraries on tumor cells is a potent tool to discover new therapeutic opportunities. Immunotoxins are cytotoxic antibody- toxin gene fusion proteins engineered to target cancer cells via antibody binding to surface antigens. Immunotoxins derived from Pseudomonas exotoxin inhibit protein synthesis via the ADP-ribosylation of the eukaryotic elongation factor (eEF2). Clear clinical benefit has been achieved in patients treated with an immunotoxin targeting CD22 expressed on B-cell malignancies. However, success has not been universal and, when treating solid tumors, immunotoxins have performed less well in producing complete responses. Therefore we conducted a screening of 2000 compounds in combination with an immunotoxin targeting the human transferrin receptor on two different epithelial tumor cell lines. Our goals included identifying agents that enhanced the cytotoxic activity of the immunotoxin and also to discern differences in cellular responses to the drug-immunotoxin combinations to identify biomarkers for susceptibility to immunotoxin-mediated killing. Among the most active hits in the screen, we found that the inhibitors of the IAPs proteins, Birinapant or SM164, strongly synergized with the immunotoxin in MDA-MB-468 cells (triple negative breast cancer) but not in A431 cells (epidermoid carcinoma). We sought to understand the different responses in each cell line.

Materials and Methods: Viability was determined using the CellTiter-Glo Luminescence Cell Viability Assay kit (Promega, Madison WI). For immunoblot analysis, the following primary antibodies were used: caspase 8, caspase 3 and cleaved caspase 3 (Cell Signaling), cIAP1 (Enzo), actin (BD Biosciences). TNFa in the cell medium was detected using the hTNFa-DuoSet ELISA (R&D System). TNFa transcript level was monitored in real time on a StepOnePlus Real Time PCR system.

Results: We confirmed the results of the drug screening. Combinations of the immunotoxin and Birinapant or SM164 greatly increased the cytotoxic activity on MD-MBA-468 cells, but exhibited only modest effects on A431 cells. We found that the level of TNFa and cIAP1 proteins was higher in MDA-MB-468 than A431. We noted that in 24 hours the immunotoxin drastically reduced the level of cIAP1 and the protein is completed degraded when Birinapant is added in both cell lines. In MDA-MB-468, because of the presence of TNFa, this leads to the activation not only of caspase 3 but also caspase 8 while in A431 we were able to detect only caspase 3 cleavage.

Conclusion: We suggest that TNFa and cIAP1 are critical elements of the synergy between the immunotoxin and IAP inhibitors. The rapid degradation
J.A. Hassell 1.
inhibited the oxidation of NADH catalyzed by complex I.

In the respiratory system to discover new OXPHOS inhibitors based on bioenergetic profiles: oxygen consumption rate (OCR) and extracellular acidification rate (ECAR); and characteristic proteomic changes. This system was used in investigating small molecules that target OXPHOS.

Material and Methods: Bioenergetic profiles were analyzed by using the XF24 Analyzer. Permeabilized cells and the following substrates and ADP to interrogate specific respiratory activity for each complex: malate and pyruvate for complex I; succinate for II; duroquinol for III; TMPD/ascorbate for IV.

Results: Using the XF24 Analyzer, we characterized the contribution of glycolysis and OXPHOS to cellular energy metabolism in several cancer cell lines, and chose HeLa cells for screening, which averagely uses both metabolic processes. In the course of screening 559 compounds from the chemical library of NPDepo, we found that NPL40330 remarkably decreased OCR value. Moreover, the proteome profiling revealed that NPL40330 upregulated the expressions of glycolytic enzymes, suggesting that it is an OXPHOS inhibitor. Next, we examined which complex does NPL40330 selectively inhibited malate/pyruvate-driven respiration; while no inhibition was observed in the respirations activated by other substrates. These perturbation profiles suggest that NPL40330 is classified under complex I inhibitors.

Conclusions: To find new OXPHOS inhibitors, we constructed a screening system based on bioenergetics and proteomic profiling and found that NPL40330 inhibits mitochondrial respiration. Furthermore, the semi-TC assay for OXPHOS established that the target of NPL40330 is mitochondrial complex I.

No conflict of interest

196 (PB-047)  
Expression of monoamine oxidase a is correlated with three-dimensional tumoursphere-formation by human breast tumor cells lines

W. Gwynne 1, J. Wu 1, A. Girgis-gabardo 1, A. Dvorkin 1, R. Hallett 1, J.A. Hassell 1.  
McMaster University, Biochemistry and Biomedical Sciences, Hamilton, Canada

Background: Breast tumors comprise a phenotypically heterogeneous tumor cell population, wherein a minor fraction of cells, termed breast tumor initiating cells (BTIC), can initiate and sustain tumorigenesis. These observations have therapeutic implications. Traditional cytotoxic anticancer therapies inhibit tumor growth but not the non-tumorigenic tumor cells, allowing BTIC to remain dormant before ultimately driving disease relapse. Culturing breast tumor cell lines as clonal, three-dimensional tumourspheres (TMS) using a chemically-defined, serum-free medium maintains a high fraction of BTIC in vitro. We previously used TMS-formation as a surrogate assay for BTIC in a high-throughput phenotypic screen and found that antagonists of serotonin biosynthesis and function inhibit BTIC activity. Herein, we report a role for monoamine oxidase A (MAO-A), which plays a role in 5-HT degradation, on BTIC activity.

Methods: The sensitivity of breast tumor cells to pharmacological inhibition of MAO-A was determined using the TMS-formation assay and Prestoblue™ cell viability assays. RNA and protein were isolated from breast tumor cells propagated in serum-containing medium or as TMS in serum-free medium. MAO-A transcript expression levels were detected by Nanostring™ nCounter analysis and at the protein level by Western blotting. To determine whether MAOA transcripts are required for breast tumor cell proliferation in vivo, we mined the results of a recent genome-wide shRNA dropout screen in 68 human breast tumor cell lines.

Results: 196 (PB-047)  
NPL40330: A novel oxidative phosphorylation inhibitor identified by bioenergetic and proteomic profiling

Y. Futamura 1, M. Muroi 1, H. Aono 1, M. Kawatani 1, H. Osada 1.  
RIKEN, Chemical Biology Research Group, Wako, Saitama, Japan

Backgrounds: Glycolysis and oxidative phosphorylation (OXPHOS) are two key metabolic processes. Due to Warburg’s discovery, glycolysis has so far gained much attention in anticancer therapeutics. However, the recent recognition of metabolic reprogramming as a major cancer hallmark led to an increased interest in OXPHOS as an attractive target against cancer stem cells and certain cancer cells as well. Here we constructed a screening system to discover new OXPHOS inhibitors based on bioenergetic profiles: oxygen consumption rate (OCR) and extracellular acidification rate (ECAR); and characteristic proteomic changes. This system was used in investigating small molecules that target OXPHOS.

Material and Methods: Bioenergetic profiles were analyzed by using the XF24 Analyzer. Permeabilized cells and the following substrates and ADP to interrogate specific respiratory activity for each complex: malate and pyruvate for complex I; succinate for II; duroquinol for III; TMPD/ascorbate for IV.

Results: Using the XF24 Analyzer, we characterized the contribution of glycolysis and OXPHOS to cellular energy metabolism in several cancer cell lines, and chose HeLa cells for screening, which averagely uses both metabolic processes. In the course of screening 559 compounds from the chemical library of NPDepo, we found that NPL40330 remarkably decreased OCR value. Moreover, the proteome profiling revealed that NPL40330 upregulated the expressions of glycolytic enzymes, suggesting that it is an OXPHOS inhibitor. Next, we examined which complex does NPL40330 selectively inhibited malate/pyruvate-driven respiration; while no inhibition was observed in the respirations activated by other substrates. These perturbation profiles suggest that NPL40330 is classified under complex I inhibitors. Finally, the enzymatic assay in vitro confirmed that NPL40330 inhibited the oxidation of NADH catalyzed by complex I.

Conclusions: To find new OXPHOS inhibitors, we constructed a screening system based on bioenergetics and proteomic profiling and found that NPL40330 inhibits mitochondrial respiration. Furthermore, the semi-TC assay for OXPHOS established that the target of NPL40330 is mitochondrial complex I.

No conflict of interest

197 (PB-048)  
Broad kinome profiling of drug candidates: single point vs. IC50 – profiling

D. Muller 1, F. Totzke 1, T. Weber 1, M. Pathe 1, C. Schachttele 1, M.H.G. Kubbata 1.  
ProQinase GmbH, Biochemical Drug Development, Freiburg, Germany

Protein kinases constitute one of the largest families of evolutionary related proteins. Approximately 500 human genes code for kinase proteins, corresponding to about 2% of the human genome. Over the past decade, deregulation of numerous protein kinases has been shown to contribute to many human diseases. Today, more than 30 protein kinase inhibitors have been approved for the treatment of cancer emphasizing the significance of kinases as drug targets.

However, most of these kinase inhibitors are ATP-competitive compounds. Due to the structural similarity of kinases especially within the ATP-binding site, many of these kinase inhibitors show limited selectivity. Still, sufficient selectivity within the human kinases is of critical importance e.g. to reduce the risk of adverse side effects during treatment.

Therefore, measuring and improving selectivity of a compound within the kinome already in early drug discovery as well as in the later optimization phase is of pivotal importance in the development of therapeutically relevant kinase inhibitors.

Broad profiling of kinase inhibitors in biochemical activity assays of several hundred kinases is nowadays well established. Typically, kinase profiling is done at one concentration of a test compound and measurement of the relative inhibition of the kinase activity compared to a high and low control. However, due to the limited dynamic range of this approach, and the challenge to select the most appropriate compound concentration, the resulting data are often of limited information with respect to the differences in the potency of compounds against On-target- and Off-target kinases.

We set up an IC50 kinase profiling approach that consists of measuring the effect of a compound on the activity of 320 human protein kinases at six different compound concentrations in logarithmic dilution steps. We will present data showing the effect of compound concentration on the selectivity score of a traditional one concentration profiling setting compared to IC50 profiling. IC50 profiles of kinase inhibitors in different stages of development with a focus on CDKs will be presented. The data demonstrates that an IC50 based profiling allows an improved determination of selectivity of a compound compared to single concentration profiling, and provides significantly improved guidance in the development and optimization of drug candidates.

Conflict of interest: Other Substantive Relationships: Employees of ProQinase GmbH.

Poster Session (Wednesday, 14 November 2018)
198 (PB-049) Poster A high-throughput pharmaceutical screening identifies compounds with specific toxicity against SMARCA4-deficient tumors

A. Wrobel1, A. Mazan1, M. Mikula1, M. Kulecka1, M. Dabrowska2, K. Pijzjak1, A. Groka-Porada1, M. Ogorek1, A. Droz1, B. Duda1, M. Milik1, O. Stosowska1, K. Wiatrowska1, N. Lewandowska1, M. Milik1, J. Ostrowski1, K. Brzozka1, T. Rzymski1, 1Sevitia S.A, R&D, Krakow, Poland; 2Maria Sklodowska-Curie Memorial Cancer Center, Department of Oncological Genetics, Warsaw, Poland; 3Medical Center for Postgraduate Education, Department of Gastroenterology, Warsaw, Poland

Background: SMARCA4 gene which encodes ATP-dependent chromatin remodeler BRG1 is mutated in virtually all cases of small cell carcinoma of the ovary hyperplastic type (SCOHT) and inactivating mutations have been reported in 15% to 35% non-small cell lung cancers (NSCLC) as well as in 5% to 10% of lung adenocarcinomas. High occurrence of inactivating mutations prompted several screening projects focused on synthetic lethality interactions with other proteins. This led to the identification of SMARCA2 (BRM) as an essential gene in SMARCA4 mutated cancers. Several studies have shown that the ATPase domain of SMARCA2 is the therapeutic target for SMARCA4-deficient cancer. We have used phenotypic screening of a library of small molecule compounds with biological activity in order to identify compounds active in synthetic lethality manner in the context of SMARCA4-inactivating mutations.

Material and Methods: We have generated cell lines carrying knockout (KO) or overexpression (OE) of SMARCA4 focus using CRISPR/Cas9 technology. Applicability of the cellular model for the synthetic lethality screening was validated in a series of SMARCA2 gene knockdown experiments. Miniaturization and automation of seeding and growth conditions enabled rapid screening of small molecule probes and tool compounds.

Results: SMARCA2 knockdown leads to rapid and irreversible loss of viability in SMARCA4 KO cells. Gene expression changes induced by the knockdown of SMARCA2 in this model recapitulated many changes previously observed in cells with inactivating mutations of SMARCA4. In the next step, we used SMARCA4 KO cell line to screen the LOPAC library (The Library of Pharmacologically Active Compounds) of drug-like compounds by measuring viability as a surrogate readout of antitumor activity. Compounds active in SMARCA4 KO cells were validated by measuring a dose response curves and compared with the activity on SMARCA4 WT cells. We identified several compounds showing differential cytotoxicity effect in SMARCA4 mutant cell lines.

Conclusions: Phenotypic screening is a feasible alternative to identification of potent leads for diseases driven by virtually undruggable targets. Identified compounds could be promising candidates for further development as drugs targeting tumors with loss-of-function mutations in SMARCA4.

Conflict of interest: Board of Directors: Krzysztof Brzozka.

199 (PB-050) Poster Curcumin treatment alone or in combination of imatinib may circumvent the side effects associated with imatinib therapy in chronic myeloid leukaemia

S.A. Guru1, M.A. Bhat1, M.P. Sumi1, P. Yadav1, N. Gupta1, A. Saxena1, 1Maulana Azad Medical College, University of Delhi, Biochemistry, New Delhi, India

Background: Despite the outstanding survival benefits of imatinib in CML patients, a significant number of patients encounter some major side effects of the imatinib treatment viz., neutropenia and thrombocytopenia bearing significantly. The molecular mechanisms of these imatinib side effects are associated with its ability to inhibit other tyrosine kinases such as PDGFRα. Here, in this study, we tried to explore the role of PDGFRα and its downstream signalling components in the development of imatinib induced thrombocytopenia in imatinib treated CML patients.

Methodology: We studied PDGFRα mRNA expression and its activation (Tyr754α, p-PDGFRα) in K562 cells and CML patients. The effect of deregulated mRNA expression and its dephosphorylation on the expression of PDGFRα’s downstream signalling molecules like PI3K, AKT1 and AKT2 was also studied and compared in thrombocytopenic and nonthrombocytopenic CML patients.

Results: The effect of imatinib, curcumin and imatinib+curcumin exposure on mRNA expression of PDGFRα, PI3K, AKT1 and AKT2 genes was analyzed in vitro in K562 cells. A significant down regulation of expression of these genes in all the three groups of treatment was observed compared to untreated group, except for AKT2 gene expression in case of curcumin treatment. Analysis of effects of imatinib, curcumin and imatinib+curcumin exposure in K562 cell line on PDGFRα protein (Tyr754α – p-PDGFRα)

Phosphorylation/activation showed that these treatments led to significantly reduced levels of PDGFRα protein (Tyr754α – p-PDGFRα) phosphorylation/activation compared to untreated group. However, we observed a higher concentration of curcumin inhibiting PDGFRα than that of imatinib.

Conclusion: Thus it is suggested that curcumin or its derivatives could be used in combination with imatinib to avoid the development of side effects associated with imatinib therapy.

No conflict of interest

200 (PB-051) Poster A Novel TACC3 inhibitor as an anti-cancer agent in breast cancer

O. Akbulut1, D. Lengeri2, B. Caliskan3, E. Banoglu2, 1Department of Molecular Biology and Genetics, Faculty of Science, Bilkent University, Ankara, Turkey; 2Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, Ankara, Turkey

Background: Transforming Acidic Coiled-Coil Containing Protein 3 (TACC3) is an essential mitotic gene which is centered at centrosomes where it ensures chromosomal segregation and microtubule stability. Being amplified or mutated in a broad spectrum of cancers, TACC3 has a potential to be a therapeutic target. There is a wide variety of in vitro and in vivo studies together with patient data about the oncogenic profile of TACC3, but there is currently no inhibitor of TACC3 used in clinics. KHS101, a small molecule inhibitor against TACC3, has been shown to reduce GBM xenografts tumor growth; similarly, SPL-B, another TACC3 inhibitor, was found to suppress tumor growth in ovarian cancer xenografts. However, none of these TACC3 inhibitors is being tested in clinics due to low systemic stability or high IC50 (50% inhibitory concentration) values. Hereby, by combining rational drug design approaches with screening, we aimed to identify a novel TACC3 inhibitor which is more effective in vivo and in vitro systems and can be used as a mitotic blocker in breast cancer (BC).

Material and Methods: A classical design approach was combined with screening to develop new TACC3 inhibitors where some chemical fragments of already available TACC3 inhibitors changed with their isosteric equivalents with the aim of improved potency as well as drug-like properties. Then, compounds were tested in vitro settings where different subtypes of BC cells were screened for the effects on viability. Moreover, these candidate agents were compared with the other TACC3 inhibitors in terms of the general cellular processes that TACC3 involved. Finally, the most promising agents were tested in vivo settings to test their anti-tumorigenic effects.

Results: High level of TACC3 is found to be associated with worse survival in BC patients. Due to the urgent need for a more potent TACC3 inhibitor, we developed and screened dozens of compounds. In vitro assays with BC cells belonging to different subtypes showed the lowest IC50 value for BRP-OZG-264 (our novel TACC3 inhibitor) and the superior effects on the cellular processes such as mitotic arrest, DNA damage and apoptosis when compared to the other available TACC3 inhibitors. Importantly, we found that oral administration of this novel inhibitor significantly suppressed the tumor growth in breast cancer xenografts better than the already reported compounds. Currently, we are testing the pharmacokinetics and toxicity of this inhibitor as well as detailed molecular characterization of TACC3 inhibition-mediated anti-tumor effects.

Conclusions: Overall, our preclinical studies suggest that this new compound (BRP-OZG-264) presumably acting as a TACC3 inhibitor, has a potential to be developed as a novel mitotic blocker for the treatment of breast cancer.

No conflict of interest
Wednesday, 14 November 2018

POSTER SESSION

Drug Synthesis

203 (PB-054)
Poster
Synthesis and evaluation of imidazo[1,2-α]pyridine analogues of the phosphatidylinositol 3-kinase inhibitor ZSTK474
G. Newcastl1, S. Gamage1, J. Spicer1, J. Flanagan1, W. Denny1, P. Shepherd1, W. Lee2. 1University of Auckland, Auckland Cancer Society Research Centre and Maurice Wilkins Centre for Molecular Biodiscovery, Auckland, New Zealand; 2University of Auckland, Department of Molecular Medicine and Pathology, Auckland, New Zealand

Background: The phosphatidylinositol 3-kinase (PI3K) pathway is frequently activated in cancers, although several small molecule inhibitors of the pathway have now been identified. Our earlier work showed that replacement of one of the morpholine groups of the PI3K inhibitor 2-(difluoromethyl)-1-(4,6-di-4-morpholinyl-1,3,5-triazin-2-yl)-1H-benzimidazole (ZSTK474) with sulfonamide-containing substituents, coupled with the addition of a methoxy group at the 4-position of the benzimidazole group, produced an active class of potent PI3Kα and dual PI3Kα/mTOR inhibitors. This work culminated in the clinical trial of our lead candidate PWT33597 in 2011. Here we describe our investigation of analogues of this class of inhibitors where the benzimidazole portion of the molecule is replaced by an imidazo[1,2-α]pyridine group.

Material and Methods: Compounds were prepared using a heteroaryl Heck reaction procedure that involved the palladium-catalysed coupling of 2-(difluoromethyl)imidazo[1,2-α]pyridines with chloro, iodo or trifluoromethanesulfonyl fluoride ( trifluorobromo trifluoromethyl) substituted 1,3,5-triazines or pyrimidines. Compounds prepared included imidazo[1,2-α]pyridine analogues of the known inhibitors ZSTK474, PWT33597, SN32976, MIFS-9922 and AS2541019. The compounds were tested for their inhibitory activity against the p110α, p110β, and p110δ isoforms of PI3K using Homogeneous Time Resolved Fluorescence (HTRF) assays.

Results: The imidazo[1,2-α]pyridine compounds followed their benzimidazole analogues in terms of selectivity for the PI3K isoforms, but in general showed less potency in the HTRF assays.

Conclusions: Using a scaffold-hopping approach we have investigated imidazo[1,2-α]pyridine analogues of the ZSTK474 class of PI3K inhibitors. The new compounds maintain the isoform selectivity of their benzimidazole analogues, but in general show less potency.

No conflict of interest
Hanover, USA; 11Novartis Pharmaceuticals Corporation, Global Drug Development, East Hanover, USA; 12Brigham and Women’s Hospital, Harvard Medical School, Center for Cardiovascular Disease Prevention, Boston, USA; 17Novartis Pharmaceuticals Corporation, Precision Medicine, Oncology, East Hanover, USA; 18Novartis Institutes for BioMedical Research, Translation Medicine, Cardiovascular, Cambridge, Massachusetts, USA; 19Novartis Institutes for BioMedical Research, Precision Medicine, Diagnostics, Cambridge, Massachusetts, USA

Background: Chronic inflammation plays key roles in lung cancer development and progression. In the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS), inhibition of the interleukin-1β (IL-1β) inflammatory pathway by canakinumab, an anti-IL-1β antibody, significantly reduced the lung cancer incidence and mortality. To gain further insights, a molecular characterization of the lung cancer patients from CANTOS was undertaken.

Methods: Serum levels of C-reactive protein (CRP), IL-6, IL-18, IL-1RA, TNF-α, leptin, adiponectin and plasma levels of fibrinogen and plasminogen activator inhibitor-1 from patients diagnosed with lung cancer in the CANTOS trial (n = 116) were determined. Circulating tumor DNA (ctDNA) in plasma samples collected at the baseline visit and/or time closest to lung cancer diagnosis from patients diagnosed with lung cancer (n = 71) were assayed using the Guardant360 platform.

Results: Of both baseline and 3 months, patients in the highest quartile of CRP or IL-6, or both downstream of IL-1β signaling, showed a trend toward shorter time to lung cancer diagnosis compared to patients from the lowest quartile. Of ctDNA mutation markers at baseline did not correlate with time to lung cancer diagnosis. Circulating tumor DNA was detected in 83% of patients, with 73% (48 of 66) of patients having detectable ctDNA at time closest to diagnosis and 66% (44 of 67) having detectable ctDNA at baseline. Patients with ctDNA baseline had a median time to lung cancer diagnosis of 628 days (n = 44), compared to 942 days (n = 23) in patients without baseline ctDNA. A typical driver mutation profile was observed in ctDNA from lung cancer patients in CANTOS. At baseline, p53 mutations were found in 47% of patients with detectable ctDNA, while mutations in NF1 and EGFR were found in 14% and 12% of patients, respectively. Importantly, there was no evidence of treatment-specific enrichment in any mutation following treatment with canakinumab.

Conclusion: Taken together, these results provide further evidence for the importance of IL-1β-mediated inflammation in lung cancer. In addition, since 66% of patients diagnosed with cancer had ctDNA present at entry into the CANTOS was, these results suggest canakinumab’s effect may be mediated by delaying the progression of diverse molecular subtypes of lung cancer.


205 (PB-056) Poster Insights on the regulation of immune checkpoint genes – potential implications for cancer immunotherapy

R. Leibowitz-Ami1, 2, A. Layani1, P. Dobosz1, K. Slabodnik Kaner2, 2, Y. Sidi2, D. Armit2, 2, Tel-Aviv University, Medicine, Tel-Aviv, Israel; 3Sheba Medical Center, Cancer Research Center, Tel-Hashomer, Israel

Background: The interface between T lymphocytes and cancer/antigen presenting cells (the “immunological synapse”) comprises of both co-inhibitory and co-stimulatory molecules ("checkpoints proteins") that modulate the signal transmitted to T lymphocytes, leading to either activation or exhaustion. Monoclonal antibodies against checkpoint proteins have anti-neoplastic activity in many malignancies, but not all cancers and not all patients with a given cancer respond. Our aim is to study the factors regulating the expression of checkpoint genes at the immunological synapse at both the transcriptional and post-transcriptional level, and their relationship with tumor immunogenicity and response to immunotherapy.

Materials and Methods: We analyzed all 33 cancers of the “tumor cancer genome atlas (TCGA)” for the expression of 22 checkpoint mRNAs known from the literature to be expressed at the cancer side of the synapse, focusing on co-expression of checkpoint mRNAs and micro-RNAs in melanoma, a highly immunogenic cancer, and in bladder cancer, a less immunogenic cancer. Co-expression was calculated with Spearman rho correlations, and expression results were corroborated in-vitro. Survival was assessed by Kaplan-Meier curves. Direct targeting of mRNAs by miRNAs was assessed by luciferase reporter assays. Transcription factor binding motifs were analyzed on the putative promoter sequences of checkpoint mRNAs using tools from MEME suite.

Results: In all cancers analyzed, we found networks of co-expressed checkpoint mRNAs that differed in their components and extent. In melanoma, we found that the two co-stimulatory checkpoint mRNAs are directly regulated by mir-16. High expression of mir-16 and low expression of checkpoint mRNAs was associated with worse survival, suggesting that mir-16 affects the immunogenicity of the synapse. In bladder cancer, there was a negative correlation between checkpoint mRNA expression and mir-15b (sharing an identical seed sequence with mir-16). Additionally, in bladder cancer all co-expressed checkpoint mRNAs have, in their putative promoter regions, binding motifs for the transcription factor BACH2, which is also co-expressed with the checkpoint mRNAs and associated with survival.

Conclusions: Our work suggests that there are networks of checkpoint genes that are co-regulated at both the transcriptional and post-transcriptional levels. We hypothesize that immunogenicity of tumors partly stems from the specific components of these co-expression networks. Moreover, our work may help point to novel checkpoints at the cancer side of the synapse that must be co-targeted with the PD-1/PD-1 pathway in order to further potentiate the activation of the immune response against cancer.

No conflict of interest

207 (PB-058) A Canadian Cancer Trials Group phase IIb study of durvalumab plus tremelimumab given concurrently or sequentially in patients with advanced, incurable solid malignancies (IND.226)

M. Smoragiewicz1, P. Bradbury2, P. Ellis1, J. Laskin1, C. Kollmannsberger1, D. Hao1, R. Juergens2, G. Goss3, P. Wheatley-Price1, S. Hotte1, K. Gelmon1, A. Tinker1, P. Brown-Walker1, I. Gaultier1, D. Tu1, X. Song1, L. Seymour1, T. Truquet1, V. Calvert1, F. Trotier1, T. Daniels1, A. Bouchard1, E. Jez1, C. Brastas1, 1Queen’s University, Canadian Cancer Trials Group, Kingston, Canada; 2Princess Margaret Cancer Centre, Division of Medical Oncology, Toronto, Canada; 3Jubavski Cancer Centre, Department of Oncology, Division of Medical Oncology, Hamilton, Canada; 4BC & A Vancouver Cancer Centre, Division of Medical Oncology, Vancouver, Canada; 5Tom Baker Cancer Centre University of Calgary, Department of Oncology, Section of Medical Oncology, Calgary, Canada; 6The Ottawa Hospital Cancer Centre, Division of Medical Oncology, Ottawa, Canada; 7Precision Medicine, Diagnostics, Cambridge, Massachusetts, USA; 8Precision Medicine, Pharmacometrics & DMPK, Gaithersburg, USA

Background: Immune checkpoint inhibitors are established therapies in many advanced cancers. The primary objective of this study was to evaluate the safety and tolerability of durvalumab (Durva), a PD-L1 inhibitor, plus tremelimumab (Treme), a CTLA-4 inhibitor, given either sequentially (SEQ) or concurrently (CON). Sequential administration of multiple agents increases total chair time adding costs overall and inconvenience for patients. The serum pharmacokinetic (PK) profile and safety of SEQ vs CON of Durva and Treme administration was evaluated in a cohort of patients (pts).

Methods: Pts with advanced solid tumours, regardless of tumour PD-L1 status or number of prior therapies, were enrolled and randomized to either SEQ Treme 75 mg IV over 1 hr followed by Durva 1500 mg IV over 1 hr q4Wks, or CON administration over 1 hr. PK samples were drawn on cycle (C) (1pt)/1(13pts) were randomized to SEQ (7pts/24 cycles) and CON (7 pts/23 cycles). There were no infusion related reactions. Drug related adverse events (AEs) were mainly Grade (G) 2 and manageable, and comparable in frequency between SEQ/CON-fatigue (43/43%), rash (43/43%), pruritus (43/ 20%) and nausea (25/29%). Potentially related ³ grade 3 AEs included 2 in SEQ (G3 diarrhea, G3 ALT/AST (complicated by steroid induced psychosis)) and 3 in CON (G3 rash, G3 pneumonia, G3 respiratory failure). Of note, the pts with the G5 AE (C1D30) had G2 dyspnea, lymphangitic carcinomatosis and pleural effusions requiring frequent drainage at baseline which gradually worsened on study, but contribution from Durva/Treme could not be excluded. One pt was not fit for further investigation. One pt in each cohort developed drug related treatment due to toxicity. The PK profiles of Durva and Treme were similar between CON and SEQ (Table 1) and to historical reference data.

Conclusions: Concurrent administration of Durva and Treme over 1 hr is safe in a comparable PK profile to sequential administration.

Conflict of interest: Ownership: Dr. Song owns stock in AstraZeneca. Advisory Board: Dr. Goss has received honoraria from the following organizations: Astrazeneca, Boehringer Ingelheim, Pfizer, BMS and Celgene. Dr. Juergens has received honoraria from the following
Concurrent Sequential Tregs in cancer patients and thereby represent a new immunotherapeutic approach. Pharmacological targeting of MALT1 may therefore reduce the numbers of FoxP3+ Tregs in the tumour, tumour-draining lymph nodes (TDLN) and distal lymph nodes (LN) and only 1 patient had grade 3 or 4 immune-related adverse event (pneumonitis, grade 3). The safety profile of pembrolizumab was very favorable and only 1 patient had grade 3 or 4 immune-related adverse event (pneumonitis, grade 3). The safety profile of pembrolizumab was very favorable and only 1 patient had grade 3 or 4 immune-related adverse event (pneumonitis, grade 3).

**Background:** Adrenocortical carcinoma (ACC) is an orphan endocrine malignancy with poor prognosis and limited response to chemotherapy. Targeting select pathways has not resulted in any meaningful response in ACC and clinical trials using small molecule kinase inhibitors did not result in any treatment breakthroughs. However, in a limited set of ACC tumor samples, programmed death-ligand 1 (PD-L1) expression was seen in about 11% of primary ACC tumors after surgical resection. This finding opens new avenues in the treatment of ACC as several studies have demonstrated correlation between PD-L1 expression and treatment outcomes in patients treated with programmed death-1 (PD-1) signaling pathway inhibitors. So far, there is no published clinical trial about using immunotherapy in ACC.

**Methods:** We are currently enrolling patients in a phase II study of pembrolizumab in patients with rare tumors that includes a pre-specified ACC cohort (http://ClinicalTrials.gov identifier: NCT02721732). Patients received pembrolizumab 200 mg intravenously once every 3 weeks. Response was assessed every 9 weeks using RECIST1.1. The primary objective of the trial was to evaluate efficacy by evaluation of non-progression rate (NPR) at 27 weeks (9 cycles), defined as the percentage of patients who are alive and progression-free at 27 weeks as assessed by RECIST 1.1. The primary end point is progression-free survival at 27 weeks (PFS27wk). Mandatory biopsies are taken at baseline, on cycle 1 day 15–21, and at the time of progression.

**Results:** At the time of analysis, a total of 15 patients were enrolled and treated in the ACC cohort. The patients remained on the study for a median duration of 18 weeks (range, 5–95 weeks). Among the 14 patients evaluable for response, the NPR at 27 weeks was 29% (4/14 patients). Of the 4 patients who were progression-free at 27 weeks, 2 patients had partial response. The first patient had 37% tumor reduction and is still continuing on therapy at 95 weeks and the second patient had a partial tumor reduction of 41%, and duration of response was 42 weeks. The remaining 2 patients had stable disease. Ten patients had evidence of disease progression within 27 weeks of starting the study (median duration 17 weeks; range, 8–27 weeks). Three of the 4 patients with PFS27wk had no evidence of hormonal excess at the time of their initial diagnosis. The safety profile of pembrolizumab was very favorable and only 1 patient had grade 3 or 4 immune-related adverse event (pneumonitis, grade 3).

**Conclusion:** Single agent pembrolizumab may be an effective option for a subset of ACC patients. As less response is seen in patients with cortisol-producing tumors further study is required to confirm this initial observation. Translational data will be presented at this meeting.

no conflict of interest

**Table 1 (abstract 207 PB-058):** The serum pharmacokinetic profile of sequential vs concurrent durvalumab and tremelimumab administration

<table>
<thead>
<tr>
<th>Dosing Regimen</th>
<th>Cmax (μg/mL)</th>
<th>AUCl0–28d (Day*μg/mL)</th>
<th>Cmax (μg/mL)</th>
<th>AUCl0–28d (Day*μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concurrent administration</td>
<td>23.4 (3.90) (n = 7)</td>
<td>3.55 (0.82) (n = 6)</td>
<td>243 (43.2) (n = 6)</td>
<td>450 (89.4) (n = 7)</td>
</tr>
<tr>
<td>Sequential administration</td>
<td>21.1 (5.42) (n = 7)</td>
<td>2.96 (1.29) (n = 6)</td>
<td>233 (102) (n = 6)</td>
<td>377 (94.4) (n = 6)</td>
</tr>
</tbody>
</table>

**Organizations:** Bristol-Myers Squibb, Boehringer Ingelheim, AstraZeneca, Roche Canada, Merck Sharp & Dohme, Lilly, Amgen, EMD Serono, Novartis Canada Pharmaceuticals Inc. She has also acted in a consulting/advisory role for AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Lilly, Merck Sharp & Dohme, Novartis, Pfizer, Amgen, Roche Canada and Takeda. Dr. Tinker has received an honoraria and grant from AstraZeneca.

**Corporate-sponsored Research:** Dr. Seymour and the Canadian Cancer Trials Group received funding from AstraZeneca for this trial. Other Substantive Relationships: Dr. Song is an employee of Medimmune.
210 (PB-061) Poster Efficacy of pembrolizumab in patients with cutaneous squamous cell carcinoma


Background: Cutaneous squamous cell carcinoma (cSCC) is the second most common form of skin cancer in the US. While the majority of patients with localized lesions are cured with surgery, the prognosis is poor in the metastatic setting as the role of chemotherapy in the treatment of metastatic cSCC is unknown. The median survival is less than 2 years and up to 70% of patients die as a consequence of the disease in the metastatic setting. Furthermore, the incidence of cSCC has risen dramatically in the last 3 decades in the US because of lifestyle changes leading to increased voluntary exposure to sunlight. However, the high-mutational burden, the presence of tumor-infiltrating lymphocytes, and evidence of direct immunosuppressive effects of UV radiation suggest that patients with cSCC may benefit from treatment with immune checkpoint inhibitors.

Methods: This is a phase II study of pembrolizumab in patients with rare tumors including a pre-specified cohort for patients with cSCC (http://ClinicalTrials.gov identifier, NCT02721732). Patients received pembrolizumab 200 mg intravenously once every three weeks. The response was assessed every nine weeks using RECIST1.1. The primary end point was progression-free survival at 27 weeks (PFS27wk), defined as the percentage of patients who are alive and progression-free at 27 weeks (9 cycles) as assessed by RECIST 1.1. Mandatory biopsies are taken at baseline, on cycle 1d and 5–15 days, and at the time of progression.

Results: At the time of analysis, a total of 18 patients were enrolled and treated in the cSCC cohort. The patients remained on the study for a median duration of 16 weeks (range, 4–93 weeks). Among the patients evaluable for response, the NBR at 27 weeks was 33% (5/15 patients). Three of the 5 patients had complete resolution of target lesions and are still continuing on treatment. The other 2 patients had 80% and 65% reduction in the size of target lesions from the baseline. The median duration of response was 67 weeks (range, 55–93 weeks). The remaining 10 patients had evidence of progressive disease within 27 weeks of starting the study. The safety profile of pembrolizumab was very favorable and only 1 patient had grade 3 or 4 immune-related adverse event (arthralgia, grade 3).

Conclusion: Single agent pembrolizumab may be an effective therapeutic option for patient with advanced cSCC. Translational data will be presented at this meeting.

No conflict of interest

211 (PB-062) Poster Immune-related pneumonitis in non-small cell lung cancer (NSCLC) patients treated with anti-PD(L)1: the impact of previous thoracic radiotherapy

A. Botticella1, T. Ibrahim2, L. Mezquita2, L. Hendriks2, J. Le Pavec2, E. Deutsch1, B. Bessé2, C. Le Pechoux1,2,1Institut Gustave Roussy, Department of Radiation Oncology, Villejuif, France;2Institut Gustave Roussy, Department of Medical Oncology, Villejuif, France;3Centre Chirurgical Marie Lannelongue, Laboratoire de Chirurgie Experimentale-UPRES EA-2705, Le Plessis Robinson, France;4Val d’Hebron University Hospital, Department of Medical Oncology, Barcelona, France;5Institut Gustave Roussy, Département D’Innovation Thérapeutique et des Essais Précoces DITEP, Villejuif, France

Background: Pneumonitis is a life-threatening adverse event of anti-PD(L)1, occurring in 1–5% of patients enrolled in clinical trials. The risk factors for the occurrence of pneumonitis are unknown. Many NSCLC patients treated with anti-PD(L)1 for metastatic disease had previous thoracic radiotherapy (RT), but little is known about the interactions between anti-PD(L)1 and thoracic RT. The main aim of this study is to assess the correlation between the onset of immune-related pneumonitis and previous thoracic RT.

Materials and Methods: Between December 2012 and November 2017, 318 consecutive non-small cell lung cancer (NSCLC) patients received ICI in our institution and their charts were retrospectively analyzed. Primary endpoint was to determine whether previous radiotherapy had an effect on the occurrence of immune-related pneumonitis. Pneumonitis was assessed by Common Terminology Criteria for Adverse Events version 4.0.

Results: Median age at ICI start was 63 years. 205 patients (64.5%) were males, 103 (32.4%) smokers and 250 (78.6%) with PS ≤ 1; 206 (64.8%) had adenocarcinoma and 76 (23.9%) squamous; 79 (24.8%) were KRAS mutated, 18 (5.5%) EGFR mutated and 5 (1.6%) were positive. PDL1 was ≥1% by immunohistochemistry in 86 (27%), negative in 37 (11.6%) and unknown in 196 (61.3%) patients. ICI treatment was median 3 line (range: 1–12), 89,4% monotherapy PD-(L)1 inhibition. Median follow-up was 32.8 months [95%CI: 5–190].

72 patients (22.6%) received a thoracic RT: 62 out of the 72 RT patients (87.5%) were irradiated with a curative intent. 53 patients (73.6% of the RT patients) received thoracic 3D-conformal RT or intensity modulated RT (normo- or mildly hypofractionated), whereas 9 received SBRT.

The occurrence of all grades was higher in previously irradiated patients compared to non-irradiated patients (16.7% versus 2.4%, p = 0.001).

Conclusions: Prior thoracic RT with curative-intent is associated with a higher risk of immune-related pneumonitis in NSCLC patients treated with anti-PD(L)1.

No conflict of interest

213 (PB-064) Poster Characterization of tumor-infiltrating immune cells and the efficacy of pembrolizumab in preclinical models of primary and bone metastatic triple-negative breast cancer

T. Kahkonen1, M. Suominen1, J. Maki-Jouppila1, J. M. Hallinen1, A. Tanaka2, M. Seiler2, J. Bernoul1, Pharmatest Services, Oncology, Turku, Finland;2Taconic Biosciences, Taconic, Rensselaer, USA

Background: High number of tumor-infiltrating lymphocytes (TILs) is associated with improved survival, and targeting of programmed cell death protein 1 (PD-1) has shown promising results in treatment of triple-negative breast cancer (TNBC). TNBC patients typically have a high incidence of bone metastasis. As immune regulation in bone is different compared to other organs, it is essential to understand immune cell infiltration also to metastatic location. The aim of this study was to assess the efficacy of anti-PD-1 therapy (pembrolizumab, Keytruda®) in the growth of primary and bone metastatic TNBC in preclinical models, and to characterize immune cell infiltration into the tumors to support immune-oncology drug discovery.

Materials and Methods: MDA-MB-231(SA)-lac human TNBC cells were inoculated orthotopically into the mammary fat pad (primary tumor model) or tibia bone marrow (bone metastasis model) of female huNOG mice (Taconic Biosciences). Treatments with pembrolizumab or IgG4 isotype control (5 mg/kg, i.p., QSD, n = 8) were started at day 3. Tumor growth was monitored by caliper measurements or X-ray imaging of tumor-induced bone lesions for 24–21 days in the primary tumor and bone metastasis models, respectively. Immunohistochemical stainings were performed at endpoint for human CD4, CD8, CD163, Granzyme B, PD-1 and PD-L1. TILs and tumor-associated macrophages (TAMs) were assessed by 4-scale immunoscorening system and PD-L1 by tumor proportion score (TPS).

Results: Pembrolizumab decreased tumor growth in the primary tumor model but had no effects in the bone metastasis model. About 38% of the mice responded to treatment. PD-1 expression was low in the control-treated orthotopic tumors and absent in the pembrolizumab treated mice due to antibody blocking of the epitope. PD-L1 expression was moderate in both tumor types (TPS 1–49%). Moderate number of CD4+ and CD8+ TILs (scoring 2–3) was observed and granzyme B expression correlated with CD8 positive TILs. In these tumors, corresponding number of CD4+ and granzyme B+ TILs were observed. However, in the bone microenvironment less CD8+ TILs (scoring 0–1) were observed and PD-1 expression was variable. Generally, intratumoral and peritumoral variation of expression and localization of TILs and TAMs was observed, especially in the bone tumors.

Conclusions: Orthotopic tumors responded to pembrolizumab treatment but bone metastatic growth was not inhibited. Bone marrow has a unique immune cell microenvironment that is different from primary tumor. The lack of efficacy of pembrolizumab could be explained by low number of CD8+ and PD-1 positive cells in tumor growing in bone. These results highlight the importance of validation of immune cell infiltration to metastatic location, and
using preclinical metastasis models to have predictive data before entering clinical trials.

No conflict of interest

214 (PB-065) Poster Development of AO-176, a next generation humanized anti-CD47 antibody with novel anti-cancer properties and negligible binding to red blood cells

M.N. Bouchlaka1, R. Puro1, B. Capocchia1, M. Donio1, R. Hiebsch1, A.J. Carter1, K.S. Crowley1, W.C. Wilson2, P. Chakraborty1, P.T. Manning1, R. Karr1, V. Sund2, D.S. Pereira1, 1Arch Oncology, Research, St. Louis, USA; 2Arch Oncology, Translational Research, San Francisco, USA

To date, inhibitors of immune checkpoints have shown significant advances as cancer treatments and have focused mainly on enhancing adaptive immune responses. CD47, a cell surface glycoprotein, is an innate immune checkpoint receptor broadly expressed on normal tissues and over-expressed on several tumors. Binding of CD47 to signal regulatory protein alpha (SIRPα) on macrophages and dendritic cells triggers a “don’t eat me” signal that inhibits phagocytosis. Several hematologic and solid tumors escape innate immune surveillance by overexpression of surface CD47 preventing engulfment and clearance of tumor cells by phagocytes. Blocking the interaction between SIRPα and CD47 has been shown to promote phagocytosis leading to reduction in tumor burden in numerous xenograft and syngeneic animal models.

We have developed a next generation humanized anti-CD47 antibody, AO-176, that not only blocks the CD47/SIRPα interaction and induces tumor cell phagocytosis, but also exhibits several unique functional properties. The first property is the ability of AO-176 to induce direct tumor cytotoxic cell death in hematologic and solid human tumor cell lines by a cell autonomous mechanism (not ADCC). Secondly, AO-176 exhibits preferential binding to tumor versus normal cells, including red blood cells (RBCs). T cells, endothelial cells, skeletal muscle cells and epithelial cells, AO-176 also does not affect the function of any of these primary cells when assayed ex vivo. The negligible binding of AO-176 to RBCs versus Jurkat tumor cells (∼ 30–50 fold lower Bmax and ∼ 16–88 fold lower EC50) is particularly profound and different from other reported anti-CD47 antibodies. AO-176 also does not induce hemagglutination of RBCs. These properties are expected not only to decrease the antigen sink, but also to minimize on-target clinical adverse effects observed following treatment with other reported RBC-binding anti-CD47 antibodies. When tested in cynomolgus monkeys, AO-176 was well tolerated with no adverse effects. A third novel property of AO-176 is its enhanced binding to tumor cells at acidic pH. AO-176 binds to human tumor cell lines ∼ 3-30-fold higher at an acidic pH of 6.5 compared to a physiological pH. Because the microenvironment of solid tumors has an acidic pH of 6.4-7.2, this enhanced binding of AO-176 at low pH has the potential added advantage of tumor-specific targeting. Lastly, we show that AO-176 demonstrates dose-dependent anti-tumor activity in tumor xenograft models. Taken together, the unique properties and activity of our next generation anti-CD47 antibody, AO-176, bodes well as this candidate progresses to clinical development.

Conflict of interest: Other Substantive Relationships: Employees of Arch Oncology.

215 (PB-066) Poster Prognostic and predictive biomarkers of efficacy for Durvalumab in patients with metastatic or advanced head and neck cancer

A. Bawer1, J. Bartelink1, T. Griep2, T. Zheng2, T. Salehi1, L. Shi1, J. Buenconsejo1, C. Morehouse1, B. Higgs1, N. Standifer1, J. Engler1, S.C. Chang1, L. Roskos1, R. Narwal1, 1Immune, Clinical Pharmacology Department, Cambridge, United Kingdom; 2Immune, Clinical Pharmacology Department, San Francisco, USA; 3Immune, Clinical Pharmacology Department, Gaithersburg, USA; 4AstraZeneca, Green, 5Medicines Development, Gaithersburg, USA; 6Immune, Clinical Statistics, Gaithersburg, USA; 7Immune, Translational Medicines, Gaithersburg, USA; 8Immune, Development, Gaithersburg, USA; 9Immune, Immuno Oncology Franchise, Gaithersburg, USA

Background: Durvalumab (Imfinzi™), an anti-PDL1, fully human IgG1 mAb, is currently being investigated for the treatment of metastatic or recurrent head and neck squamous cell carcinoma (HNSSC) and in other indications as monotherapy and immunomodulating agent. We aimed to characterize the relationship between tumor kinetics and survival, and to evaluate prognostic and predictive biomarkers for efficacy outcomes in HNSSC patients.

Methods: Pooled data from 232 HNSSC patients treated with single-agent durvalumab at 10 mg/kg Q2W IV from 3 clinical studies (CD-ON-MEDI4736-1108 [NCT01693562], CONDOR [NCT02319044], and HAWK [NCT02207530]) were used to develop population tumor kinetic, dropout and survival models based on nonlinear mixed-effects modeling approach, including a multivariate covariate analysis.

Results: Tumor kinetic modeling revealed that tumor shrinkage was associated with 2 predictive biomarkers that were statistically significant and deemed clinically relevant: low tumor burden and high PD-L1 expression in the tumor. Similarly, survival benefit (after accounting for individual tumor kinetic profiles) was associated with (p < 0.01) low neutrophil/lymphocyte ratio (NLR), high albumin levels (both known prognostic biomarkers) and high PD-L1 scores on either immune cells or tumor cells.

Conclusions: A tumor kinetic model coupled with dropout and survival models adequately described clinical outcomes in HNSSC patients treated with durvalumab and enabled identification of disease specific risk-factors and biomarkers potentially predictive of immune therapy. The modeling framework can be a useful tool to guide patient selection/enrichment strategies for immune-oncology therapies.

No conflict of interest

216 (PB-067) Poster Converting PD-L1-induced T-lymphocyte inhibition into CD137-mediated costimulation via PD-L1xCD137 bispecific DART™ molecules

A. Berezhnoy1, L. Huang2, K. Shah3, D. Liu4, J. Dichiaro5, C.Y. Lam5, L. Liu6, R. La Motte-Mohs7, J. Li7, V. Ciccarone7, J. Tamura7, R. Alderson7, G. Diedrich8, E. Borvini9, S. Johnson9, P. Moore10, 1MacroGenics INC, Cell Biology and Immunology, Rockville, USA; 2MacroGenics INC, Protein Engineering, Rockville, USA; 3MacroGenics INC, Protein Engineering, Brisbane, USA; 4MacroGenics INC, Cell Biology and Immunology, Brisbane, USA; 5Macrogenics INC, Research, Rockville, USA

Background: PD-1/PD-L1 axis blockade is a clinically proven cancer therapeutic strategy, but can be insufficient to fully activate tumor-specific T cells. CD137 co-stimulation synergistically increases the activity of PD-1 blockade in mouse tumor models. Clinical application of such an approach, however, may be limited by toxicity associated with the systemic administration of CD137 agonists. Here we demonstrate that bispecific DART molecules comprising anti-PD-L1 and CD137 mAb specificities provide PD-1 axis blockade concomitantly with PD-L1-dependent CD137 co-stimulation.

Materials and Methods: PD-L1xCD137 bispecific DART molecules were constructed based on PD-L1 blocking mAbs and CD137-engaging mAbs and evaluated for binding to their respective antigens and in reporter assays, as well as in CD3 or SEB-driven T-cell activation and MLR assays. Anti-tumor redirected T-cell activity was evaluated in combination with anti-CD3 based DART molecules. RNAseq was performed to characterize T-cell gene expression.

Results: PD-L1xCD137 DART molecules bind and block PD-L1, reversing PD-1-mediated T-cell inhibition equipotently to the effect of approved PD-L1 benchmark mAbs. They also bind CD137, but, without secondary cross-linking or clustering induced by PD-L1+ cells, fail to induce CD137 signaling. In the presence of PD-L1-expressing cells, however, PD-L1xCD137 DART molecules drive CD137 activation and immune cell co-stimulation. Robust T-cell activation and cytokine secretion was induced by PD-L1xCD137 DART proteins, with significantly greater activity than that observed with the combination of PD-L1 blocking and CD137 agonist mAbs. Notably, when combined with tumor targeted anti-CD3 based DART molecules, PD-L1xCD137 bispecific molecules enhance activation of effector cells in the presence of tumor cells and increase tumor growth inhibition. Transcriptome studies revealed a gene expression profile unique induced by the PD-L1xCD137 bispecific protein but not by the mAb combination.

Conclusions: These data show that PD-L1xCD137 bispecific DART molecules can switch on CD137 co-stimulation in a PD-L1-dependent fashion. While tumor adaptive resistance via PD-L1 induction promotes immune escape, PD-L1xCD137 DART molecules can exploit the checkpoint ligand up-regulation and further amplify checkpoint blockade by contributing a co-stimulatory signal. Further investigations as a potential therapeutic approach to overcome limitations of existing PD-1/PD-L1-targeting strategies is warranted.

Conflict of interest: Other Substantive Relationships: Full-time employee of MacroGenics, INC.
Distinct immunological properties of the two histological subtypes of adenocarcinoma of the ampulla of vater

M.H. Kim, M. Jang, H. Kim, W.J. Lee, C.M. Kang, H.J. Choi. Yonsei University College of Medicine, Division of Medical Oncology, Department of Internal Medicine, Seoul, Korea; Yonsei University College of Medicine, Department of Pathology, Seoul, Korea; Yonsei University College of Medicine, Department of Hepatobiliary and Pancreatic Surgery, Seoul, Korea

Background: Adenocarcinoma of the ampulla of vater (AOV) is classified into intestinal type (IT) and pancreatic type (PB), but the immunological properties of these subtypes remain to be characterized. Here, we evaluated the clinical implications of PD-L1 expression and CD8+ T lymphocyte density in adenocarcinoma of the AOV tumors, and their potential association with the clinical implications of PD-L1 expression and CD8+ T lymphocyte density into intestinal type (IT) and pancreatobiliary type (PB), but the immunological immune checkpoint pathway in the tumor microenvironment and potentiated immunological memory.

Materials and Method: We analyzed 123 adenocarcinoma of the AOV patients who underwent surgical resection, and tumors were classified into IT type and PB type. Tumor or inflammatory cell PD-L1 expression, CD8+ T lymphocyte density in the cancer cell nest (intratumoral) or in adjacent stroma, and YAP localization and intensity were analyzed by immunohistochemical staining.

Results: PB type tumors showed higher tumoral PD-L1 expression than IT type tumors, and tumoral PD-L1 expression was associated with shorter disease-free survival (DFS; hazard ratio [HR], 1.77; p = 0.045) and overall survival (OS; HR, 1.99; p = 0.030). Intratumoral CD8+ T lymphocyte density was higher in IT than in PB type and associated with favorable DFS (HR, 0.47; p = 0.022). The nuclear staining pattern of YAP in tumor cells, compared to non-nuclear staining patterns, was more frequently associated with PB type and increased tumoral PD-L1 expression. Nuclear YAP staining was a significant prognostic factor for OS (HR, 2.21; p = 0.022).

Conclusions: These results show that the two subtypes of adenocarcinoma of the AOV exhibit significant differences in tumoral PD-L1 expression and intratumoral CD8+ T lymphocyte density, which might contribute to their distinct clinical features.

No conflict of interest

A novel immunomodulatory strategy of targeting glyco-immune checkpoints with EAGLE technology

L. Peng. Pfizer Pharmaceuticals, Biotherapeutics Discovery, Waltham, USA

Cancer therapy has been revolutionized by inhibiting immune-checkpoints to harness the power of the immune system in fighting cancer. However, the majority of patients are resistant to the current immuno-oncology drugs. There is a strong need to identify novel mechanisms of cancer immune evasion and explore novel therapeutic modalities. Glyco-immune checkpoints axis plays a critical role in modulating innate and adaptive immune responses against cancer. However, this pathway is underexplored for therapeutic interventions of cancer, because the complexity and heterogeneity of glycan-ligands on tumor cells pose grand challenges for conventional therapeutic modalities. Here we described a multi-functional antibody-like novel therapeutic modality, named EAGLE (Enzyme-Antibody Glyco-Ligand Editing), which can overcome the heterogeneity and complexity problems and specifically edit tumor-specific glycans. We evaluated the efficacy of EAGLE molecule and studied its mechanism of actions in a breast cancer EMT6 syngeneic tumor model. Systematic delivery of the EAGLE molecule decreased the amount of immunosuppressive glycan-ligands on tumor cell surfaces, and increased T-cell infiltration and activation in the syngeneic tumor models. EAGLE treatment led to 50% complete regressions of established tumors as a monotherapy and 100% cures in combination with an anti-PD1 mAb. Furthermore, cured mice from EAGLE treatment completely rejected the rechallenge of tumor cells, suggesting that EAGLE induced anti-tumor immunological memory.

In summary, the novel therapeutic modality, EAGLE, blocked the glyco-immune checkpoint pathway in the tumor microenvironment and potentiates innate and adaptive antitumor immunity, offering a novel immunomodulatory strategy to treat cancer.

No conflict of interest

Targeting myeloid-associated immunosuppression with a novel immunoglobulin-like transcript 4 (ILT4)-specific monoclonal antibody


Background: Immunotherapy has dramatically improved efficacy in human malignancies, especially melanoma and lung cancer. However, current approved immunotherapies targeting T lymphocytes are not effective in many cancers, as tumors can employ additional mechanisms to limit host immune responses and escape immunosurveillance. Myeloid-derived suppressor cells are an important factor mediating immunosuppression in the tumor microenvironment and are considered a prime target for therapeutic intervention. ILT4/ILT2 is an inhibitory member of the leukocyte immunoglobulin-like receptor (LIR) family that is expressed primarily by myeloid cells, including monocytes, granulocytes, and dendritic cells. ILT4 interacts with major histocompatibility (MHC) class I complexes and angiopoietin-like (ANGPTL) ligands, and its signaling is associated with the induction of a tolerogenic phenotype in antigen presenting cells. Although ILT4 has been proposed as a putative target for cancer immunotherapy, the utility of targeting this pathway for anti-tumor efficacy has not been previously demonstrated in vivo.

Material and Methods: Here we describe the discovery and characterization of antibody clone 1E1 – a fully human monoclonal antibody of the IgG4 subclass that specifically binds ILT4, primary human myeloid cells, and angiopoietin-like (ANGPTL) ligands, and its signaling is associated with the induction of a tolerogenic phenotype in antigen presenting cells. Although ILT4 has been proposed as a putative target for cancer immunotherapy, the utility of targeting this pathway for anti-tumor efficacy has not been previously demonstrated in vivo.

Results: We show that clone 1E1 is specific to ILT4 and does not bind to other LIRL-family receptors. Clone 1E1 blocks ILT4 binding to Human Leukocyte Antigen (HLA)-G, other MHC class I molecules (HLA-A-, B, and F), as well as ANGPTL ligands. Moreover, clone 1E1 reverses ILT4-mediated suppression of signal transduction. Blocking ILT4 dependently enhances proinflammatory cytokine expression of GM-CSF and TNFα in LPS-stimulated human PBMC cultures. Utilizing a novel human immune system SK-MEL-5 tumor mouse model, we demonstrate the...
presence of ILT4+ myeloid cells both in the periphery and in the tumor infiltrate. Treatment with clone 1E1 results in approximately 50% reduction in tumor growth, alterations in the both splenic and tumor myeloid subset distributions, as well as changes in myeloid-centric chemokine and cytokine profiles.

Conclusions: These data support a role for ILT4 antagonism as a new strategy for enhancing anti-tumor immune responses by targeting a myeloid-associated immunomodulatory pathway.

Conflict of interest: Corporate-sponsored Research: All authors are current or former employees of Merck & Co (also known as MSD in Europe/EU). This body of work was financially supported by Merck & Co (also known as MSD in Europe/EU).

221 (PB-072) Poster
Identification of novel inhibitors of Arginase-1 for cancer immunotherapy by high-throughput screening
Y. Grobben 1, J.C.M. Utdehaag 1, N. Willemsen-Seegers 1, W.W.A. Tabak 1, J. van Groningen 1, J. Friesen 2, H. Rutjes 3, J. de Man 1, R.C. Buissman 4, G.J.R. Zaman 5. Netherlands Translational Research Center B.V., Biology, Oss, Netherlands; 2Pivot Park Screening Centre, Oss, Netherlands

Background: Arginase-1 (Arg-1) is an important drug target for cancer immunotherapy. Expression of Arg-1 by tumor-infiltrating myeloid-derived suppressor cells induces local L-arginine depletion, which results in reduced T cell and natural killer cell proliferation. Despite increased insight in the role of Arg-1 in tumor immune suppression, there is still a lack of drug-like Arg-1 inhibitors. To identify novel inhibitors, a new high-throughput screening (HTS) assay was developed (Arginase Gold®) and used to screen a 93,000-com-pound diversity library.

Materials and Methods: ABH and (3R,4S)-3-Amino-1-((S)-2-amino-propanoyl)-4-(3-boronoephosphoryl)morpholine-3-carboxylic acid (AABC) were synthesized and shown to be active in vitro. Arginase Gold is a homogeneous mix-and-match assay based on a novel and proprietary chemical probe. In contrast to previously reported arginase assays, this assay requires only two addition steps, short incubation times and mild reaction conditions. Moreover, it is a high-throughput fluorescence assay and allows for kinetic measurements. Binding of compounds to Arg-1 was further studied by thermal shift assay, surface plasmon resonance and protein crystallography.

Results: The Arginase Gold assay technology was validated by testing a set of published Arg-1 inhibitors (i.e., ABH, nor-NOHA and AABC), resulting in potencies consistent with those in the classical urea detection assay. Nevertheless, we found that inhibitor potencies varied considerably depending on the exact conditions in both assays, demonstrating the importance of Arg-1 stability and a minimal incubation time with L-arginine. Kinetic experiments performed with the screening assay showed that ABH does not stably inhibit Arg-1 over time, which explains why IC50 values of ABH vary significantly in scientific literature. Furthermore, we observed that AABC has slow association kinetics at pH 9.5, but not at pH 7.4, which was confirmed with surface plasmon resonance. The pH-dependent binding character of AABC could be related to the boronic acid-borate equilibrium.

Conclusions: To demonstrate the use of the assay for HTS, a library of 93,000 compounds was screened. Of the initial 621 hits, 297 were confirmed at a character of AABC could be related to the boronic acid-borate equilibrium.

222 (PB-073) Poster
Bispecific CD40/FAP DARPin® molecule for tumor-restricted immune activation
N. Rigamonti 1, A. Schlegel 2, S. Barzin 1, J. Schwestermann 1, J. Krieg 3, S. Mangold 4, M. Paladino 5, S. Bruckmaier 6, V. Calabro 7, V. Levitsky 8, S. Pfle 9, M. Stumpf 10, C. Metz 11. Molecular Partners, Biology, Schlieren, Switzerland; 2Molecular Partners, Lead Generation, Schlieren, Switzerland

CD40 is a member of the tumor necrosis factor receptor (TNFR) superfAMILY which can activate both innate and adaptive tumor immunity, making it an attractive target for cancer immunotherapy. Systemic administration of agonistic CD40 antibodies (Ab) has shown signs of activity in cancer patients, where the results hint at the clinical efficacy. New therapeutic approaches to achieve tumor-restricted activation through intratumoral administration of CD40 Ab are now in early clinical development, aiming to reduce systemic toxicity and increase efficacy. However, there are clear limitations, such as tumour accessibility. Here, we present a novel approach based on systemic administration of a bispecific DARPin® molecule targeting human CD40 and a tumor antigen (TA), enabling CD40 pathway activation exclusively in the presence of TA-expressing cells. Using fibroblast activation protein (FAP) alpha, a glycoprotein abundantly expressed in many solid tumors, as a TA, we generated a bispecific CD40/ FAP DARPin® molecule able to functionally activate the human CD40 receptor in (1) a reporter cell assay, (2) primary lymphocytes and (3) primary monocytes. The CD40 activation only took place in the presence of FAP-expressing cells, confirming a mechanism of action strictly dependent on FAP-mediated cross-linking. In order to properly address the in vivo activity, a surrogate mouse-specific CD40/FAP DARPin® molecule was also generated and tested in different in vitro assays showing a FAP-dependent activation and similar results of the human counterpart. Experiments are ongoing to assess the efficacy and mechanism of action of a tumor-restricted CD40 agonistic DARPin® molecule. In conclusion, we have generated bispecific agonist CD40/FAP DARPin® molecules able to activate the CD40 pathway in cellular assays with a targeting-dependent mechanism of action, supporting the hypothesis that these DARPin® molecules could lead to a tumor-localized immune activation in vivo. Data are ongoing in vivo cocultures in mouse tumor models to test this hypothesis will be also shown at the meeting.

No conflict of interest

223 (PB-074) Poster
MAPK pathway activity plays a key role in programmed death ligand 1 expression of EGFR wild-type non-small cell lung adenocarcinoma cells
T. Stuvel 1, A. Kol 2, E. De Vries 3, M. de Bruyn 4, R. Fehrman 5, A. Terwisscha van Scheltinga 6, S. de Jong 7. Cancer Research Center Groningen, University of Groningen, Medicine Oncology, Groningen, Netherlands; 2Pivot Park Screening Centre, Oss, Netherlands; 3Cancer Research Center Groningen, University of Groningen, Obstetrics and Gynaecology, Groningen, Netherlands; 4University of Groningen, University Medical Center Groningen, Clinical Pharmacy and Pharmacology, Groningen, Netherlands

Background: Immune checkpoint inhibitors targeting the programmed cell death protein 1 (PD-1) programmed death-ligand 1 (PD-L1) interaction have improved survival of patients with epidermal growth factor receptor (EGFR) wild-type non-small cell lung cancer (NSCLC). Still, many patients do not respond to these inhibitors. The aim of the present study is to improve understanding of PD-L1 regulation in EGFR wild-type NSCLC, which may provide a rationale for combination therapy of immune checkpoint inhibitors with other (targeted) agents.

Materials and Methods:
1. Publicly available RNA-seq data of EGFR wild-type lung adenocarcinoma (n = 197) and squamous cell lung carcinoma (n = 172) from The Cancer Genome Atlas (TCGA) was used to investigate the correlation between PD-L1 gene expression and RAS, MEK, and PI3K pathway activity scores, or interferon gamma (IFNγ) signaling related gene expression.
2. Next, the influence of EGFR, an activator of the wild-type EGFR signaling pathway, and IFNγ on PD-L1 mRNA (by qPCR), total protein (by Western blotting) and membrane expression (by flow cytometry) was determined in a panel of 5 lung adenocarcinoma cell lines after treatment for up to 72 hours. Small molecule inhibitors and siRNAs of the JAK/STAT, MAPK and PI3K pathway were used to modulate activity of their respective targets. Results were validated using in vivo cocultures of tumor cells with peripheral blood mononuclear cells (PBMCs).

Results: Analysis of TCGA RNA-seq data revealed that gene expression of transcriptional targets of IFNγ signaling correlated with PD-L1 gene expression in EGFR wild-type lung adenocarcinoma and squamous cell lung carcinoma, but inferred MAPK activation correlated with PD-L1 gene expression only in lung adenocarcinoma. In our tumor cell line panel, stimulation with EGFR or IFNγ increased PD-L1 mRNA levels and membrane expression levels. These were further enhanced by combining EGFR and IFNγ, raising PD-L1 mRNA levels 66–88 fold and membrane expression levels 3.5–31 fold compared to untreated controls across our cell line panel. Similarly, coculture with PBMCs increased tumor cell PD-L1 membrane expression. Inhibition of the MAPK pathway, using EGFR-inhibitors cetuximab and erlotinib or mitogen-activated protein kinase kinase...
Novel, small molecule PD-L1 inhibitors for cancer immunotherapy

D. Sivanandan1, S. Garapaty1, G.P. Seerapur1, R. Das1, R. Kar1, A.K. Singh1, V. Ashok Kumar1, C. Venkateshappa2, R. Putta2, M. Pendyala3, T. JadHAV2, A.E. Wan1, S. Ghana1, S. Birudukota1, H. Gogoi1, H. Nadig1, R. GangaiNath1, Z. Mohd1, J. Athisayanathan1, S. Rajagopal3, 1Jubilant Biosys Ltd, Biology, Bangalore, India; 2Jubilant Biosys Ltd, Medical Chemistry, Bangalore, India; 3Jubilant Chemsys Ltd, Chemistry, Noida, India; 4Jubilant Biosys Ltd, ADME/Tox, Bangalore, India

Background: The PD-1/PD-L1 molecular pathway is one of the primary mechanisms of immune evasion deployed by cancer cells. Activation of PD-1/PD-L1 pathway induces apoptosis of activated T-cells, inhibits their proliferation, facilitates T-cell anergy and exhaustion and enhances the function of regulatory T-cells. Therefore, blocking this pathway restores the proliferation and cytotoxicity of CTls, inhibits the function of Tregs and results in decreased T-cell apoptosis. Few mAbs targeting PD-1/PD-L1 have been approved for a number of malignancies. But, these approved therapies require bolus intravenous injections, are administered in high dose and have a long half-life, which could contribute to the well-documented drug-related adverse effects. Small molecule inhibitors, can therefore, provide increased oral bioavailability, increased bio-efficiency and shortened half life activity for a more controllable treatment, particularly in the case of auto-immune or other adverse effects.

Methods: Rational design approaches were used to design novel PD-1/PD-L1 pathway modulators; potency of these inhibitors was assessed in an in-vitro TR-FRET assay. Checkpoint signaling assays as well as ex-vivo co-culture assays were used to assess the ability of the compounds to restore T-cell proliferation and function. Syngeneic cancer models were used to assess tumor growth inhibition in vivo.

Results: Three novel series of potent PD-L1 inhibitors are being developed for the treatment of cancer. One of the leads, JBI-426 exhibited an IC50 of <0.1 μM on PD1:PD-L1 binding and no cytotoxicity against cancer cell or immune cells. JBI-426 showed good in vitro ADME properties in terms of aqueous solubility and metabolic stability and excellent oral bioavailability in mouse pharmacokinetics. JBI-426 restored IFN-g level that was depleted due to co-culture of cancer cells and immune cells. Oral administration of JBI-426 at 50 mg/kg resulted in a strong tumor growth inhibition, in RENCA and CT26 syngeneic models and was well tolerated. This tumor growth inhibition was associated with an increase in CD4+ as well as CD8+ cytotoxic T lymphocytes in this tumor. Similar effect was observed with leads from other two chemical series as well.

Conclusions: Since the therapeutic benefit of PD1/PD-L1 pathway has already been well established with mAbs, orally bioavailable small molecule inhibitors certainly provide a significant therapeutic benefit in treating cancer and in enhancing the quality of life of these patients.

No conflict of interest

222 (PB-077) Poster Generation of human immune checkpoint double knock-in mice (diKI HuGEMM) for preclinical efficacy assessment of combinatorial therapeutic antibodies

L. Zheng1, D. He1, R. Sun2, A. An1, J. Fei2, H. Li1, D. Ouyang1, 1Crown Bioscience Inc., Translational Oncology, San Diego, USA; 2Shanghai Model Organisms Inc., Research & Development, Shanghai, China

Introduction: Immune checkpoint inhibitors (ICls), i.e. PD1, PDL1 and CTLA4 therapeutic antibodies, have led to long-term survival in many late stage solid tumor patients. Despite their revolutionary clinical impact, the overall response rate is still low. One major roadblock is that compensatory immune inhibitory pathways are turned on to protect tumor cells from being attacked by T cells. Various combinatorial ICI treatments have now been investigated in clinical trials to tackle this challenge. We have previously reported the development of immune checkpoint target humanized mice (HuGEMM) through CRISPR-Cas9 knock-in. We have generated PD1 and CTLA4 HuGEMM mice and used them for efficacy assessment of corresponding therapeutic antibodies. We have also generated human PD1 expressing MC38 cells and PD1 HuGEMM, and demonstrated robust efficacy of several PD1 therapeutic antibodies.

Methods: Here we report the establishment of iCI double knock-in (Ki) mice, i.e. PD1/PDL1 and PD1/CTLA4 diKI HuGEMM, and use them as tools to test immunotherapies with PD1/PDL1 and PD1/CTLA4 combinatorial ICI treatments.

Results: Similar to clinical benefit of PD1 and CTLA4 combo shown in the melanoma and NSCLC patients, we found combined treatment of Nivolumab and ipilimumab antibody leads to 103% TGI in the corresponding PD1/CTLA4 diKI HuGEMM, with complete tumor remission in 80% of treated mice. These mice remained disease free throughout a tumor re-challenge study for over 40 days. We have also developed PD1/PD1 diKI HuGEMM and tested combined treatment of Atezolizumab and Ipilimumab antibody leads to 103% TGI in the corresponding PD1/PD1 therapeutic antibodies.

Conclusion: Our diKI HuGEMM models offer robust tests on ICI combinations, as well as combos of ICIs with other anti-tumor therapeutic modalities.

Conflict of interest: Corporate-sponsored Research: Crown Bioscience Inc., Shanghai Model Organisms Inc.

227 (PB-078) Poster Association of immune checkpoint expression, tumor STING expression and DNA damage repair deficiency in breast cancer

E. Gilmour1, F. OrfilaIdia1, S. Mcquaid1, L. Mcilveen2, G.W. Irwin3, L. Knight4, M. Salto-Tellez4, R. Kennedy1, N. McCabe1, E. Parkes1

1Queen’s University Belfast, Centre for Cancer Research and Cell Biology, 2Organis Inc., Shanghai Model Organisms Inc.
Background: We previously developed a 44-gene expression assay capable of identifying a subgroup of breast cancer with DNA damage repair deficiency due to loss of the Fanconi Anemia/BRCA repair pathway. This assay was retrospectively validated as predictive of improved relapse-free survival following DNA damaging chemotherapy. This 44-gene assay is independent of adenosine concentrations, and rescued cytokine production (p = 0.0008) compared with anti-PD-L1 alone, with a 6-fold decrease in tumor volume compared to anti-PD-L1 alone (median tumor volume = 105 ± 79 mm³ vs 696 ± 427 mm³ on day 23, respectively).

EOS100850 represents a novel, potent, insumountable and best-in-class A2AR blocker, specifically optimized for immuno-oncology indications, that deserves to be studied.

Conflict of interest: No conflict of interest

229 (PB-080) Poster Chemotherapy beyond immune checkpoint inhibitors in patients with metastatic colorectal cancer


Background: Immune checkpoint inhibitors (ICI) have changed the landscape of cancer treatment, yet only a small subset of patients (pts) with microsatellite instability-high (MSI-H) metastatic Colorectal Cancer (mCRC) seem to benefit from ICI therapy. Thus, most mCRC patients with microsatellite stable (MSS) tumors are refractory to CPI. Many clinical trials are evaluating different approaches to overcome primary immunoresistance of mCRC patients. We aimed to assess whether sequential CPI followed by chemotherapy (CT) may be an alternative therapeutic approach in this subset of patients.

Material and Methods: We retrospectively evaluated response upon chemotherapy after immunotherapy failure. All pts with mCRC treated by chemotherapy after failure of anti-PD(L)1 alone or in combination at the early Drug Development Department at Gustave Roussy were included. Primary endpoints were RECIST objective response rate (ORR), progression-free survival (PFS), and overall survival (OS).

Results: Twenty-three mCRC patients were treated with CT between 2014 and 2018. Median number of previous lines including CPI were 4 (range, 2–7). Median age was 58 years old (25–88), 19 pts (83%) were male and 87% had MSS tumors. CT regimens included FOLFOX (1 pt), FOLFIRI (2 pts), and FOLFOX-bevacizumab (6 pts). Median interval between CPI and CT was 0.5 months (range, 0.3–3.2) for 23 pts were assessed for the analysis. Overall response rate was PR in 4 pts (19%), SD in 9 pts (43%) and PD in 8 pts (38%). Patients with PR had received FOLFOX-bevacizumab (1 pt); FOLFOXIRI (1 pt), FOLFOXIRI plus targeted therapy (4 pts; 1 pt Bevacizumab; 3 pts Cetuximab), FOLFOX plus targeted therapy (3 pts; 1 pt Panitumumab; 2 pts Bevacizumab), Regorafenib (2 pts), and Carboplatin (1 pt BRCAM). Median PFS and OS post-CPI were 2.7 months (95% CI, 2.1–3.5) and 5.5 months (95% CI, NA), respectively.

Conclusion: Chemotherapy after CPI failure is feasible in patients with immunorefractory mCRC. The results of this small retrospective cohort need to be validated in independent retrospective cohorts as well as prospectively. The role of immunotherapy as a modifier of both tumor cells and microenvironment in mCRC deserves further research.

Conflict of interest: Other Substantive Relationships: Pr Soria is full time employee of Medimmune since sep 2017.
Advances in harnessing the immune system for cancer treatment have been spectacular in the recent years with the achievement of highly durable clinical responses with antibodies to checkpoint receptors such as CTLA4 and PD1. Apart from PD-1 and CTLA-4, there are several other checkpoint proteins in the tumor microenvironment that play a role in dampening the anti-tumor immune response. In addition to PD1 and CTLA4, upregulation of inhibitory pathways prevent effective immunity. T cell Ig and ITIM domain (TIGIT) is a recently identified co-inhibitory receptor expressed by activated T cells, Tregs, and NK cells. TIGIT binds two ligands, CD112 (PVR/2, nectin-2) and CD155 (PVR), and these ligands are expressed on T cells, APCs, and tumor cells. TIGIT is upregulated on tumor antigen-specific (TA-specific) CD8 T cells and CD8 tumor-infiltrating lymphocytes (TILs) in various cancer types and TIGIT receptor/poliovirus receptor (PVR) ligand interaction signaling inhibits cytotoxicity mediated by NK and CD8 T cells. Interestingly, TIGIT-expressing CD8 T cells often co-express the inhibitory receptor PD-1. Therefore simultaneously blocking of both TIGIT and PD-1 pathway could potentially result in better anti-tumor activity.

We sought to discover and develop small molecule immune checkpoint antagonists capable of simultaneously targeting TIGIT and PD-1 pathways. We reasoned that such therapeutic agents will be amenable for oral dosing, likely show greater response rate due to dual antagonism and allow better management of irAEs due a shorter pharmacokinetic profile. Herein we report the pharmacological evaluation of the first-in-class small molecule antagonists capable of targeting both PD-1 and TIGIT immune checkpoint pathways. The design hypothesis for generating a dual antagonist is primarily based on approach of truncating high affinity peptides or critical fragments from the interface of TIGIT/PVR interactions to arrive at the shortest pharmacophore. Considering the pockets of sequence similarity of PDL1 and TIGIT proteins a focused library of small molecule compounds, based on shortest pharmacophore, mimicking the interaction of checkpoint proteins was designed and synthesized to achieve compounds exhibiting dual antagonism towards TIGIT and PD-1 pathways.

We have identified new novel antagonists demonstrating dual TIGIT and PD-L1 inhibition with potent rescue of PVR-mediated inhibition of IL-2 production from T cells and PD-L1 mediated IFN-γ production. The SAR optimized lead compounds exhibits desirable invitro ADME and DMPK profile including oral bioavailability and better tumor distribution. The lead compounds exhibit significant anti-tumor activity in a syngeneic tumor model and demonstrated profound immune PD in vivo on both T and NK cells. Additional biomarker characterization and efficacy studies in additional tumor models are ongoing.

No conflict of interest

231 (PB-082) Poster Targeting TNFR2 – A key regulator of the tumor immunosuppressive microenvironment

A. Raue1, R. Fulton1, J. Sampson1, A. Camblin1, J. Richards1, Y. Jiao1, L. Xu1, C. Wong1, A. Koskikaryen1, V. Paragas1, L. Luus1, S. Ghassemifar1, D. Francesca1, C. Carland1, K.E. Paulson1, 1Tufts University School of Medicine, Developmental. Molecular and Chemical Biology, Boston, USA; 2Tufts University School of Medicine, Graduate Program in Pharmacology and Experimental Therapeutics, Boston, USA; 3Tufts University School of Medicine, Graduate Program in Pharmacology and Drug Development, Boston, USA

Background: Despite the dramatic anti-tumor responses observed for immune checkpoint inhibition in subsets of patients, there remains an unmet medical need for improving larger patient populations. Resistance to checkpoint inhibitors include multiple immunosuppressive mechanisms that are present in the tumor microenvironment, many of which are linked to regulatory T cells (Tregs). Here, we have identified TNF receptor 2 (TNFR2) as a key regulator of the immunosuppressive microenvironment and have observed significant anti-tumor responses using a monoclonal antibody.

Materials and Methods: Human and mouse tissues were profiled for TNFR2 expression in the tumor versus periphery. Antibodies against murine TNFR2 were generated by screening an antibody library and by rabbit immunization. Antibodies were characterized for affinity, ability to block TNF and for developability. A selected number of antibodies were expressed as murine IgG1 and evaluated for efficacy in multiple syngeneic mouse tumor models. The mechanism of action of the most potent murine surrogate antibody was investigated further both in vitro and in vivo.

Results: TNFR2 was highly expressed on human and murine intratumoral Tregs compared to other T cell subpopulations. Notably, TNFR2 expression in the periphery was low for all T cell populations. The murine surrogate antibody Y9 showed robust anti-tumor activity at a single injection of 200 μg/mouse. In addition to T cell and murine tumor models, this antibody anti-PD-1 resistant models. Y9 treatment broadly downregulated several suppressive markers such as PD-1, GARP/LAP, and CTLA-4 on tumor infiltrating T cells. Treg depletion was not consistently observed across responding tumor models, suggesting it may not be the dominant mechanism. The mechanism of action required CD8 T cell and NK cell responses and led to long-term memory in re-challenge experiments.

Conclusion: Treatment with our murine surrogate anti-TNFR2 antibody led to robust responses in multiple syngeneic mouse tumor models both alone and in combination with checkpoint inhibition. The effect was specific to the tumor microenvironment, as no effect on Tregs in the periphery was observed. A human anti-TNFR2 antibody was modeled on our murine surrogate and is being developed to potentially become an efficacious and safe treatment option for patients that are refractory to, or relapsing on, currently approved immune checkpoint inhibitors.

No conflict of interest

232 (PB-083) Poster Green tea and decitabine in the treatment of triple negative breast cancer: pre-clinical studies on alterations in tumor wnt signaling and immune recognition properties

A. Yee1, M. Alamoudi2, M. Chipman1, K. Li1, A. Applebaum1, D.F. Francesca1, C. Carland1, K.E. Paulson1, 1Tufts University School of Medicine, Developmental. Molecular and Chemical Biology, Boston, USA; 2Merrimack Pharmaceuticals, Inc., Research, Cambridge, USA

Background: Triple negative breast cancer (TNBC) is an aggressive subtype with extensive metastases. The incidence of fatal brain metastases has dramatically increased with improved clinical management of non-brain metastases. A major barrier to improving TNBC patient outcome is the lack of most treatments to cross the blood brain barrier (BBB). We have identified a pre-clinical combination therapy of epigallocatechin-3-gallate (EGCG; active ingredient of green tea) and decitabine (DAC; DNA demethylation). EGCG/DAC is effective at reducing primary tumors and brain metastases. Both compounds cross the BBB and are in cancer clinical trials or FDA approved for other malignancies. Mechanistically, EGCG/DAC alters the signaling landscape of the treated TNBC xenograft tumor and unexpectedly predicts new susceptibility to immune checkpoint inhibitors.

Results: Increased Wnt signaling is associated with TNBC and brain metastases, often due to epigenetic silencing of Wnt pathway inhibitors. Wnt signaling inhibitors might be increased by EGCG/DAC, because DAC reactivates SFRP1, etc. and EGCG increases the HBP1 transcriptional repressor/Wnt pathway inhibitor (our work). We determined the maximum tolerated dose (MTD) for EGCG/DAC efficacy in a pre-clinical model of human TNBC xenographs in immune compromised mice. The clinically relevant EGCG/DAC MTD reduced primary tumors and brain and non-brain metastases. We demonstrated reduced Wnt signaling in tumors, concomitant with the re-induction of SFRP1, HBP1, etc. We next used RNA seq with bioinformatics analyses to comprehensively delineate the molecular mechanisms. In addition to reduced Wnt signaling, there was significant up-regulation in (1) antigen-presenting pathways (numerous genes; direct MHC staining); (2) cancer antigens (e.g. NY-ESO1, MAGEA); and (3) interferon/JAK-Stat pathway (numerous genes; P<stat3). Notably, these were tumor-intrinsic changes. Lastly, treatment of TNBC tumors in a syngeneic mouse model (with an intact immune system). EGCG/DAC resulted in (1) reduced syngeneic tumor size; (2) decreased Wnt signaling; (3) increased antigen presentation machinery; and (4) increased interferon/JAK-Stat signaling. There was increased CD8+ T cell infiltration—a biomarker that predicts efficacy for anti-PD1 and other immune checkpoint inhibitors.

Conclusion: Studies in other cancers revealed that increased Wnt signaling and a disabled interferon pathway are impediments to immune checkpoint inhibitor efficacy. EGCG/DAC treatment altered both Wnt and interferon signaling and predict sensitization to immune checkpoint inhibitors: (1) Heightened tumor interferon signaling causes increased antigen presentation capability; (2) increased immunogenicity upon induction of cancer antigens; (3) decreased Wnt signaling; (4) CD8+T-cell infiltration. These studies may advance the use of immune checkpoint inhibitors to improving breast cancer patient outcome.

No conflict of interest
Wednesday, 14 November 2018
POSTER SESSION
New Therapies with Pleiotropic Activity

233 (PB-084)
Poster
Characterization of MP1000, a unique bioactive lipid nanoparticle targeting multiple cancers
J. Duex1, C. Johnson1, G. Miknis1, M. Kandasamy1, C. Hudson1, J. Staszak-Jirkovsky1, N. Goodman1. Machaivert Pharmaceuticals, Biology, Aurora, USA

Background: Lipids and their associated pathways are increasingly found to be critical to cancer etiology. In particular, intestinal, gastric, colon, hematological and other KRAS-dependent cancers have been shown to be dependent upon lipid metabolism for disease progression. We have pursued the identification of bioactive lipid compounds that simultaneously target multiple metabolic pathways and subsequently disrupt tumor progression. From our investigations we have identified MP1000, a unique mixture of bioactive lipids, that has undergone lead optimization and formulation as stable nanoparticles and is being pursued as a new anti-cancer agent.

Materials and Methods: MP1000 nanoparticles formed by thin film hydration and extrusion methods. It has been extensively characterized including dynamic light scattering (DLS), zeta potential, liquid chromatography mass spectrometry (LC-MS), and electron microscopy analytical methods and shown to be comprised of 90 nm diameter particles that can be stably stored frozen long-term. MP1000 was tested in immunocompromised mice for in vivo efficacy and in a non-GLP acute safety study in rats.

Results: MP1000 has demonstrated monotherapy efficacy in vitro in 3D tumor cell culture assays including colorectal and pancreatic cancer cell lines (P < 0.05). MP1000 is being evaluated for monotherapy efficacy in multiple in vivo xenograft models and has shown efficacy when administered orally or injected intravenously in immunocompromised mice xenografted with pancreatic carcinoma models (P < 0.05). MP1000 has demonstrated high potential for loading with small molecule therapeutic payloads to form synergistic nanoparticles that have multiple anti-tumor mechanisms of action (MOA). To date MP1000 has successfully been loaded with immune cell activating payloads and cell cycle arrest payloads.

Conclusions: Based on preliminary efficacy and its single-agent and combination efficacy in multiple tumor xenograft models, MP1000 and multiple payload formulations of MP1000 are in preclinical development in preparation for clinical trials.

Conflict of interest: Other Substantive Relationships: All authors are employees of Machaivert Pharmaceuticals, LLC.

234 (PB-085)
Poster
Imprime PGG, a soluble yeast beta-glucan PAMP, converts the immunosuppressive myeloid tumor microenvironment into an immunoreactive one: translation of preclinical findings to melanoma and triple-negative breast cancer (TNBC) patients
M. Uhlik1, A. Chan1, B. Harrison1, A. Jonas1, K. Gordon1, X. Qiu1, N. Otton1, R. Walsh1, M. Gargano1, M. Chisamore1, J. Lowe1, B. Osterwalder1, N. Bose1, J. Graff1. Biothera Pharmaceuticals Inc., Research and Development, Eagan, USA; 3Biothera Pharmaceuticals Inc., Program Management, Eagan, USA; 4Merk & Co. Inc., Oncology Early Development, Kenilworth, USA; 5Biothera Pharmaceuticals Inc., Clinical Development, Eagan, USA; 6B.O. Consulting GmbH, Clinical Development, Riehen, Switzerland

Background: Imprime PGG (Imprime), an IV administered soluble yeast β-glucan PAMP, converts the immunosuppressive myeloid tumor microenvironment into an immunoreactive one: translation of preclinical findings to melanoma and triple-negative breast cancer (TNBC) patients

Methods: Imprime was tested in combination with anti-PD1 mAb in a MC38 colon cancer model. Imprime’s M1-polarization effects were evaluated by RNA expression and flow cytometric analyses of spleens and tumors from the different treatment groups. T cell activation effects were assessed by anti-CD3/CD28 proliferation assay. Clinically, the activation of monocyte/macrophage population was assessed in samples collected in an ongoing Phz clinical trial (NCT029861303) combining Imprime with Pembrolizumab in metastatic melanoma patients (prior CPI-naive) and for TNBC patients (prior chemoTx-failed, but CPI naive). From these patients, we obtained pre- and on-treatment PBMCs and biopsies to assess biological changes within the periphery (flow cytometry) and tumor site (multispectral imaging).

Results: Imprime combined with anti-PD-1 elicited a robust control of tumor growth which was greater than either agent alone. Macrophages evaluated from Imprime-treated tumors downmodulated M2-related genes (Arg-1, CCL17, IDO etc.) and upregulated M1 genes (INOS, TNF-α, IL-12B etc.). Flow cytometry revealed increased M1 markers (CD86, PD-L1, iNOS, and MHCI) with Imprime treatment compared to vehicle. Furthermore, macrophages from Imprime/PD-1-treated tumors showed significantly less T cell immunosuppressive functionality than those from PD-1 treated tumors. In clinical samples, higher frequency of monocytes and enhanced expression of CD86 and HLA-DR were observed in the periphery with Imprime/Pembrolizumab. Compared to pre-Tx samples, tumor biopsies from Imprime/Pembrolizumab on-Tx samples showed increased myeloid infiltration and polarization to M1 phenotype (CD80+/CD206-), as well as consistently increased T cell infiltration and activation (Ki67+ and/or GranzymeB+).

Conclusions: Collectively, these data show consistency between preclinical and clinical observations that demonstrate that Imprime/Pembrolizumab remodels the immunosuppressive myeloid tumor microenvironment and triggers the infiltration and activation of immune cells in preclinical and patient tumors.

Conflict of interest: Ownership: All authors with the exception of Michael Chisamore and Bruno Osterwalder are employees of Biothera and own Biothera company shares or/and stock. Bruno Osterwalder owns Biothera share options and/or stock. Michael Chisamore is an employee of Merck and owns Merck stock.

235 (PB-086)
Poster
Target discovery of natural product inspired phyllanthusmins for treatment of high grade serous ovarian cancer
A. Young1, S. Kurina1, A. Huntsman2, R. Rathnayake2, M. Korkmaz3, A.D. Kinghorn2, L. Aldrich3, S. Cologna3, J. Fuchs2, J. Burdette1. University of Illinois at Chicago, Medical Chemistry & Pharmacognosy, Chicago, IL, USA; 2Ohio State University, Medical Chemistry & Pharmacognosy, Columbus, OH, USA; 3University of Illinois at Chicago, Chemistry, Chicago, IL, USA

Background: High grade serous ovarian cancer (HGSOC) is a lethal gynecological malignancy with a need for new therapeutic agents. Many of the most widely used chemotherapeutic drugs are either derived from or are semi-synthetic derivatives of natural products. We developed potent synthetic analogs (PHYs) of the phyllanthusmins class inspired by prior natural product isolated from Phyllanthus poilanei Beille.

Materials and Methods: HGSOC cell lines, OVCAR3 and OVCAR8, and non-tumorigenic controls, ISE80 and FT33, were used in this study. Cytotoxicity assays included sulforhodamine B assay, and annexin X/Pi staining and Western blotting for confirmation of apoptosis induction. A photo affinity labeling method was used to attach PHY analogs to solid phase support. Targets were isolated using a pulldown technique and mass spectrometry. CRISPR-Cas9 genome editing was used to knock out and confirm putative targets.

Results: The most potent analog, PHY34, has nanomolar potency in HGSOC cell lines in vitro and displayed cytotoxic activity through late-stage autophagy inhibition and activation of apoptosis. PHY34 was readily bioavailable through intraperitoneal administration in vivo where it significantly reduced HGSOC tumor burden. Targets were identified using photo affinity labeling-aided protein pulldown and mass spectrometry, and confirmed by generating knockout cell lines of targets.

Conclusions: This class of compounds holds promise as a potential, novel, direct-chemotherapeutic approach and demonstrates the effectiveness of pleiotropically targeting autophagy and apoptosis as a viable strategy for combating high grade serous ovarian cancer.

No conflict of interest
Recent well-designed preclinical studies have demonstrated that KRAS mutations can activate the canonical NF-κB signaling pathway, which is a pivotal role in tumor progression. Furthermore, inhibition of this KRAS-NF-κB axis would be a promising target to develop a new molecular targeted anticancer agent. Curcumin, a naturally occurring polyphenol derived from the plant Curcuma longa, has been shown to block NF-κB signaling pathway via at least two mechanisms; inhibition of IκB phosphorylation and inhibition of ubiquitin-dependent degradation of IκB. Several preclinical studies have clearly demonstrated that curcumin exhibited anticancer effects in colorectal pancreatic cancer models through NF-κB inhibition. However, since curcumin is highly lipophilic and orally administered curcumin has very low bioavailability, its clinical application in cancer patients has been hampered.

To overcome this problem, we focused on developing a synthetic water-soluble curcumin mono-β-D-glucuronide (CMG) to administer this promising agent intravenously. First CMG was chemically synthesized from a starting material and in three steps. Then CMG was injected intravenously into SD rats at 30 mg/kg and measured a concentration of active free-form curcumin. As a result, the level of active free-form curcumin was more than 1,000-higher than those with orally administered curcumin. Finally we found that the intravenous injection of CMG exhibited the in vivo antitumor effects in the mouse xenograft model bearing human colorectal cancer cells HCT116 without loss of body weight.

These results suggested that CMG could be developed as an anticancer agent targeting KRAS-NF-κB axis without the serious side effects. The combination chemotherapy regimens of CMG with conventional anticancer agents are currently undertaken.


No conflict of interest

Thursday, 15 November 2018

POSTER SESSION

Animal Models

Poster 240 (PB-001)

Establishment and application of a panel of PBMC-humanized mouse tumor models in immune-oncology and targeted cancer immunotherapy

L. Zhang1, S. Qi1, Y. Jin1, H. Wu1, F. Chen1, L. Zhao1, X. An1, W. Tan2, X. Fu3, M. Qiao1, S. Shi1, W. Yang1, 2Crown Biosciences, In Cancer Biology, Taicang, Suzhou, China; 2Crown Blood Center, Taicang Blood Center, Taicang-Suzhou, China

Background: To meet the rapidly growing I/O market, the demands for fast, relevant and cost effective mouse tumor model systems are also increasing. We developed a panel of straightforward humanized tumor models, designated as MiXeno platform. Validation and characterization of MiXeno models is essential and will help to apply these models in the I/O field.

Method: CrownBio has established a sizable collection of MiXeno models where human PBMCs were reconstituted in the mouse system for the evaluation in vivo activity of immune checkpoint inhibitors or immune-modulators. These MiXeno models were characterized with tumor response to anti-PD-1 and anti-CTLA4 antibodies, and onset of possible graft versus host disease (GVHD) or graft versus tumor response (GVT) under different conditions.

Conclusions: This data shows that although loss of cerebellum does not affect in vitro angiogenesis, there appears to be a link between cerebellum and AGO2 in endothelial cells, and a potential role for cerebellin in leiomadid’s angiogenic activity. Overall, our data indicates that additional factors/conditions or a multi-gene coordinated effort is needed for CRBN to mediate the anti-angiogenic effects of IMiDs.
improve capacity and consistency of MiXeno platform, we are conducting studies to characterize and validate commercialized frozen PBMCs for MiXeno model establishment. In addition, we are comparing T cells reconstitution in immunocompromised B2M mice with NOG or NCG mice by profiling specific human/mouse antigens, such as CD45 and CD3.

Results: Models with over-expression of a variety of tumor antigens (e.g., EGFR, CD47, Brat, PD-L1, etc.) were used to develop specific MiXeno tumor models. Meanwhile, in order to overcome the limitation of PBMC shortage, commercialized PBMC has been purchase and implanted into several xenograft models, and exhibit consistent tumor growth with fresh PBMC, as well as human immune component reconstitution. Up to date, a variety of test articles of different categories, including checkpoint inhibitors, T cell modulators and bispecific T cell engagers (e.g. EpCam-CD3, CD47/CD3, BCMA/CD3) have been evaluated using this platform. Commercialized I/O drugs, such as Pembrolizumab, are being tested in commercialized PBMC implanted immunocompromised mice. Evaluation of immunocompromised B2M application for better reconstitution are also in progress.

Conclusions: MiXeno platform are valid model system for the human immuno-modulatory drugs including bispecific antibodies evaluation and will be optimized by introduction of specific MiXeno tumor models, commercial PBMC and B2M mice. Further studies are needed to expand model collections and to extend their applications in I/O space. Several outstanding questions remain to be further addressed, e.g. PBMC donor recruitment limitation, feasibility of in vitro PBMC donor screening, and selection of suitable animal strain for better reconstitution, etc.

No conflict of interest

241 (PB-002) Poster Characterization of a large panel of syngeneic tumor models and validation of an in vivo screening platform optimized for efficacy or immune-modulation evaluation for novel I/O drugs

Y. Jin¹, L. Zhang¹, H. Wu¹, B. Mao², A. An³, S. Guo², W. Yang³, Q. Shi¹.
¹Crown Bioscience Inc, Cancer Pharmacology, Taicang, Suzhou, China; ²Crown Bioscience Inc, Bioinformatics, Taicang, Suzhou, China; ³Crown Bioscience Inc, Biomarker Technology, Taicang, Suzhou, China; ⁴Crown Bioscience Inc, Cancer Pharmacology, Taicang, Suzhou, China

Background: Syngeneic model is a useful tool for evaluating the anti-tumor effect of cancer immunotherapy. Crownbio’s MuScreen is the first in vivo screening tool to test novel I/O drug candidates utilizing multi-syngeneic models. To better understand interplay between immune-modulation and anti-tumor phenotype in an in-depth fashion, better model characterization is required to capture dynamic changes of immune cell presence and infiltration within different compartments of tumor-bearing mice upon treatment with checkpoint inhibitors.

Method: Anti-PD-1 antibody was tested in 12–13 murine syngeneic models to evaluate the antitumor efficacy and the profiling of tumor infiltrated lymphocytes, as well as the percentage change of immune cell population in spleen and whole blood were evaluated. Anti-PD-1 antibody at 10 mg/kg was dosed twice weekly via intraperitoneally injection. Both of PBS and isotype PBS or hIgG groups. Comprehensive and dynamic immune cell populations were determined at basal level or at different time points post treatment, revealing trend of immune status change in tumor bearing animals. Highly variable data was observed in APD-1 Ab treated groups. Data analysis with more sophisticated bioinformatics methods is in process. Correlational analysis was attempted to reveal dynamic relationship between immune-profiling changes and anti-tumor effects.

Conclusion: Muscreen represents a powerful screening platform for I/O drug evaluation using a collection of well-characterized syngeneic tumor models. The advantage from a large scale validation study using syngeneic models reveals dynamic changes of both immune-profiling and anti-tumor response, which will help to make strategic decisions not only on the preclinical model selection but also on combinatorial drug selection.

No conflict of interest

243 (PB-004) Poster Development and characterization of a palbociclib-resistant luminal A breast PDX model from a clinically resistant patient

A. Moriarty¹, M. Quinn¹, M. Rundle¹, A. Patnaik², M. J. Wick¹. ¹START, Preclinical Research, San Antonio, USA; ²START, Clinical Research, San Antonio, USA

Background: Palbociclib is a CDK 4/6 inhibitor approved in combination with letrozole in hormone receptor-positive breast cancer. Although this combination therapy has been found effective in some patients, resistance often develops. To aid in developing new therapies for palbociclib-resistant breast cancer and better understand resistance mechanisms, we established a PDX model from a patient with luminal A breast cancer at time of progression, following response to palbo/letrozole therapy for six months. This model, designated ST3164B, was developed in athymic nude mice and characterized for receptor expression, genomic alterations and in vivo drug sensitivity.

Methods: ST3164B was established from pleural fluid taken from a 63 year old Asian woman pretreated with various therapies including eribulin, an investigational therapy and palbociclib/letrozole. The resulting model was passaged and challenged with palbociclib at various doses to confirm resistance. Receptor expression was determined immunohistochemically. Genomic analysis including NGS and RNAseq were tested at early and later passages to identify mechanisms of resistance.

Results: The ST3164B model retained high ER/PR staining (2+) expression over tested passages with similar histology compared with an archival clinical sample. DNA/RNA sequencing identified a number of confirmed variants; however, none have been currently identified as known mechanisms for palbociclib resistance.

Conclusion: We have established and characterized a palbociclib-resistant breast PDX model designated ST3164B which can be utilized as a valuable tool in better understanding CDK4/6 resistance and in developing new therapies for CDK4/6 inhibitor resistant patients.

No conflict of interest

244 (PB-005) Poster Assessment of functional signal transduction pathway activity in patient-derived tumor xenografts to predict and evaluate therapy response

W. Verhaegh¹, H. van de Stolpe¹, L. Holtzer¹, B. De Regt¹, J. Wrobel¹, M. Posch², N. Caushaj², A. Maier², T. Metz². ¹Philips Research, Precision and Decentralized Diagnostics, Eindhoven, Netherlands; ²Charles River, Discovery Research Services Germany, Freiburg, Germany

Background: Patient-derived xenograft (PDX) models are becoming the cornerstone of preclinical profiling of anti-cancer agents, facilitating efficacy tests of agents targeting cellular signal transduction pathways. Selecting the right PDX models, usually based on molecular characteristics, is key for the success of such preclinical studies. However, molecular alterations found in PDX models do not necessarily imply activation of the respective signal transduction pathways, a prerequisite for sensitivity of a PDX model to a targeted drug. Hence better tools are needed of functional pathway activity in individual PDX and also clinical samples.

Method: We developed computational models to assess functional activity of signal transduction pathways, using Bayesian networks that look at mRNA levels of pathway target genes resulting from activation (Verhaegh et al, Cancer Res 2014:2936–45), for the AR, ER, FOXP-PI3K, Hedgehog, TGFbeta, Wnt, and NFκB pathways. Calibration of network parameters was done on samples with known ground truth pathway activity. Gene and protobet selection was fine-tuned for application on PDX models to avoid undesired effects of the tumor microenvironment when compared to patient samples. After calibration, biological validation was performed on various healthy and diseased cell and tissue types.

Pathway activity was inferred for PDXs from public data sets and from the Charles River PDX collection. Activity scores were compared between PDXs representing different tumor stages, following different treatments and carrying various genetic aberrations, and were correlated to response data of PDXs and corresponding 3D cell cultures.

Results: Pathway activity clearly varied between different PDXs. For example, AR pathway activity differed between androgen-dependent, castration-induced and castration-resistant prostate PDXs. Comparison of WNT pathway activity with alterations of the APC gene in the same PDX model revealed which gene alterations affect pathway activity and which ones do not. Even in the absence of genic alterations, sensitivity of ex vivo 3D PDX cultures to PI3K inhibitors correlated with FOXP-PI3K pathway activity, and sensitivity to porcine inhibition correlated with WNT pathway activity. Pathway activity reduction was generally observed in PDXs following
exposure to agents targeting a given pathway, demonstrating the effectiveness of such compounds on a biological level. Taken together, PDX models displaying a high pathway activity score trend to be sensitive to agents targeting this pathway, irrespective of genetic alterations.

**Conclusion:** Signal transduction pathway activity scores are an excellent tool to select PDX models for preclinical profiling of targeted anti-cancer agents. They complement standard molecular characterization of PDXs and consequently have been incorporated in the Charles River PDX collection database.

**No conflict of interest**

245 (PB-006) Poster

Immunocytometry and diet-variants of a stage-defined, transgenic immunocompetent mouse model of HCC (ASV-B)


1AFR Oncology, Preclinical and Translational Department, Paris, France; 2Inserm U965-Lariboisiere University Hospital, Digestive and Cancer Surgery Department, Paris, France; 3Inserm U955-Monod University Hospital, Medical Oncology and Clinical Pharmacology Department, Creteil, France; 4Inserm U965-Institut des Vaisseaux et du Sang, Preclinical Department, Paris, France; 5Inserm U965, Lariboisiere University Hospital, Clinical Physiology-Functional Investigations Department, Paris, France; 6Inserm U1149-Beaumont University Hospital, Pathology Department, Clichy, France; 7Saint-Joseph Paris Hospital, Medical Oncology Department, Paris, France; 8Beaumont University Hospital, Medical Oncology Department, Clichy, France

**Background:** Hepatocellular carcinoma (HCC) is a complex multistep malignancy often arising on underlying chronic liver disease and is the main cause of death in patients with non-alcoholic steatohepatitis (NASH). Due to the obesity epidemics, NASH incidence is rising and is predicted to become the leading cause of HCC in the next decades. Therefore, there is an urgent need for robust animal models fully recapitulating the NASH-related HCC carcinogenesis. In this study, we further characterize and develop our transgenic HCC mouse model, focusing on immune landscape and specific diet-induced variants.

**Methods:** ASV-B is a transgenic mouse model (C57BL/6J) that spontaneously develops, upon SV40 T-Ag oncogene expression in hepatocytes, a reproducible stage-defined HCC: hyperplasia at week(W)8, nodular stage at W12, and diffuse carcinoma at W16-20. DNA was extracted from frozen livers at W20 for immune markers analysis using qRT-PCR (LightCycler, Roche). Immune populations were also assessed using automated immunohistochemistry (IHC) (Bond Max, Leica). To mimic NASH, we exposed mice to 5 different diet variants: classic diet (yellow), a high-fat diet (blue), a diet enriched with saturated fatty acids + 1.25% cholesterol (green), a diet containing 22% of vegetal oil + 0.2% cholesterol (red), and a 1.25% cholesterol diet containing 21% of milkfat (red).

**Results:** In ASV-B, we showed an increase of liver volume and angio genesis using ultrasound and Doppler, compared to control. Moreover, ASV-B livers harbor marked arteriolarization (increased arterial flow) and capillarization (tortuous and dilated sinusoids, surrounded by activated hepatic stellate cells). We further assessed 40 immune markers on 7 tumor specimens at W20, using qRT-PCR. As frequently observed in human inflammatory HCC, we observed an increase of CD8/Foxp3, INOS, CD11b, PD-1, PD-L1, IL17a, TNF-α, IL17a and IL17f mRNA expression. In addition, IHC staining showed intratumoral infiltration of CD8+ lymphocytes and F4/80+ macrophages.

To develop NASH, we fed 10 ASV-B and 5 control mice with 5 different diet variants. All mice fed with special diets also received 30% fructose in the water. ASV-B mice receiving yellow, blue, and green regimens seemed to have similar liver volumes and weights, whereas red and orange regimens appear to be associated with increased liver volume, liver weight and higher morbidity and mortality. At the conference, we will show the morphologic changes of the livers using HPS staining (necrosis, steatosis, fibrosis, etc.), and the immune landscape in the livers of treated and compared with the parent line using IHC staining and genomic analysis. In the ASV-B model, we have been able to develop specific-diets variants aimed at mimicking NASH.

**No conflict of interest**

246 (PB-007) Poster

Patient-derived xenograft (PDX) models from metastatic castration-resistant prostate cancer (mCRPC) patients reflect clinical disease and therapeutic outcomes

D. Ciznadjia1, P. Kipp2, M. Kohli3, S. Tati4, M. Mancini5, A. Davies2.

1Champions Oncology, Research, Hackensack, USA; 2Champions Oncology, Medical Affairs, Hackensack, USA; 3Mayo Clinic, Oncology, Rochester, USA

**Background:** Prostate cancer (PC) is a leading cause of cancer-related mortality in men and prognosis for mCRPC remains poor despite the availability of novel therapies including androgen receptor (AR) pathway inhibitors. Integration of drug screening and sequencing in PDX models may allow for precision medicine in mCRPC by advancing our understanding of resistance mechanisms (de novo and acquired) and identification of clinical response biomarkers. Here we highlight a cohort of PDX models developed from the bone metastases of mCRPC patients and demonstrate alignment of genomic features with specific clinical genetic elements as well as recapitulation of patient tumor histology and patient responses to therapy.

**Methods:** A total of 9 mCRPC PDX models, along with matched clinical specimens, were evaluated by next-generation sequencing for genomic alterations (mutations, amplifications/deletions, fusions, and gene expression). PDXs were also analyzed by immunohistochemistry to determine expression and localization patterns for the androgen receptor (AR) and prostate-specific membrane antigen (PSMA). Drug screening of models was performed against the AR inhibitor enzalutamide, with tumor regression (TR) and RECIST criteria determined and correlated with patient outcomes.

**Results:** Nine PDX models developed from 7 mCRPC patients, including matched models from two patients pre- and post-abiraterone progression, were interrogated. Strong and extensive nuclear AR and membrane PSMA staining was observed in each PDX evaluated to date, correlating with clinical tumor grade. The common TMPRSS2-ERG and TMPRSS2-ETV4 fusions were identified in 4/9 models and the expression profile of one model suggested possible neuroendocrine features. In matched patient models, 94% of mutations from the pre-abiraterone model were retained in the model developed post-treatment. Two mCRPC models developed from patients with acquired de novo resistance to enzalutamide also demonstrated a lack of response to the same agent in vivo.

**Conclusion:** Our study demonstrates alignment of genomics between PDX models, and clinical metastases, as well as reflecting our understanding of resistance mechanisms to clinical therapy, highlighting the application of these models for translational modeling and in vivo clinical trial simulation. The availability of matched PDX models developed from single patients enables insights into the molecular changes governing disease resistance. Comprehensive sequencing (WES and RNA) and standard of care drug testing of these and additional PC models developed from tissue and circulating tumor cells (CTCs) under our two-year NCI award (N44CA170013) is planned to gain further under standing of such mechanisms, as well as permit biomarkers of responses to be identified. In this context, application of mCRPC models to support future drug development will remain important and continue to evolve.

**Conflict of interest:** Ownership: Daniel Ciznadjia, Peter Kipp, Swetha Tati, Maria Mancini, Angela Davies.

247 (PB-008) Poster

Establishment and characterization of the HCC78 NSCLC tumor xenograft model

A. Moriarty1, L. Gamez1, M. Quinn1, M. Rundle1, K. Papadopoulos1, D. Rasco2, A. Patnai2, M.J. Wick1.

1START, Preclinical Research, San Antonio, USA; 2START, Clinical Research, San Antonio, USA

**Background:** The HCC78 NSCLC cell line is widely used in vitro for targeted drug development in cancers harboring ROS1-fusions including non-small cell lung cancer (NSCLC). However, lack of an accessible, matched xenograft has limited in vivo evaluation of potential therapies. To this end, we established the HCC78 NSCLC xenograft model in athymic nude mice. The resulting model was characterized and compared with the parent line using immunohistochemistry and genomic analysis. In vivo studies were performed using the xenograft model comparing various therapies targeting ROS1, ALK and EGFR and relevant chemotherapeutics.

**Materials and Methods:** The HCC78 NSCLC xenograft model was established and serially passaged in athymic nude mice. Receptor expression was determined using immunohistochemistry and compared with the parent cell line. For single agent in vivo studies, mice were dosed following targeted therapies: crizotinib, ceritinib, alecrtinib, brigatinib, erlotinib, afatinib, osimertinib, cetuximab and the chemotherapies docetaxel and cisplatin. Dosing was initiated on Day 0 and continued until control groups

**Abstracts, 30th EORTC-NCI-AACR Symposium**
reached designated tumor volume endpoint and drug response reported as %T/C value.

Results: Immunohistochemistry on the cell line and model passages reported intensity values of (+) for ROS1 receptor expression. The following vivo %T/C values were reported: Crizotinib = (−11%), Ceritinib = (35%), Alectinib = (100%), Brigatinib = (−51%), Erlotinib = (72%), Afinatinib = (30%), Osimertinib = (100%), Cetuximab = (100%), Docetaxel = (8%), and Cisplatin = (60%). Tumor regressions were reported with brigatinib treatment.

Conclusion: We have established a reproducible xenograft model of the HCC78 cell line which can be used for developing new therapies to ROS1-fusion positive cancers.

No conflict of interest

248 (PB-009)  Poster
Whole exome sequencing of canine cancer cell lines identifies common oncogenic mutations
D. Duval1, S. Das1, R. Idate1, D. Gustafson1, Colorado State University, Clinical Sciences, Fort Collins, USA

Background: Cancer cell cultures have served as a backbone in research exploring cancer cell biology and have informed the vast majority of preclinical studies to test cancer therapeutics. Preclinical analysis of human cell lines in conjunction with whole exome sequencing has helped to evaluate the addiction and sensitivity to drugs that target oncogenic pathways. Similarly, we have conducted whole exome sequencing in a panel of canine cancer cell lines to identify somatic variants that contribute to the pathogenesis of canine cancers and to determine the prevalence of common human oncogenic mutations.

Material and Methods: Genomic DNA was isolated from 33 canine cell lines, exonic regions were captured using the Agilent Sure Select XT Canine V.2.0 Exome Capture (43.45 Mbp), and the Illumina HiSeq 2500 platform was used to generate 151 bp paired end reads. Reads were mapped to CanFam3.1 and variants were called using FreeBayes. Germline variants were removed by screening against 3 canine germline databases and 13 local normal samples. Somatic variants were further screened to eliminate synonymous variants and variants that fell outside the coding region and scored for functional impact using Sift4G. Somatic variants were filtered for known drivers of human cancers from the COSMIC database and cross referenced for known oncogenic variants listed on cBioPortal.

Results: Mean on-target coverage for these 33 samples was 181x. Somatic mutation profiles identified in this screen were on average 62% SNPs which were dominated by C/G < T/A changes. Mutation frequency ranged from 22 to 129 mutations/captured MB. A total of 9,849 variants in 5554 genes were identified across 33 cell lines. Sift scoring categorized 34.8% of the missense mutations as deleterious. Filtering for COSMIC mutated cancer genes identified 232 genes with 447 variants in the 33 cell lines ranging from 3 cancer gene variants in CML10C2 canine melanoma to 43 in OSW canine lymphoma cell line. The most frequently mutated cancer gene was TP53 (11 mutations in 10 cell lines). By cross-referencing the identified variants with cBioPortal, we identified 60 candidate oncogenic mutations in 30 genes including activating mutations in BRAF, E1F1AX, ERBB2, KIT, KRAS, MET, NRAS, PIK3CA, PPM1D, PTEN(1), RAC1, and inactivating mutations in ARID1A, ASXL1, ATRX, CCND3, CDKN1A, CIC, EP300, FBXW7, KDM6A, KMT2C, KMT2D, MED2, NF1, PSEN, SETD2, SPEN, SRGAP3, TET2 and TP53. Oncogenic drivers were identified in 30 out of 33 cell lines. Selected mutations were confirmed using Sanger sequencing. Microarray and Western blot analysis revealed decreased expression in the 4 cell lines with NF1 frameshifts.

Conclusions: Whole exome screening of canine cancer cell lines has identified driving mutations that can be targeted for the treatment of canine cancer and development of novel therapies.

No conflict of interest

249 (PB-010)  Poster
Prevention of tumor ulceration by tumor cell inoculation into the mammary fat pad
C. Obodozie1, J. Beshay1, S. Ruf1, G. Bijelic1, S. Moor1, B. Giesen1, U. Leisegang1, H. Weber1, ProQinase GmbH, In vivo Pharmacology, Freiburg, Germany

Currently, cancer research focus on development of novel cancer immunotherapies. In several tumor models tumor has been limitedly successful. Inability of subcutaneous tumor growth occurs very often in an early stage of tumor growth. Ulcerated tumor-bearing mice must be euthanized according to animal ethical rules before the potential highest tumor volume can be reached. Hence, the number of existing/remaining animals is too low to get statistical significant calculations at study end and study duration is too short to study the effects on the immune system, which needs time to react after test substance exposure.

We observed for implanted 4T1 and EMT6 cells, both originated from breast cancer, that tumor ulceration appeared with lower frequency and at a later timepoint when implanted into the mammary fat pad in comparison to subcutaneous implantation. This brought us to the idea to implant also tumor cells other than breast cancer cells into the mammary fat pad (MFP). Both methods (subcutaneous and MFP inoculation) are heterocytic and can be calibrated.

We compare 8 syngeneic tumor cell lines from various entities when inoculated into the MFP and the subcutaneous space, respectively, with respect to tumor volume, occurrence of tumor ulceration and potential therapeutic window. In detail, in the CT26wt tumor model, tumor volume was >2-fold increased reaching 1500 mm3 and tumor ulceration was reduced >50% by MFP compared to subcutaneous inoculation. Likewise, study duration for the LL2 tumor model was prolonged by 4 days after MFP implantation compared to subcutaneous. Here, a 5-fold increase in tumor volume and a 3-fold decrease in tumor ulceration was observed when cells were inoculated into the MFP. Implantation of MC38-CEA tumor cells into the MFP did not lead to any tumor ulceration, whereas MC38-CEA s.c. implantation resulted in 7 tumor ulcerations out of 12 tumors. The outcome for additional syngeneic cell lines A522, Hepa-1,6, RENCA, B16F10, Clone3 is listed. Pathohistological examinations verified the absence of ulcerations in MFP-tumors and revealed the “protecting” effect of MFP. Additionally, examples for the impact of immune checkpoint inhibitors like anti-PD-1, anti PD L1 and/or anti-CTLA-4 on MFP implanted tumor growth are presented as well as the analysis on immune cell populations determined by flow cytometry analysis.

In summary, tumor ulceration could be prevented up to 100% by using the mammary fat pad as an alternative injection site. Thus, using MFP implantation extends study duration which is now mainly limited by tumor size. In addition, tumor growth appears better with less heterogeneity. In conclusion, the heterotypic MFP implantation of tumor cells is superior to the traditional subcutaneous implantation resulting in an improved meaning- and powerful tumor model.

Conflict of interest: Other Substantive Relationships: All authors work at ProQinase GmbH.

250 (PB-011)  Poster
Establishment of mouse prostate homograft tumor models for efficacy evaluation of combinatory immunotherapies
J. Wang1, H. Sun1, A. An1, L. Zhang1, H. Li1, D. Ouyang1, C. Bioscience Inc., Translational Oncology, San Diego, USA

Introduction: Despite the early approval of Sipuleucel-T for metastatic castration-resistant prostate cancer, which often perceived as a milestone achievement in cancer immunotherapy, subsequent progress in prostate cancer immuno-therapy development has been limitedly successful results with tumor vaccines and its resistance to immune checkpoint inhibitors, such as PD1 & PD-L1. It is now generally accepted that we need to tackle prostate cancer by combinatorial approaches of chemotherapeutic agents and immuno-therapeutic agents. Specifically, genetically engineered mouse models (GEMM) recapitulate some aspects of human prostate cancer in both histo-/molecular pathology. Among those, Pten loss in mouse prostate leads to hyperplasia at 4 weeks. PIN at 6 weeks, and fully invasive adenocarcinoma from 12 weeks of age. PTEN null tumors are also resistant to androgen depletion. In the meantime, although KRAS mutation are not often seen in human prostate cancer, activation of MAPK pathway often happens in advanced tumor. Mutant KRAS or BRAF can robustly promote mouse prostate cancer progression. Pten null and Kras G12D, Pten null mouse prostate cancer models have been well characterized by a number of labs. However, parental GEMM models are difficult to be used for pharmacological studies due to the spontaneous nature of tumor onset and progression. The compound mutant mice are also costly to breed.

Method: We have generated transplantable mouse prostate cancer homograft models by passing the primary tumor subcutaneously in the C57BL/6 mice GEMM. We have transplanted mouse prostate cancer homograft models by passing the primary tumor subcutaneously in the C57BL/6 mice GEMM.

Results: These mouse tumors bearing Pten null or Pten null mouse prostate cancer models have been well characterized by a number of labs. However, parental GEMM models are difficult to be used for pharmacological studies due to the spontaneous nature of tumor onset and progression. The compound mutant mice are also costly to breed.

Poster Session (Thursday, 15 November 2018)
Monitoring changes in biochemical elements within tumors is crucial to understanding cancer biology and to helping with the development of novel therapies. Yet to date, experimental techniques enabling sensitive and quantitative measurement of the levels of small molecules contributing to tumor development have been limited in preclinical oncology models.

**Materials and Methods:** We implemented in vivo microdialysis to measure signaling molecules and oncometabolites in the tumor microenvironment of freely moving rodents. To this aim, a small dialysis probe is implanted in syngeneic tumors in mice. Probes are perfused with artificial dialysate fluid and dialysate containing molecules from the tumor microenvironment is collected at regular intervals. Levels of biochemicals in the dialysate are then quantified by liquid chromatography-mass spectrometry (LC-MS).

**Results:** First, we tested whether implantation of dialysis probe affects in vivo tumor growth. To do so, tumor volume from probe-implanted and non-implanted MC38 allografts in mice was monitored daily for up to 10 days following probe implantation. We found that the growth curve from probe-bearing MC38 tumors was similar to probe-free tumors, revealing that probe implantation did not influence tumor size progression. Next, the levels of metabolites that are known to be involved in tumor development were measured in tumor allografts of freely-moving mice using in vivo microdialysis. Relevant levels of arginine, ornithine and putrescine were quantified by LC-MS. Levels of key molecules of the adenosine signaling and tryptophan/kynurenine pathway metabolites in MC38 tumors were also quantified. To better understand the homogeneity of this particular allograft model, we measured the concentration of the above-mentioned molecules in different regions of a tumor. To this end, two separate probes were implanted in the center and the peripheral area of the same 300 mm³ MC38 allograft. We found that levels of measured molecules were similar in dialysates collected from both probe locations, suggesting homogenous levels of biochemicals throughout the microenvironment of these non-necrotic tumors.

**Conclusions:** Our proposed experimental approach allows for a quantitative and sensitive measurement of key biochemical mediators of tumor progression. In vivo microdialysis in murine tumor models may be used to elucidate the mechanisms by which therapies, such as chemotherapy and immune checkpoint inhibitors, modulate the tumor microenvironment. Our efforts in preclinical cancer research has the potential to bring new insights on the mechanisms underlying cancer development and help the discovery of next-generation therapies for cancer.

**No conflict of interest**

**Thursday, 15 November 2018**

**POSTER SESSION**

**Chemoprevention**

**254 (PB-015) Poster**

**Biochemical targets and biomarkers of liver cancer and therapeutic strategies with leaf extracts of justicia adhatoda L**

S. Arora¹, Guru Nanak Dev University Amritsar, Botanical and Environmental Sciences, Amritsar, India

Liver cancer or hepatocellular carcinoma (HCC) is one of the commonest cancers in the world especially in countries like India which have a high incidence of Hepatitis B infection. Apart from Hepatitis B, it may be caused by other diseases that lead to cirrhosis of the liver such as hepatitis C infection, and alcohol abuse. Unfortunately, like many other cancers, liver cancer may go undetected until a late stage. To explore the targets and biomarkers of liver cancer, in vivo anticancer studies were done in rats using various biochemical markers. The leaves of the plant Justicia adhatoda were extracted serially with the solvents of increasing polarity and vasicine was isolated from most active n-butanol fraction by column chromatography and structure elucidation was also done. The effect of vasicine was analyzed on Phase I, Phase II, antioxidant enzymes and serum parameters in rats treated with CCl4. However, it has been found that rats treated with lower and higher doses of vasicine along with CCl4 reduced the specific activity in a dose dependent manner. These findings reveal that administration of vasicine modulates both phase I and phase II enzymes as well as antioxidative enzymes in rats. Phase I enzymes which mediate oxidation, reduction or hydrolysis reaction converts lipophilic chemical compounds into hydrophilic products, so it can be effectively eliminated by the kidneys. One of the most common modifications is hydroxylation catalysed by the cytochrome P-450-dependent mixed-function oxidase system. In the present study, CCl4 administration resulted in significant elevation in the level of both SGOT and SGPT in a dose dependent manner. Administration of vasicine for 12 days along with the CCl4 administration restored the activities
Poster 257 (PB-018)
Natural antibiotic Tetrocarcin-A downregulates Functional Adhesion Molecule-A in conjunction with HER2 and inhibits apoptosis proteins and inhibits tumour cell growth
S.H. Veilani1, R. Cruz1, H. Jahmz2, L. Hudson1, G. Settel1, A. Eramo2, A. Hopkins3. 1RCSI, Department of Surgery, Dublin, Ireland; 2University College Dublin, UCD School of Veterinary Medicine, Dublin, Ireland; 3Istituto Superiore di Sanità, Department of Oncology and Molecular Medicine, Rome, Italy

Background: Overexpression of the adhesion protein Functional Adhesion Molecule-A (JAM-A) has been linked to aggressive disease in breast and other cancers, but JAM-targeting drugs remain elusive. The purpose of the study was to identify JAM-A-targeting compounds by means of a natural compound chemical screen.

Materials and Methods: The effects of Tetrocarcin-A on cell viability, colony formation and semi-in-vivo tumor xenograft growth were examined in MCF-7 HER2 and BT474-Trastuzumab-resistant breast cell lines, in addition to breast cancer primary cultures and lung cancer stem cells. The impact of Tetrocarcin-A on the protein expression of signalling effectors controlling cell fate was examined by Western blotting, and compared with that of transient JAM-A gene silencing. Finally, apoptotic pathways were investigated downstream of Tetrocarcin-A treatment.

Results: Screening of a natural compound library identified the antibiotic Tetrocarcin-A as a novel downregulator of JAM-A and the human epithelial growth factor receptor-2 (HER2) protein expression in breast cancer cell lines. Lysoosomal inhibition partially rescued the downregulation of JAM-A and HER2 caused by Tetrocarcin-A, and attenuated its cytotoxic activity. Tetrocarcin-A inhibited c-FOS phosphorylation at Threonine-232, its transcriptional regulation site, and downregulated expression of the inhibitor of apoptosis proteins (IAP). This translated into Tetrocarcin-A-induced caspase-dependent apoptosis. To begin evaluating the potential clinical relevance of our findings, we extended our studies to other models. Encouragingly, Tetrocarcin-A downregulated JAM-A expression and caused cytotoxicity in primary cultures derived directly from breast cancer patients and in lung cancer stem cells. It also inhibited the growth of breast cancer cell xenografts in a semi-in vivo model involving invasion across the chicken egg chorioallantoic membrane.

Conclusions: Taken together, our data suggest that Tetrocarcin-A warrants future evaluation as a novel cancer therapeutic by virtue of its ability to downregulate JAM-A expression, induce apoptosis and reduce tumorigenic signalling.

This work was financially supported by Science Foundation Ireland (grant 132/1994 to AMR). Tetrocarcin-A was provided by the NCI/ITP Open Chemical Repository https://dtp.cancer.gov. (NSC 333856).

No conflict of interest

Poster 257 (PB-018)
Natural antibiotic Tetrocarcin-A downregulates Functional Adhesion Molecule-A in conjunction with HER2 and inhibits apoptosis proteins and inhibits tumour cell growth
S.H. Veilani1, R. Cruz1, H. Jahmz2, L. Hudson1, G. Settel1, A. Eramo2, A. Hopkins3. 1RCSI, Department of Surgery, Dublin, Ireland; 2University College Dublin, UCD School of Veterinary Medicine, Dublin, Ireland; 3Istituto Superiore di Sanità, Department of Oncology and Molecular Medicine, Rome, Italy

Background: Overexpression of the adhesion protein Functional Adhesion Molecule-A (JAM-A) has been linked to aggressive disease in breast and other cancers, but JAM-targeting drugs remain elusive. The purpose of the study was to identify JAM-A-targeting compounds by means of a natural compound chemical screen.

Materials and Methods: The effects of Tetrocarcin-A on cell viability, colony formation and semi-in-vivo tumor xenograft growth were examined in MCF-7 HER2 and BT474-Trastuzumab-resistant breast cell lines, in addition to breast cancer primary cultures and lung cancer stem cells. The impact of Tetrocarcin-A on the protein expression of signalling effectors controlling cell fate was examined by Western blotting, and compared with that of transient JAM-A gene silencing. Finally, apoptotic pathways were investigated downstream of Tetrocarcin-A treatment.

Results: Screening of a natural compound library identified the antibiotic Tetrocarcin-A as a novel downregulator of JAM-A and the human epithelial growth factor receptor-2 (HER2) protein expression in breast cancer cell lines. Lysoosomal inhibition partially rescued the downregulation of JAM-A and HER2 caused by Tetrocarcin-A, and attenuated its cytotoxic activity. Tetrocarcin-A inhibited c-FOS phosphorylation at Threonine-232, its transcriptional regulation site, and downregulated expression of the inhibitor of apoptosis proteins (IAP). This translated into Tetrocarcin-A-induced caspase-dependent apoptosis. To begin evaluating the potential clinical relevance of our findings, we extended our studies to other models. Encouragingly, Tetrocarcin-A downregulated JAM-A expression and caused cytotoxicity in primary cultures derived directly from breast cancer patients and in lung cancer stem cells. It also inhibited the growth of breast cancer cell xenografts in a semi-in vivo model involving invasion across the chicken egg chorioallantoic membrane.

Conclusions: Taken together, our data suggest that Tetrocarcin-A warrants future evaluation as a novel cancer therapeutic by virtue of its ability to downregulate JAM-A expression, induce apoptosis and reduce tumorigenic signalling.

This work was financially supported by Science Foundation Ireland (grant 132/1994 to AMR). Tetrocarcin-A was provided by the NCI/ITP Open Chemical Repository https://dtp.cancer.gov. (NSC 333856).

No conflict of interest

Poster 258 (PB-019)
An improved formulation of GLG-302, a STAT3 antagonist, with chemopreventive activity in preclinical models of mammary cancer
R. Shoemaker1, J. Fox1, H. Gomez2, J. White3, F. Moinpour4, C. Grubbs4. 1National Cancer Institute Division of Cancer Prevention, Chemopreventive Agent Development Research Group Division, Bethesda, MD, USA; 2GLG Pharma, Headquarters, Jupiter, FL, USA; 3MRGlobal, DCP Repository, Kansas City, USA; 4UAB, Chemoprevention, Birmingham, AL, USA

Background: GLG-302 was initially identified as a potential STAT3 antagonist using in silico screening. Subsequent in vitro and in vivo mechanistic studies verified the mechanism. Because of the potential role of this signaling pathway in mammary carcinogenesis, we evaluated GLG-302 in preclinical models of mammary cancer.

Materials and Methods: We first tested a carboxymethylcellulose suspension of GLG-302 in mice and rats to establish the ability of orally administered compound to affect STAT signaling in normal mammary tissue and to determine tolerated doses for chronic administration. In both species, after two weeks of treatment immunohistochemical studies indicated the pharmacodynamic markers pSTAT3 and Ki67 were reduced in normal ductal epithelium. In both species the compound was well tolerated at doses as high as 500 mg/kg/day.

Results: Preliminary cancer prevention efficacy testing was performed in mice using daily oral administration of a suspension of GLG-302 in carboxymethylcellulose. Cancer prevention efficacy was indicated in the spontaneous MMTV/Her2 mouse model and in rat DMBA-induced tumor model with compound administered in the feed. No body weight loss or other toxicity was observed. We then evaluated alternative approaches to formulation of GLG-302 and demonstrated that a Trizma salt form could be produced with aqueous solubility to 50 mg/mL. While subject to rapid
elimination from plasma, this formulation demonstrated better bioavailability than the suspension. We tested this formulation in the rat and mouse mammary tumor models and verified retention of activity on pharmacodynamic markers. Striking preventive activity was observed in the MMTV/Her2 mouse model.

**Conclusion:** GLG-302 given orally to mice and rats can modulate STAT3 signaling and proliferation in mammary epithelium. Additional studies, especially toxicology studies, will be needed to assess the potential of this class of compounds for chemopreventive use.

**Conflict of interest:** Ownership: Hector Gomez and GLG Pharma are owners of GLC-302. A joint patent exists on the Trizma salt formulation of GLG-302 with NCI, MRIGlobal and GLG staff as inventors.

---

**Materials and Methods:** In present study, we have evaluated the role of IGFBP-3 of oral daily doses 0,1

---

**Conclusion:** The overall results suggested the possible role of IGFBP-3 in promoting the migration and invasion of prostate cancer cells under the hypoxic condition. Western blotting, immunocytochemistry, overexpression and knock-down systems were used.

**Results:** Under the hypoxic conditions (1% Oxygen), prostate cancer PC3 cells showed increased levels of IGFBP-3 when compared to normoxic conditions (21% Oxygen) in a time-dependent manner. Hypoxia is a major regulator of prostate cancer progression through stimulating the angiogenic, migratory and invasive potential of cancer cells. We down-regulated IGFBP-3 using ShRNA construct against it in PC3 cells. The knock-down of IGFBP3 did not considerably affect the growth pattern of PC3 cells as compared to scrambled control but addition of fisetin (25–100 μM) decreased the cell viability in both normoxic and hypoxic conditions for time period up to 72 h. The down-regulation of IGFBP-3 resulted in the increased expression of E-cadherin, a biomarker of epithelial phenotype. The wound scratch assay showed the promigratory role of IGFBP-3 in cancer cells and treatment with fisetin inhibited the migration of these cells under the hypoxic condition. The knockdown of IGFBP3 resulted in the decreased invasive potential of cancer cells in comparison to cells in hypoxic state.

**Conclusions:** The first 3 h after administration AMP was absorbed in the tumor significantly faster than in the normal organs/tissues. The AMP complex was standardized by the anti-(porcine) AFP complex with therapeutic agents showing antitumor activity during drug development and preclinical study.

**Abstracts, 30th EORTC-NCI-AACR Symposium**

**Thursday, 15 November 2018**

**Drug Delivery**

**Poster Session**

**261 (PB-022)**

**Poster**

**Tofacitinib enhances delivery of antibody-based therapeutics to tumor cells through reduction of tumor-associated inflammatory cells**

D. Fitzgerald 1, N. Simon 1, A. Antignani 1, S. Hewitt 2, M. Gadina 3, S. Tsurkan 1, J. Tcherkassova 1, H. Treshalina 2, E.Y. Grigor’evaa, I. Treshchalin 1, E. Penevezeva 1, 1Pharmaceutical Research Center "PharmAccess", lab. Moscow, Russian Federation; 2Federal Budgetary Institution, N.N. Blokhin Medical Research Center of Oncology of the Ministry of Health of Russian Federation, Lab. Moscow, Russian Federation; 3Gause Institute of New Antibiotics, Lab. Moscow, Russian Federation

**Background:** Antibody-based therapeutics play important roles in treating cancer. However, the routes by which tumor-targeted antibodies gain access to tumor cells vary. The complex environment of an esthesur tumour are not well defined. Compounds that increase the efficiency of delivery to malignant cells are likely to constitute important avenues of advancement. Here, we report on the action of tofacitinib, an FDA-approved JAK3/JAK1 inhibitor.

**Materials and Methods:** Tumor cells were injected into mice, treated with tofacitinib or vehicle and then treated with an antibody-based therapeutic agent. Tumor growth was measured following one of four treatments: vehicle only, tofacitinib only, antibody agent alone or a combination of antibody and tofacitinib. Tumors from treated mice were analyzed by flow cytometry for the uptake of fluorescently-labeled antibodies into malignant cells. Further, tumors were removed from treated animals and stained by immunohistochemistry with antibodies inflammatory cells. Immune-related transcripts from tofacitinib-treated mice were quantified via Nanostring analysis. Likewise, serum cytokines were measured by ELISA.

**Results:** Tofacitinib altered the tumor microenvironment by reducing the number of tumor-associated inflammatory cells and allowed increased delivery of antibody-based agents to malignant cells. Alone, tofacitinib exhibited no antitumor activity, but combination treatments with recombinant immunotoxins (IT) or an antibody drug conjugate (ADC) resulted in increased anti-tumor responses compared to either antibody-based drug alone, confirming in vivo synergy. Further, fluorescently-labeled antibody-based agents accumulated in a higher percentage of malignant cells following tofacitinib treatment. The action of tofacitinib was microenvironment-dependent as there was no enhancement of killing or antibody uptake in tissue culture. Nanostring profiling of tofacitinib-treated tumors indicated that the levels of several cytokine transcripts involved in immune cell chemotaxis were reduced. Reductions in arginase, IL6, CCL2, CCL4, and CXCL2 were confirmed at the protein level. Histological analysis of tumor tissue revealed that tofacitinib reduced the tumor size, neutrophils, monocytes, and macrophages within the tumor microenvironment thereby allowing greater access of therapeutic antibodies to malignant cells.

**Conclusions:** Present findings serve as a rationale for designing human trials in cancer patients where short-term treatments with tofacitinib could be administered in combination with antibody-based therapies to increase their uptake into prostate cancer cells under hypoxic conditions.
faster than in the MG. The mean rate of AMP elimination from T47D and MG was similar between 6 and 9h after drug administration, after which it was slower, and by 24h the IDg in the tumor was 5 times higher than in the MG (IDg = 0.87 vs 0.18). The retention period was longer for the tumor as well (MRT = 12.3h vs 9.5h).

**Conclusions:** High efficacy of low doses orally administrated AMP was achieved due to the targeting and receptor mediated endocytosis yielding retention of AMP in T47D. Oral treatment of AMP showed intestinal absorption and sufficient bioavailability.

**No conflict of interest**

263 (PB-024) Poster

**Pre-beta HDL discoidal mimetic, CER-001®, and novel Apolipoprotein-A-I (ApoA-I) multimers, Cargomer®, as new targeted delivery vehicles for therapeutic cancer medicines**

V. Albini†, E. Lacoste‡, R. Baron§, R. Palmantier¶, J.L. Dasseux∥, †Imvita SAS, Research & Development, Toulouse, France; ‡Imvita SAS, Chief Executive Officer and CSO, Toulouse, France; §RND Pharma, President, Toulouse, France; ¶ImmThera Consulting SAS, President, Toulouse Area, France; ∥Cerenis Therapeutics Holding SA., President and CEO, Balma, France

**Background:** Despite the huge potential of nanoparticles, the delivery efficiency of their payload to tumors has plateaued at 0.7% in the last decade. The ability to produce and manufacture recombinant human (rh)ApoA-I to design a new type of nanoparticles, CER-001® and Cargomer®, deploying the HDL natural features of targeted delivery system opens the way for improved means of drug delivery.

ApoA-I is the major protein of HDL that plays an essential role in reverse cholesterol (Chol) transport, in particular via the scavenger receptor SR-B1 that is expressed on hepatocytes and macrophages and is highly over-expressed on cancer cells. Based on HDL capabilities to transport lipophilic molecules as well as micro-RNA and deliver esterified-Chol into the cytosol of SR-B1-expressing cells, CER-001® and Cargomer® have been developed to optimize the delivery of cytotoxic drugs, siRNA or radiotracer to tumors. CER-001® is a negatively charged pre-b HDL mimicetic, which is being evaluated in phase III clinical trial (TANGO) in patients with genetic LDL deficiencies and in phase II (TARGET) as 89Zr-nanocarrier for tumor imaging in patients with esophageal cancer. Cargomer® are innovative rhApoA1 multimeric nanocomplexes developed for targeted drug delivery.

**Methods:** Paclitaxel, a poorly soluble cytotoxic drug was complexed in CER-001® (CER-Pacl) through a proprietary production process. MDA-MB-231, a human breast cancer cell line, was engrafted orthotopically into NOD-SCID mice. At d25, mice were administered IV with either CER-Pacl, paclitaxel protein-bound (Abraxane®) as positive control, both groups at 265mg/kg, or vehicle, 1 x qd for 5d, then, 2x qw until euthanasia at d57. 24h after last administration.

Cargomer® have been loaded with the siRNA oncogenic KRAS mutant G12D or STAT-3 DNA oligo antisense both coupled to modified Chol with a Zr-nanocarrier for tumor imaging in patients with esophageal cancer. Cargomer® are innovative rhApoA1 multimeric nanocomplexes developed for targeted drug delivery.

**Results:** In contrast to the mice in the vehicle group, tumor growth was inhibited in the mice treated with CER-Pacl as well as with Abraxane®.

<table>
<thead>
<tr>
<th>groups</th>
<th>tum vol (mm³)</th>
<th>tum vol (mm³)</th>
<th>paclitaxel : tumor (ng : mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vehicle</td>
<td>150</td>
<td>864</td>
<td>1.2</td>
</tr>
<tr>
<td>Abraxane®</td>
<td>143</td>
<td>105</td>
<td>23.5</td>
</tr>
<tr>
<td>CER-pacli</td>
<td>143</td>
<td>141</td>
<td>31.7</td>
</tr>
</tbody>
</table>

The prepared Cargomer®-siRNA or -DNA elute in a single major peak by SEC. Preliminary data show a reduction of KRAS expression in Panc-1 treated by Cargomer®-siRNA compared to Cargomer®-scrambled siRNA.

**Conclusions:** CER-001-Pacl is at least as efficacious as Abraxane® nanoparticles to deliver and treat tumor-bearing mice. The Cargomer® is a flexible targeting nanoparticle platform with promising delivery activities.

**Conflict of interest:** Other Substantive Relationships: Consulting services paid by Cerenis Therapeutics.

264 (PB-025) Poster

**Liposomal Gemcitabine, FF-10832, Overcomes Gemcitabine resistance by improving pharmacokinetics**

T. Matsumoto†, T. Ioroi‡, T. Komori§, Y. Yoshino†, H. Kitahara#, Y. Izumi#, S. Shimoyama†, K. Takada†, S. Hagiwara†, T. Hara†, †FUJIFILM Corporation, Pharmaceutical & Healthcare Research Laboratories, Kanagawa, Japan

**Background:** Gemcitabine (GEM) based therapies are standard treatment for advanced pancreatic cancer. However, it is thought that rapid clearance of GEM is one of the reasons for poor clinical outcomes or drug resistance. We developed FF-10832 (F832) that achieved long circulation in plasma, high accumulation and payload release in tumors. F832 demonstrated anti-tumor effects in both Capan-1[GEM-sensitive] and BxPC-3[GEM-resistant] human pancreatic cancer xenograft models.

The aims of this study are to demonstrate the mechanism of F832 to overcome GEM-resistance and the potential of combo therapy for maximizing the efficacy.

**Materials and Methods:** Expression levels of GEM activation related molecules were compared between Capan-1 and BxPC-3 tumors by qRT-PCR.

The active form of GEM [dFdCTP] in F832 or GEM-treated tumors were quantified by LC/MS to analyze the correlation of anti-tumor effects and Cmax, AUC or mean residence time (MRT) which represents the average time of dFdCTP staying in tumors.

For the identification of F832 internalized cells, FACS and IHC with fluorescent dye labeled F832 were used.

The anti-tumor effects of F832 and GEM in combo with immune checkpoint inhibitor (ICI) were examined in a syngeneic GEM-resistant mouse tumor model (EMT6).

All animal studies were approved by the FUJIFILM animal committee.

**Results:** We found that mRNA levels of ENT1, a major transporter for GEM, was significantly low in the BxPC3 model, compared to the Capan-1 model. The lower expression of ENT1 is thought to result in the reduced Cmax, AUC and MRT of dFdCTP, and GEM resistance in the BxPC3 model.

On the other hand, MRT of dFdCTP in F832 treated tumors was comparable to that in the Capan-1 model, although Cmax and AUC were reduced. These results suggested that the efficacy of F832 in the BxPC3 model can be attributed to longer MRT of dFdCTP than that after GEM administration.

In addition, we clarified the mechanism of F832 uptake and payload release in tumors. FACS and IHC data showed that F4/80 positive tumor-associated macrophages preferentially internalized F832. Macrophages that internalized F832 completely released GEM. These results indicated that macrophages would act as a reservoir of GEM and contribute to longer exposure of tumors to dFdCTP.

Then, we examined the combo effect with ICI in the EMT6 model. F832 + ICI demonstrated statistically significant anti-tumor effects compared to GEM + ICI. Several animals of F832 + ICI showed complete regression of tumors, though the others didn’t.

**Conclusions:** We indicated that F832 could overcome GEM-resistance through longer exposure (MRT) of tumors to dFdCTP, presumably by the EPR effect and the following tumor macrophages related reserve function.

Then, the potential of combo therapy with ICI showed enhanced efficacy in GEM-resistant models. We have started a Phase1 study (NCT03444050).

**Conflict of interest:** Other Substantive Relationships: All authors are employees of FUJIFILM Corporation.
in vivo, in vivo, and in a clinical trial; however, its effects on tumor vasculature have not been studied yet, which was the aim of the study.

**Material and Methods:** Dense cell suspensions of B16F1 murine melanoma cells or HUVEC human endothelial cells were prepared in medium containing 1–5 mM CaCl₂, placed between electrodes (2 mm gap) and subjected to 8 square-wave electric pulses (260 V, 100 μs, 1 Hz). After in vitro treatment, cytotoxicity and ATP depletion were determined. Additionally, the anti-vascular effect was evaluated by tube formation and wound healing assay in vitro and by intravital microscopy of normal and tumor vasculature in dorsal window chamber (DWC) model in C57Bl/6 mice. Treatment was performed by intratumoral or intradermal injection of either 5 μl of MiliQ water or CaCl₂ (50 mM, 168 mM, 250 mM) then electric pulses (4+4 square-wave pulses, 520 V, 100 μs, 1 Hz). After 4 mm gap plate electrodes were applied. Treatment, DWC were imaged for 3 days, then Rhodamine B labeled dextran was injected into the orbital plexus and images of fluorescent vessels were taken. Mice were euthanized on day 3, skin in the region of DWC was excised, paraffin-embedded sections were prepared and stained with H&E.

**Results:** CaEP was cytotoxic and caused ATP depletion in a dose-dependent manner. Anti-vascular effects were observed in vitro and in vivo, suggesting that CaEP causes ATP depletion and affects both tumor and normal blood vessels. We have demonstrated that CaEP is highly cytotoxic to tumor cells and also has an anti-vascular effect on both normal and tumor blood vessels. Our study provides further evidence for the use of CaEP in clinical settings for the treatment of cutaneous tumors.

**Conflict of interest:** Ownership: Competing financial interests: A patent has been submitted – PCT/DE2012/050498 Therapeutic applications of calcium electroporation to effectively induce tumor necrosis (co-inventors: S. Frandsen and J. Gehl).

267 (PB-028) Poster
**FF-10850, a novel liposomal topotecan, dramatically improved pharmacokinetics, anti-tumor effects, and the safety profile of topotecan**


**Background:** Topotecan has been approved and used as a treatment option for ovarian, cervical, and small cell lung cancers. The major clinical issues of topotecan are rapid clearance from plasma and serious hematotoxicity. These issues significantly limit the clinical use of topotecan. In order to maximize the therapeutic benefit of topotecan while overcoming these issues, we designed FF-10850, a novel liposomal topotecan, expecting preferential distribution in the tumor tissue through enhanced permeability and retention (EPR) effect.

**Material and Methods:** FF-10850 was formulated with a proprietary and optimized composition of dihydrosphingomyelin (DHSM), cholesterol, and polyethylene glycol. The anti-tumor effects of FF-10850 were compared to topotecan in the ES-2 ovarian cancer xenograft mouse model. The anti-tumor effects of FF-10850 in combination with the immune checkpoint blockade were also examined in a syngeneic mouse tumor model. Hematotoxicity and systemic toxicity of FF-10850 were evaluated in rats and compared to the toxicity of topotecan. All animal experiments were approved by the FUJIFILM animal experiment committee.

**Results:** The introduction of DHSM as a lipid component for the liposomal shell of FF-10850 allowed stable encapsulation of topotecan in the interior of the liposomal shell and prolonged the plasma half-life compared to the conventional liposomal formulation with hydrogenated soy phosphatidylcholine. Weekly administration of FF-10850 at 0.5 mg/kg demonstrated almost equivalent anti-tumor effects to topotecan at the maximum tolerated dose (2 mg/kg, daily for five consecutive days) in the ES-2 mouse xenograft model. Higher concentrations of FF-10850 up to 4 mg/kg were tolerated and induced stronger anti-tumor effects resulting in tumor regression. In addition to the monotherapy treatment, FF-10850 achieved an induction of robust and durable anti-tumor effects in combination with the immune checkpoint blockade even after completion of treatment in a syngeneic mouse model.

Toxicity studies in rats demonstrated that FF-10850 improved the toxicity profile of topotecan, including reduction in hematotoxicity and prolongation of the plasma half-life, whereas the target organs of toxicities identified in FF-10850 treated rats were similar to those identified in topotecan treated rats. Improvement of the safety profile was supported by pharmacokinetics and pharmacodynamic analysis using measuring phospho-HAX2, which is a DNA damage marker induced by topotecan.

**Conclusions:** FF-10850 dramatically improved the anti-tumor effect and toxicity of topotecan by modulating the pharmacokinetic profile. FF-10850 also demonstrated the potential of combination therapy with the immune checkpoint blockade. Based on these results, IND-enabling studies are currently underway to move FF-10850 forward to the Phase 1 clinical trial in 2019.

**Conflict of interest:** Other Substantive Relationships: All authors are employees of FUJIFILM Corporation.

269 (PB-030) Poster
**Dual hydrophobization formulation strategies improve intravenous half-life, safety, and tumor delivery of siRNA nano-polyplexes**

M. Jackson, S. Bedingfield, F. Yu, M. Stokan, R. Miles, E. Hoogenboezem, E. Curvino, T. Giorgio, C. Duvall, Vanderbilt University, Biomedical Engineering, Nashville, USA

**Background:** While RNA-based drugs hold great promise for targeted, personalized medicine, they generally suffer from delivery challenges due to rapid clearance from circulation and poor tissue biodistribution. Encapsulation of siRNA in nano-sized, electrostatic polymer complexes (si-NPs) can improve pharmacokinetic profiles and delivery to solid tumors in mice, but the impact of formulation parameters (i.e. ratio of carrier: cargo or cargo chemical modification) on in vivo pharmacokinetics and safety have been minimally studied. Herein, we sought to optimize polyplex si-NP formulation using a dual carrier-cargo hydrophobization strategy to improve both circulation time and safety of si-NP formulations in vivo.

**Materials and Methods:** Reversible Addition Fragmentation Chain Transfer (RAFT) polymerization was used to synthesize diblock copolymers [PMPC-DB] containing homopolymer poly(2-methacyloyloxyethyl phosphorylcholine) [PMPC] and random copolymer core block composed of polyl(dimethyl amino ethyl methacrylate-co-butyl methacrylate) [DB]. Polyplexes (si-NPs) with either normal or palmitic acid conjugated-siRNA (PA-siRNA) were formulated at polymer: siRNA ratios of 10, 15, and 20. Cy5-labeled si-NPs were injected in male CD-1 mice and their circulation times were monitored by intravital microscopy. Toxicologic properties of si-NPs were monitored upon repeat injection in mice by sampling serum liver enzymes, cytokines, complete blood counts, body weight, and tissue histology. Tumor uptake of fluorescent si-NPs was measured by flow cytometry.

**Results:** Doubling the si-NP polymer: siRNA ratio increased circulation half-life five-fold. However, as amount of polymer was increased, associated liver toxicities increased. For polyplexes formulated at polymer: siRNA ratios of 10 or 15, PA-siRNA increased si-NP half-lives to the equivalent of pentoxifylline. While further increasing polymer: siRNA ratios did not improve half-life, maintaining superior pharmacokinetic profiles. This dual hydrophobization strategy further reduced the toxicity of polyplex formulations, which were well-tolerated in a 6 injection repeat dose study in mice. Optimally formulated PMPC-DB polyplexes also exhibited greater than two-fold increased uptake in orthotopic MDA-MB-231 xenografts compared to the commercial transfection agent, in vivo Jet-PEI.

**Conclusions:** Our work demonstrates that varying polymer:siRNA ratio and employing dual hydrophobization strategies significantly impact siRNA polyplex pharmacokinetics and carrier-associated toxicities in mice. By utilizing hydrophobic moieties on both carrier polymers and cargo siRNA, large doses of siRNA can be delivered to tumors using less total polymer. These data have implications on maximizing the therapeutic index of new molecularly-targeted, siRNA-based cancer therapies.

**No conflict of interest**

270 (PB-031) Poster
**Drug delivery to malignant brain tumor by a peptide specific to Annexin A1**

M. Nonaka, K. Hideaki, M. Fukuda, Kyoto University, Graduate School of Pharmaceutical Sciences, Kyoto, Japan; 2National Institute of Advanced Science and Technology, Laboratory for Drug Discovery, Tsukuba, Japan

**Background:** Annexin A1 (Anxa1) is a ubiquitous protein which is normally expressed in the cytoplasm and nucleus in the cell. In the tumor vasculature, Anxa1 translocated onto the cell surface of endothelial cells, indicating...
Anxa1 is the most specific marker of tumor vasculature [Oh et al., Nature, 429: 629-33, 2004]. Previously, we reported that a carbohydrate mimetic peptide, designated as IF7, targets to various solid tumors through binding to Anxa1 on the tumor endothelial cells [Hatakeyama et al. PNAS108:19587–92, 2011]. IF7 rapidly crossed endothelial cells via transcytosis and was incorporated in the tumor cells. IF7 conjugated with SN38, an anti-cancer drug, efficiently regressed tumor, indicating IF7 becomes a novel drug delivery system.

Materials and Methods: Brain tumor xenograft model of rat glioma C6 cell was established. Fluorescence-tagged IF7 was injected intravenously into the brain tumor-bearing mice. For dual xenograft model, mouse melanoma B16 cells, which stably express luciferase, were injected subcutaneously and into the brain in one mouse. IF7 was conjugated with SN-38, which is an active metabolite of irinotecan, a topoisomerase I inhibitor and then IF7-SN38 was injected intravenously at a low dose (7 nmol/g/mouse) everyday.

Results: In this study, we hypothesized that IF7 crosses blood-brain barrier by transcytosis and delivers anti-cancer drug to tumor stroma. When IF7-labeled with fluorescent dye was injected into the mice of glioma xenograft model, fluorescence signal accumulated in the tumor site but not in the normal brain. We next established the dual tumor xenograft model by implanting C6 glioma cells subcutaneously and in the brain. Of note, IF7-SN38 suppressed growth of both brain and subcutaneous tumors at a low dose.

Conclusions: These results suggest that IF7 can cross blood-brain barrier and efficiently deliver anti-cancer drugs into malignant brain tumor. A new peptide with increased stability in vivo is now under developing by applying phage display technology.

No conflict of interest

271 (PB-032) Poster
The conjugation of newly developed unsymmetrical bisacridine antitumor agents with quantum dots nanoparticles to improve the drug delivery

E. Matysiak², A. Kowalczyk², P. Bujak¹, A. Nowicka², E. Augustin⁵, Z. Mazierska¹, Gdansk University of Technology, Chemical Faculty, Department of Pharmaceutical Technology and Biochemistry, Gdansk, Poland; ²University of Warsaw, Faculty of Chemistry, Warsaw, Poland; ³Warsaw University of Technology, Faculty of Chemistry, Warsaw, Poland

Background: The search of our group for the potential anticancer drugs among acridine derivatives resulted in the synthesis of new promising unsymmetrical bisacridine derivatives (UAs), EP 15461518.1, 2017, which exhibited high cytotoxic activity against a lot of tumor cell lines and high antitumor efficacy against several types of human cancer xenografts in nude mice. To improve the drug delivery conditions the nanoconjugates of newly developed UAs, compounds C-2028, C-2045, with red (QDred) and green (QDgreen) quantum dots nanoparticles were synthesized. There is known, that nanoparticles penetrate the cell membrane by endocytosis. Therefore, we aimed to answer the question, which properties are crucial in the improving of drug-conjugate transport into the cell. The stability of the synthesized nanocojugates were tested at various pH values: 7.4, 5.5 and 4.4, corresponding to the outer cell layer, endosomes and lysosomes.

Materials and Methods: UAs were non-covalently attached to Ag-In-Zn-S-MUA nanocrystals. The amounts of UAs anchored to nanocrystals were determined from voltammetric analyses using the charges of cathodic peak 

150°C. To modify the QCM electrode with QD nanocrystals, the droplet of dry. The FTIR spectra were acquired in a transmission mode with the spectral resolution of 4 cm⁻¹. The dynamic light scattering (DLS) and zeta potential (ZP) measurements were performed with a He-Ne (4 mV) laser at 632.8 nm, in buffer at 25°C. Quartz crystal microbalance with dissipation (QCM-D) analysis, was carried out in the flow system, rate 100 mL min⁻¹, temp. 21°C. To modify the QCM electrode with QD nanocrystals, the droplet of 150 μL of QD solution, 1 mg mL⁻¹ was placed on the Au surface and left to dry. The FTIR spectra were acquired in a transmission mode with the spectral resolution of 4 cm⁻¹.

Results: DLS, ZP and FTIR investigations confirmed the effectiveness of the conjugation procedure between UAs compounds and QD nanocrystals. The increase of QD diameter value at pH 7.4 evidenced that the conjugate was formed. However, at pH 4.0 the low hydrodynamic diameter indicated that the drug was released from the nanocojugate, except of QDred- C-2045 nanocojugate. The changes of ZP values in the pH range from 4.0 to 8.4 demonstrated that QDgreen are stable at whole tested pH range, while the QDred only in the pH range 5.5-6.4. The QCM-D experiment showed that the most probable way of transport of QD-UAs conjugates to the green endocytosis. In the acidic pH values (5.5 in endosomes and 4.0 in lysosomes) the decomposition of nanoconjugate took place. The release of C-2028-6 from QD nanoconjugates was more effective than C-2045 in the case of QDred at pH 5.5 and for QDgreen at pH 4.0.

Conclusions: Under physiological conditions, pH 7.4, the UAs-QD nanocojugates are stable. Inside the endosomes, pH 5.5, the nanoconjugate stability significantly decrease according to the drug structure. Under acidic conditions in lysosomes, pH 4.0, 80% of the drug is released from the nanoconjugate.

No conflict of interest

272 (PB-033) Poster
Discovery of the novel, homogeneous payload platform dolasynthen for antibody-drug conjugates

M. Kozytaska¹, A. Yurkovetskiy¹, M. Yin¹, N. Bodyak², P. Conlon², B. Du¹, C. Stevenson¹, D. Gumerov³, M. Nazzaro³, D. Lee³, W. Lee¹, L. Qin², K. Catcott¹, D. Demady³, S. Collins¹, J. Xu¹, J. Duvall¹, M. Damelin¹, D. Toader¹, T. Lowinger¹, Mersana Therapeutics, Discovery Chemistry, Cambridge, USA; Mersana Therapeutics, Analytical, Cambridge, USA; Mersana Therapeutics, Pharmacology, Cambridge, USA

Background: Dolasynthen is a novel, proprietary, homogeneous payload platform enabling the creation of antibody-drug conjugates (ADC) with drug-to-antibody ratios (DAR) ranging from 6 to 22. We previously reported a high DAR payload platform, Dolaflexin, which is a polymer-based, polydisperse scaffold incorporated into the ADCs XMT-1522 and XMT-1536 currently in clinical trials. We disclose here efficacy, safety and PK data for Dolasynthen, a second-generation Auristatin F hydroxypropylamide (AF-HPA) payload platform with a defined, fully homogenous structure that allows for the creation of ADCs with diverse, defined DAR values.

Materials and Methods: A set of payload platforms was synthesized that use AF-HPA cytotoxic warhead. These payload platforms contain highly biodegradable and biocompatible polypeptide frameworks and can be varied with respect to overall charge and the identity and characteristics of hydrophilic moieties to balance overall physicochemical properties. ADCs were prepared by conjugating the payload platforms to IgG1 antibodies through stochastic bioconjugation, or site-specifically to engineered antibodies. The resulting ADCs displayed DARs in the range of 6–22 and their overall properties and activity in xenograft animal models were determined. ADCs prepared with lead payload platforms were evaluated in mouse xenograft efficacy models, non-human primates (NHP) tolerability and TK studies.

Results: SAR data showed a strong correlation between the structure of the payload platform and observed activity in vitro and in vivo and allowed for further optimization of hydrophilic modifiers and other physicochemical enhancing components. PK evaluation in non-human primates showed a strong correlation of the ADC exposure to the net charge of the payload platform. Lead ADCs showed excellent efficacy in mouse xenograft models and desired tolerability in NHPs at doses and DARs comparable to Dolaflexin conjugates. The lead payload platform was used to generate homogenous ADCs that showed the expected pm activity in vitro and excellent efficacy in vivo. The ADCs showed exceptional plasma stability and good exposures in tumor-bearing mouse models and NHP tolerability studies.

Conclusions: The novel, fully homogenous AF-HPA based payload platform Dolasynthen showed potent in vivo antitumor activity and excellent tolerability in NHP. The Dolasynthen platform is amenable to the generation of ADCs with enhanced homogeneity including fully homogeneous ADCs. The hydrophilic nature of the structurally defined framework coupled to careful design of the payload led to identification of a platform that shows great promise for future clinical use.

No conflict of interest

Thursday, 15 November 2018

POSTER SESSION

Drug Design

273 (PB-034) Poster
Design and synthesis of benzopyrrolo[2,1-a]phthalazine hybrids as potent anticancer agents to small cell lung cancer

T.L. Chen³, Y.W. Lin³, T.L. Su³, T.C. Lee³, ³Academia Sinica, Institute of Biomedical Sciences, Taipei, Taiwan

Combination therapy that combine two or more anti-cancer drugs enhance efficacy (via synergistic or an additive mode) compared to the mono-therapy approach. Hybrid molecules conjugate two different pharmacophores and display dual mode of action via interaction with two targets. Thus, hybrid
molecules may have advantage over combination therapy on enhanced efficacy and reduced side-effects. Here, we have synthesized a series of hybrids, pyrrolo[2,1-alphathalazine, which consist of phthalazine (anti-angiogenesis moiety) and 1,2-bis(hydroxymethyl)pyrrole (DNA cross-linking moiety). We demonstrated that these hybrids possessed dual mode of action, and displayed potent therapeutic efficacy against human small cell lung cancer (SCLC) cells in mouse xenograft models. Here, we further used benzolog approach to design and synthesize novel hybrids, namely 1,2-bis(hydroxymethyl)benzol[pyrrolo[2,1-α]-phthalazines with various secondary amine side chains at C6 and their ethylcarbamate derivatives for anticancer evaluation. We found that these new hybrids exhibited significant cytotoxicity against the growth of a panel of human cancer cell lines in vitro. The values of IC_{50} of these compounds were ranged from dozens of picomolar to micro M, respectively. Similar potency was measured in cell lines (IC_{50} = 2.6 nM, respectively).

**Conclusion:** We have discovered a novel, potent and selective series of small-molecule CD73 inhibitors for cancer immunotherapy. Members of this series exhibit a favorable pharmacokinetic profile and are apt for further development.

**Conflict of interest:** Other Substantive Relationships: Employees of Arcus Biosciences.

---

**Introduction:** Extracellular adenosine (ADO) is present in high concentrations within the tumor micro-environment (TME), and ADO is a potent inhibitor of T cell and NK cell activation, resulting in suppression of immune function. The generation of ADO is dependent upon the ecto-nucleotidases CD39 (transforms ATP to AMP) and CD73 (transforms AMP to ADO). Inhibition of CD73 represents a promising therapeutic strategy for preventing ADO-mediated immunosuppression in the TME. Here we present the discovery of a novel class of small molecules that are capable of inhibiting CD73.

**Methods:** The potency of CD73 inhibitors was evaluated by measuring AMP hydrolysis by CHO-CD73 cells using a malachite green assay. Potency was also measured using human T cells and soluble recombinant CD73. Selectivity against related ecto-nucleotidases was also assessed. Pharmacokinetic parameters were determined in preclinical species.

**Results:** A novel class of small molecule CD73 inhibitors has been developed starting from a high throughput screening hit. Several potent small-molecule CD73 inhibitors have been co-crystallized with recombinant CD73. They have been found to bind to the closed form of CD73 and to occupy the adenosine binding pocket, maintaining the same π-π stacking interaction observed with the CD73 inhibitor AMPCP. This class of molecules also demonstrates a key hydrogen bond with CD73 residue Asp506 to provide a drastic improvement in CD73 inhibition. Compounds exhibit potent CD73 inhibition in multiple biochemical and cell based assays providing IC_{50} values of less than 20 nM. Inhibitors have also been screened against various NTPDases as well as the adenosine receptors and found to be highly selective for CD73. Select molecules do not show significant inhibition of the major CYP450 isozymes. Pharmacokinetic properties in rodents indicate the potential for oral bioavailability with low to moderate clearance.

**Conclusions:** Efforts have resulted in the discovery of a novel class of small-molecule CD73 inhibitors. These compounds are capable of inhibiting both soluble and membrane-bound CD73 and represent a step forward in the development of an orally bioavailable CD73 inhibitor for use in the treatment of cancer.

**Conflict of interest:** Other Substantive Relationships: Employees of Arcus Biosciences.

---

**Introduction:** Adenosine (ADO) is a potent inhibitor of T cell and NK cell activation and is present in high concentrations in the tumor microenvironment (TME) resulting in an immunosuppressed phenotype. In the TME, generation of ADO relies on the sequential hydrolysis of ATP by two ecto-nucleotidases, CD39 (ATP→AMP) and CD73 (AMP→ADO). Inhibition of CD73 eliminates a major pathway of ADO production and can reverse ADO-mediated immune suppression. Here we present the discovery, drug design, and characterization of a highly potent and selective series of small molecule inhibitors of CD73.

**Materials and Methods:** The potency of CD73 inhibitors was evaluated by measuring AMP hydrolysis by CHO-CD73 cells using a malachite green assay. Potency was also measured using soluble recombinant CD73. In the presence of human serum, CD73 inhibition was measured by quantitation of AMP hydrolysis via luminescence. Selectivity against related ecto-nucleotidases was also assessed. The pharmacokinetic properties of select inhibitors were evaluated in rodents. X-ray co-crystal structures of select inhibitors were obtained by co-crystallizing them with recombinant human CD73.

**Results:** The medicinal chemistry team at Arcus Biosciences has developed a concise series of potent and selective CD73 inhibitors via interrogation of structure activity relationships (SAR) and structure-based drug design. Key molecular interactions were identified from high resolution X-ray crystal structures for select inhibitors bound to human CD73. These inhibitors were found to occupy the adenosine pocket and form an array of hydrogen bonds with CD73 residues D506, R354, and N390. Furthermore, a strong hydrophobic π-π stacking interaction between two phenylalanine residues (F417 and F500) and coordination of the di-zinc catalytic center was integral to retain high potency. A001022, a representative member of this series, potently inhibits soluble and membrane-bound CD73 (IC_{50} = 0.86 and 2.6 nM, respectively). Similar potency was measured in cell lines (IC_{50} = 0.55 nM, SKOV-3; A001022 exhibits a favorable pharmacokinetic profile and a long half-life.

**Conclusions:** We have discovered a novel, potent, and selective series of small molecules capable of inhibiting CD73. These efforts have expanded the understanding of the structural underpinnings of small-molecule CD73 inhibition. Members of this series exhibit a favorable pharmacokinetic profile and are apt for further development.

**Conflict of interest:** Other Substantive Relationships: Employees of Arcus Biosciences.
Results: Interestingly, a moderately well-defined pocket was identified on the transmembrane domain where a Spy1 glutamate residue is bound to. This residue was found to form several electrostatic interactions with multiple positively charged residues in the identified cavity. The CDK2 pocket and the bound Spy1 residue were then screened to reveal a number of hits from various structural scaffolds that have all required features for inhibiting Spy1 binding to CDK2.

Conclusions: Potential hits obtained from this computational study will be tested in-vitro to prove their usefulness in cancer treatment.

No conflict of interest

277 (PB-038)  Poster

Pyrrolo[2,1-a]phthalazine hybrids with anti-angiogenesis and DNA crosslinking induction activities are potent anticancer agents

T.C. Lee 1, T.L. Chen 1, S.M. Chang 1, H.B. Pidugu 1, Y.W. Lin 1, M.H. Wu 2, H.C. Wu 3, T.L. Su 1, 1 Academia Sinica, Institute of Biomedical Sciences, Taipei, Taiwan; 2 Industrial Technology Research Institute, Biomedical Technology and Device Research Laboratories, Hsinchu, Taiwan; 3 Academia Sinica, Institute of Cellular and Organismic Biology, Taipei, Taiwan

Hydroceptor molecules are composed of two distinct biologically active molecules that act at different targets and thus may display dual mechanism of action. Using hybrid anticancer drug for treatment cancer can mimic the combination therapy, because hybrid molecule may have increased potential of selectivity profiles for biological targets as compared to the corresponding single drugs. While angiogenesis is as hallmark of tumor development and metastasis is a validated target for cancer treatment, the outcomes of anti-angiogenic therapy are still far away from the desired overall benefits. On the other hand, the adverse effects of DNA crosslinking agents also limit their clinical application. The development of novel cytotoxic drugs with increased efficacy but lowered in incidence of adverse events is an unmet need. Here we designed and successfully synthesized pyrrolo[2,1-a]phthalazine derivatives comprising two pharmoceptors that suppress tumor growth via different mechanisms: anti-angiogenesis and induction of DNA crosslinks. Our data demonstrated that the hybrid pyrrolo[2,1-a]phthalazine derivatives are cytotoxic to a batch of cancer cell lines by inducing DNA damage, arresting cell cycle progression at the G2/M phase, and triggering apoptotic death. These hybrid molecules also inhibit angiogenesis by targeting VEGFR-2 on endothelial cells. We also demonstrated that compound 29d, encapsulated in a liposomal formulation, significantly suppressed the growth of small cell lung cancer cells (H526) in mouse xenograft models. At the dose used, there is no body weight loss in mice, supporting its low toxicity. By immunohistochemical staining, the tumor xenografts in mice treated with compound 29d showed time-dependently decreased intensity of CD31, a marker of blood vessel, but increased that of g-H2AX, a marker of DNA damage. Our present data suggest that the conjugation of anti-angiogenic and DNA damaging agents are potential hybrid agents for the treatment of cancer.

277 (PB-040)  Poster

PROTAC small-molecule degraders of MDM2 protein

A. Aguilar 1, J. Yang 2, Y. Li 3, D. McCachern 1, S. Przybranowski 1, L. Liu 4, C.Y. Yang 1, S. Wang 1, 1 University of Michigan, The Rogel Cancer Center and Department of Internal Medicine, Ann Arbor, USA; 2 University of Michigan, The Rogel Cancer Center and Department of Pharmacology and Internal Medicine, Ann Arbor, USA; 3 University of Michigan, The Rogel Cancer Center and Departments of Medical Chemistry and Internal Medicine, Ann Arbor, USA

The tumor suppressor function of p53 is compromised in essentially all human cancers. In about half of human cancers, wild-type p53 status is retained, however, its function is effectively inhibited by the murine double minute 2 (MDM2) protein through a direct protein–protein interaction. Therefore, inhibition of MDM2 is an attractive cancer therapeutic target and this drove the discovery of several highly potent small molecule MDM2 inhibitors that have entered clinical development for cancer treatment. Since MDM2 is a direct transcriptional target of p53, MDM2 inhibitors’ activations of p53 leads to upregulation of MDM2 mRNA and accumulation of MDM2 protein limiting their potential clinical efficacy and also may cause unwanted effects due to the oncogenic activity of MDM2 protein.

We here describe our design, synthesis and evaluation of small-molecule MDM2 degraders based on the protein-ligand targeting chimera (PROTAC) concept and our investigation of their therapeutic potential and mechanism of action in vitro and in vivo. Our MDM2 degrader effectively induces rapid degradation of MDM2 resulting in accumulation of wild-type p53 protein and activates p53 transcriptional activity in leukemia cells without accumulation of MDM2 protein. Consistent with its design to effectively degrade MDM2, our MDM2 degrader potently inhibits cell growth and induces apoptosis in nano-molar concentrations in ALL and AML cell lines >10−10–100 times more potent than the best MDM2 inhibitors; strongly activates wild-type p53 in RS4;11 xenograft tumor tissue in mice; and achieves complete tumor regression in RS4;11 xenograft model in mice at well tolerated dose-schedules. Our data suggests that targeting MDM2 degradation is a novel and very exciting therapeutic approach for the treatment of ALL and AML.

Conflict of interest: Other Substantive Relationships: Shao meng Wang, Angelo Aguilar, Jiuling Yang, and Yangbing Li, are co-inventors for MDM2 degraders disclosed in this study. The University of Michigan has filed a number of patent applications related to these MDM2 degraders, which have been licensed to Oncopia Therapeutics. Shao meng Wang is a co-founder, stock holder, and consultant for Oncopia Therapeutics. Angelo Aguilar is a consultant for Oncopia Therapeutics.

280 (PB-041)  Poster

Potent anti-tumor activity correlated with inhibition of DNA damage response genes with highly selective and orally bioavailable CDK12 covalent inhibitors

R. Poddutono 1, S. Rajagopal 2, S. Mukherjee 1, S. Manoppan 2, D. Samiulla 2, S. Nayak 2, S. Sivakumar 2, M. Ravindra 1, S. Gore 1, A. Dhudhashiya 2, K. Charmananna 2, G. Daginakatte 2, S. Chelu 2, M. Ramachandra 1, S. Samadara 1, 1 Aurigena Discovery Technologies Ltd., Medicinal Chemistry, Bangalore, India; 2 Aurigena Discovery Technologies Ltd., Pre-clinical Biology, Bangalore, India

Background: Many anti-tumor agents are used in chemotherapy for cancers. However, they also have serious side effects because they express similar growth inhibitory activity in normal cells. Meanwhile, the development of selective targeted drugs inhibiting biomolecules relating to tumor growth, invasion, and metastasis has been proceeding with the goal of selectively attacking cancer cells. It has been revealed that the proliferation of cancer stem cells involved in redevelopment, invasion, and metastasis is promoted by sphinogosine-1-phosphate (S1P). We focused on the transmembrane domains of sphinogosine 1 phosphate receptor 1 (S1P1), and hypothesized that peptides which utilized these domains would exhibit a selective anti-tumor effect in cancer cells. We designed peptides based on the transmembrane domains of S1PR1 and evaluated whether the peptides showed tumor cell growth inhibitory activity against various kinds of human tumor cells.

Material and Methods: We chemically synthesized conjugation peptides of the human S1PR1 transmembrane domains and evaluated whether the synthesized peptides showed tumor cell growth inhibitory activity against various kinds of human tumor cells.

Conclusions: We demonstrated a novel strategy for discovering an anti-tumor molecule by focusing on transmembrane domains of proteins involved in the proliferation of cancer stem cells. In particular, the peptide based on the transmembrane domain 2 of S1PR1 exhibited remarkable anti-tumor activity against human melanoma, NSCLC, renal cancer, and prostate cancer by WST assay.

Results: The peptide based on the S1PR1 transmembrane domain 2 exhibited the strongest anti-tumor activity in the synthesized peptides based on the domain from 1 to 7. The result was that more than 95% of the tumor cells were inhibited in cells treated with a concentration of 25 μM for 48 hours.

280 (PB-042)  Poster

No conflict of interest

289 (PB-043)  Poster

Selective CDK12 degraders for cancer therapy

N. Bailey Kobayashi 1,2,3, M. Sawada 4,5, Y. Hasegawa 6,7, T. Yoshida 1,2,3, 1 Academia Sinica, Institute of Biomedical Sciences, Taipei, Taiwan; 2 Industrial Technology Research Institute, Biomedical Technology and Device Research Laboratories, Hsinchu, Taiwan; 3 Academia Sinica, Institute of Cellular and Organismic Biology, Taipei, Taiwan

CDK12 is a Ser/Thr kinase that regulates the cell cycle and transcriptional activity of the tumor suppressor p53. CDK12 expression is increased in a variety of human cancers. In about half of human cancers, wild-type p53 status is retained, however, its function is effectively inhibited by the murine double minute 2 (MDM2) protein through a direct protein–protein interaction. Therefore, inhibition of MDM2 is an attractive cancer therapeutic target and this drove the discovery of several highly potent small molecule MDM2 inhibitors that have entered clinical development for cancer treatment. Since MDM2 is a direct transcriptional target of p53, MDM2 inhibitors’ activations of p53 leads to upregulation of MDM2 mRNA and accumulation of MDM2 protein limiting their potential clinical efficacy and also may cause unwanted effects due to the oncogenic activity of MDM2 protein.

We demonstrated a novel strategy for discovering an anti-tumor molecule by focusing on transmembrane domains of proteins involved in the proliferation of cancer stem cells. In particular, the peptide based on the transmembrane domain 2 of S1PR1 exhibited remarkable anti-tumor activity against human melanoma, NSCLC, renal cancer, and prostate cancer, which suggests that the peptide could be a candidate for a new anti-tumor agent.

No conflict of interest
Cyclin-dependent kinase 12 (CDK12) regulates transcription and plays a critical role in DNA damage response, splicing, and pre-RNA processing. During transcription CDK12 regulates transcription elongation by phosphorylating RNA polymerase II (RNAP II) at Serine 2 in the C-terminal domain (CTD). Overexpression of CDK12 in various tumor types supports the possibility that CDK12 has oncogenic properties similar to other transcription-associated kinases. Based on its critical role in transcription and RNA processing, CDK12 is considered as an attractive therapeutic target for cancer.

Several series of potent and selective CDK12 covalent inhibitors were identified by structure-guided and iterative medicinal chemistry approaches. Compounds were further optimized towards attaining good physicochemical properties, high potency, good selectivity and desirable pharmacokinetic profile to achieve anti-tumor activity.

Optimization of two distinct chemical series resulted in very potent and highly selective CDK12 inhibitors. The covalent mode of action for these biochemically potent compounds has been confirmed by CDK12 target engagement assay in the cellular context. These selective inhibitors showed significant anti-proliferative activity in TNBC and other cancer cell lines including those with ETS fusion, which correlated with the inhibition of pS2 (RNAP II). Anti-proliferative activity also correlated well with down-regulation of a number of DNA damage response genes including BRCA1, RAD51, ATM and FANCE1. Consistent with the inhibition of genes involved in DNA damage repair, a highly synergistic anti-proliferative activity was observed when treated in combination with cisplatin. In vivo efficacy in a mouse model of TNBC as a single agent and in combination with DNA-damaging agents are currently underway and the data will be presented.

Conflict of interest: Ownership: All authors are employees of Aurigen. Discovery Technologies Limited.

281 (PB-042) Poster Natural product inhibitors of hypoxia inducible factor-2a for the treatment of renal cell carcinoma

M.M. Lee1, D.C. Rabé1, T.C. Mckee2, G.M. Woldemichael2, J.R. Vasselli1

1National Cancer Institute, Urologic Oncology Branch, Bethesda, USA; 2Graduate School of Biomedical Sciences, New York, NY, USA

Almost all sporadic ccRCC tumors harbor a loss of von Hippel Lindau (VHL) gene function, resulting in constitutive activation of hypoxia inducible factor-alpha subunits (HIF-a) which contributes to tumorigenesis. In particular, HIF-2a acts as an oncogene, and upregulation of HIF-2a in ccRCC is often associated with poor prognosis. HIF-2a turns on transcription of a large number of HIF-regulated genes, many of which contribute to RCC associated with poor prognosis. HIF-2a turns on transcription of a large number of HIF-regulated genes, many of which contribute to RCC associated with poor prognosis. HIF-2a target genes. Using ICM-Pro protein/small molecule docking software, the three lead compounds were found to bind only to the HIF-2a PAS B domain, and not to the HIF-1a protein, suggesting a mechanism of action similar to the clinical compounds in which the inhibitor prevents HIF-2a ARNT dimerization in VHL deficient cells. Additionally, cultured cell-based assays showed the compounds to be active against ccRCC proliferation, motility, and invasion. Interestingly, treatment of ccRCC cell lines with the three lead compounds resulted in significant downregulation of HIF2A levels, a phenomenon not seen with other HIF-2a inhibitors, and a potential mechanism to circumvent or overcome resistance to this newly targeted oncogenic signaling pathway.

No conflict of interest

282 (PB-043) Poster A journey of developing a new class of active and potent PI3Kalpha inhibitors

S. Gamage1, A.C. Giddens1, K.Y. Tsang1, J.U. Flanagan1, J.D. Kendall1, W.J. Lee2, B.C. Baguley1, C.M. Buchanan1, S.M.F. Jemison1, R.P. Shepherd1, W.A. Denny1, G.W. Newcaste2

1University of Auckland, Auckland Cancer Society Research Centre, Auckland, New Zealand; 2University of Auckland, Department of Molecular Medicine and Pathology, Auckland, New Zealand

Background: Phosphoinositide 3-kinase (PI3K) is a lipid kinase, a central component in the PI3K/AKT/mTOR signalling pathway. The targeting of PI3K, and more specifically the PI3Kα isomorph, with small molecule inhibitors has been widely explored as developing anti-cancer therapeutics. Several PI3Kα inhibitors are currently under evaluation in human ccRCC (2-(2-difluoromethylbenzimidazolyl)-1,3,5-triazine (ZSTK474) is a known small molecule pan Class I (PI3K) inhibitor. Using ZSTK474 as the starting point we endeavoured to design and develop a series of new analogues to inhibit PI3Kα.

Material and Methods: We used established chemistry to form various substituted 2-(difluoromethyl)-1H-benzo[d]imidazole moieties. Reacting these with 4-(1H-chloro-1,3,5-triazine-2,4-diyl)dimorpholine we synthesized a series of ZSTK474 analogues. Then we replaced one of the morpholine groups of the ZSTK474 analogue, 4-(6-(2-(difluoromethyl))-4-methoxy-1H-benzo[d]imidazol-1-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (SN 30378) with sulphonamide containing substituents obtained a new class of active and potent PI3Kα inhibitors eg: 4-(4-(1H-chloro-1,3,5-triazine-2,4-diyl)dimorpholine (SN 32539).

Results: By doing a SAR study and replacing one of the morpholine rings of ZSTK474, with sulphonamide containing substituents obtained a new class of active and potent PI3Kα inhibitors eg: 4-(4-(1H-chloro-1,3,5-triazine-2,4-diyl)dimorpholine (SN 30378) as the lead compound of the study. By changing one of the morpholine groups to different moieties we identified a new class of active and potent PI3Kα inhibitors eg: 4-(4-(4-(4-methoxy-1H-benzo[d]imidazol-1-yl))-6-(4-(methylsulfonyl)piperazin-1-yl)-1H,3,5-triazin-2-yl)dimorpholine (SN 32539).

Conclusions: The initial SAR study identified 4-(6-(2-(difluoromethyl))-4-methoxy-1H-benzo[d]imidazol-1-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (SN 30378) as the lead compound of the study. By changing one of the morpholine groups to different moieties we identified a new class of active PI3Kα inhibitors. Finally we improved the solubility of these to obtain a potent PI3Kα inhibitor ZSTK474. By changing one of the morpholine groups to different moieties we identified a new class of active PI3Kα inhibitors. Finally we improved the solubility of these to obtain a potent PI3Kα inhibitor ZSTK474.

No conflict of interest
CRISPR/Cas9 to generate an isogenic KDM6A knockout model in the RT4 bladder cancer cell line.

Results: In our paired sample analyses, KDM6A loss was always identified in the primary tumor and early non-invasive recurrences, suggesting KDM6A loss is an early oncogenic event in bladder cancer pathogenesis. KDM6A mutation often co-occurs with mutations in other chromatin modifying genes. In patients with both KDM6A and ARID1A mutation, the ARID1A mutation was exclusive to the metastatic samples in four out of nine patients, suggesting that ARID1A mutations may play an important role in metastatic progression. Further supporting the hypothesis that KDM6A mutations typically precede those in ARID1A in bladder cancer progression, analysis of the TCGA whole-exome data from primary muscle invasive tumors demonstrated that KDM6A loss is typically a clonal alteration present in all cancer cells whereas ARID1A were more likely to be subclonal. Functional analysis of KDM6A loss in the RT4 bladder cancer cell line demonstrated that knockout of KDM6A resulted in accelerated cell growth and increased colony formation, as well as a significantly altered RNA expression profile compared to parental RT4 cells. Gene Set Enrichment Analysis (GSEA) identified significant downregulation of a number of negative feedback regulators of RAS/MAPK signaling, consistent with an increase in RAS/MAPK signaling.

Conclusions: Our data show that KDM6A loss is an early event in bladder cancer development that promotes cell growth and rewiring of the RNA expression signature whereas ARID1A mutations are a later genetic event that may play a role in metastasis. In the future, we hope to develop new therapeutic strategies that target the underlying biology of these mutations.

Document not received.

284 (PB-045) Poster
Genetic alterations of driver genes as an independent prognostic factor for disease free survival in patients with resected non-small cell lung cancer
1Shizuoka Cancer Center, Division of Thoracic Oncology, Shizuoka, Japan; 2Shizuoka Cancer Center, Division of Thoracic Oncology, Shizuoka, Japan; 3Kyoto University, School of Public Health, Graduate School of Medicine, Kyoto, Japan; 4Shizuoka Cancer Center, Research Institute, Shizuoka, Japan; 5Shizuoka Cancer Center, Division of Diagnostic Pathology, Shizuoka, Japan.

Background: Shizuoka Cancer Center launched the first prospective pan-cancer molecular profiling study in Japan in January 2014, identifying patient-specific molecular signatures via multi-omics analysis, with a view to developing cancer precision medicine. By May 2018, whole-exome sequencing (WES) for over 4,800 pts had been completed across all types of tumors. This study aims to assess the association between molecular signatures and clinical information in pts with postoperative relapse-free survival (p-rfs) of non-small cell lung cancer (NSCLC) for identification of novel prognostic factors, focusing on the association with driver mutations (mt).

Materials and Methods: Between February 2014 and September 2015, 242 pts with NSCLC; including 192 pts with adenocarcinoma (Ad) and 50 pts with squamous cell carcinoma (Sq), underwent surgery and were enrolled in this study. Surgically resected tissue was subjected to WES using an ion torrent proton platform (Thermo Fisher Scientific). Mt detected in 138 cancer-related genes listed in Vogelstein torrent platform (Thermo Fisher Scientific). Mt detected in 138 cancer-related genes listed in Vogelstein torrent platform (Thermo Fisher Scientific).

Results: The relation between the quantum entanglement in theoretical physics and metastatic SBC/CRC and results can be analysed to track the development of resistance. Novel therapeutic options are needed as there are limited treatment options for these pts. The TARGET trial continues to recruit patients from all cancer types, allocating patients to clinical trials based on the results of molecular testing.

Conclusion: ctDNA may be used for routine molecular characterisation of metastatic SBC/CRC and results can be analysed to track the development of resistance. Novel therapeutic options are needed as there are limited treatment options for these pts. The TARGET trial continues to recruit patients from all cancer types, allocating patients to clinical trials based on ctDNA and tumour profiling.

No conflict of interest

286 (PB-047) Poster
The relation between the quantum entanglement in theoretical physics as a new insight into the cancer biology
S. Niknamian1, S. Zaminpira1. 1Cellular and Molecular Biology, University of Cambridge, United Kingdom, Biology, Tehran, Iran

Quantum entanglement is a phenomenon in theoretical physics that happens when pairs or groups of particles are generated in ways that the state of each particle cannot be described independently of the others, even when the particles are separated by a large distance. Instead, a quantum state for the system is described as a whole. Based on the introduction of cancer as an evolutionary metabolic disease (EMHC), each cancerous cell is an eukaryotic cell with different metabolic rate from healthy cells due to the compromised state must be described for the system as a whole. Based on the introduction of cancer as an evolutionary metabolic disease (EMHC), each cancerous cell is an eukaryotic cell with different metabolic rate from healthy cells due to the compromised state must be described for the system as a whole.
Extracellular vesicle analysis as non-invasive tool to predict and follow treatment response in bladder cancer

P. Häkä,1 C.H. Sha,1 J. Fanebo,2 P. Hybring,1 V. Arapi,3 R. Lewensohn3, K. Viktorsson1, A. Ullen3, Karolinska Institutet, Oncology/Pathology, Stockholm, Sweden; Karolinska Institutet, Physiology and Pharmacology, Stockholm, Sweden; Theme Cancer, Karolinska University Hospital, Stockholm, Sweden

Background: Urothelial cancer of the bladder is the most common cancer of the urinary tract and about 3000 patients is diagnosed each year in Sweden. About 20–30% of these patients develop muscle invasive or metastatic disease which requires platinum-based chemotherapy. However, progression and development of resistant disease is common. Effective second line treatment is a challenge and there is unmet medical need in platinum-progressive metastatic urothelial cancer due to few treatment options and short median overall survival.

Within the international, multicentre prospective Phase I trial (VINSOR trial), we have studied the standard second-line chemotherapy drug vinflunine in combination with the targeted compound sorafenib. In this study we have explored the biomarker potential of tumor-derived extracellular vesicles (EVs) in plasma from a subset of the patients with different response profiles.

Material and Methods: Plasma and urine samples were collected prior to treatment and at day 8 and prior to cycle two in the VINSOR trial and have been stored at −80°C. Plasma from patients with different length of overall survival and progression free survival was analysed for EVs and their biomarkers. For EVs/exosomes isolation plasma was filtered through a 0.45 μm filter, and around 500 μl was loaded on IONEX exosome isolation columns. 500 μl elution fractions were collected and analyzed for size and concentration of EVs/exosomes using nanoparticle tracking analysis (NTA), EV/exosome specific markers (CD9, CD63) as well as urothelial carcinoma surface proteins (VEGFR2, FGFR3, PDGFR, Ephrin A1, EphA2 were profiled using western blotting. The miRNA expression pattern of EVs was analyzed by NanoString and further processed using Ingenuity Pathway Analyses.

Results: EVs including exosomes were efficiently isolated from plasma samples from the VINSOR-trial. Their amount and size varied among the patients both at baseline and during the treatment course and are currently explored in context of treatment response and outcome. Western blot analyzes revealed expression of the exosome marker CD9 and variances among urothelial proteins prior and post treatment. RNA was isolated from fraction 8 with the most concentrated and pure exosomes and miRNA pattern was analysed using NanoString. Differences in miRNA profiles were observed both between patients and prior and post treatment indicating a putative biomarker potential of EV/exosome derived miRNA profiling.

Conclusions: EVs were efficiently isolated from plasma of urothelial carcinoma patients. Clear differences in amount and size of EVs/exosomes, protein surface markers and miRNA expression pattern is observed during treatment. Thus analyses of EVs/exosome may hold biomarker potential to predict and follow treatment response in patients with advanced urothelial cancer treated by targeted combination chemotherapy.

No conflict of interest

Clinical significance of circulating microRNA-200c expression in breast cancer

P. Yadav1, M. Mirza2, K. Nandi3, S.K. Jain4, R. Kaza1, P. Sharma1, A. Saxena1, All India Institute of Medical Sciences, Department of Biochemistry, Jodhpur, India; 2Maulana Azad Medical College, Department of Biochemistry, New Delhi, India; 3Maulana Azad Medical College, Department of Surgery, New Delhi, India

Background: Breast cancer is the leading cause of cancer-related death in the female population. MicroRNA-200c (miR-200c), a non-coding RNA, is important in the epithelial to mesenchymal transition (EMT) and metastasis in breast cancer. The present study was focused on diagnostic and prognostic role of serum miR-200c expression in breast cancer patients.

Materials and Methods: Study includes 75 histopathologically confirmed, newly diagnosed breast cancer patients and 75 healthy controls. Serum samples were collected before and after treatment from each patient. Total RNA from serum was isolated by using Trizol reagent. Total RNA was polyadenylated and reverse transcribed into cDNA. The expression level of miR-200c was detected by using miRNA qRT-PCR. Relative expression was analyzed using U6 snRNA as a reference. Total follow up period was 41 months and mean follow up period was 28 months. Kaplan-Meier survival analysis was performed for overall survival of breast cancer patients. The present study was conducted at Maulana Azad Medical College and Associated Hospitals, New Delhi, India. The Ethical approval was obtained from the Institutional Ethics Committee of Maulana Azad Medical College, New Delhi, India.

Results: In cases, more than 8 mean fold increased microRNA-200c expression was observed compared to the healthy controls. On ROC analysis for diagnosis, An AUC of 0.743 with 69.7% sensitivity and 85.33% was observed. Level of miR-200c expression was significantly decreased (5.6 mean fold) after treatment. There was a significant association seen between miR-200c expression with TNM stage (p = 0.0001), histological grade (0.04), lymph node status (p = 0.003) and distant metastasis (p = 0.001) of breast cancer patients. ROC analysis for prognosis yielded significant AUC values (0.817 and 0.866 respectively) for early vs advanced stage and distant metastasis. There was a significant association (p = 0.006) found between overall survival and expression of miR-200c in breast cancer patients.

Conclusion: Our results suggest that circulating miR-200c overexpression might be a useful non-invasive, diagnostic and prognostic indicator for breast cancer patients. A large pool study will be required to confirm our findings.

No conflict of interest
Once macro-metastases in the liver had developed, foci of accumulated dissemi-nated cells reappeared. PC339-CVM cultures remained stable in the prop-portion of color combinations upon long-term culturing. Subcutaneous injection resulted in a primary tumor with a patchy fluorescent expression pattern indicating all labeled clones to be represented. Upon removal of the primary tumor, multiple visible (macro)metastases appeared spontaneous in the liver with each single lesion showing the same color expression, indicating a monoclonal origin. In addition, single and very small groups of fluorescently labeled cells could be identified in the lung, indicating of dormant cells and occult micro-metastases.

**Conclusions:** This preclinical metastatic liver model is a novel tool to trace the lineage of (metastatic) clones and to assess the impact of therapeutic interventions on metastatic PCDAs.

**No conflict of interest**

**290 (PB-051) Poster**

**Susceptibility of BRCA associated PDAC to immunotherapy**

C. Stossel1,2,3, D. Atlas4, Y. Glick-Gorman3, S. Halperin3, E. Jourdan1, M. Raites Gurevitch7, M. Schvimer9, R. Rencoheo, S. Gallinger2,7, G. Markel2,3, R. Ortenberg6, R. Berger1,2, T. Golan1,2,5, Sheba Medical Center, Oncology institute, Tel Hashomer, Israel; 6 Tel Aviv University, Sackler Faculty of Medicine, Tel Aviv, Israel; 7 Sheba Medical Center, Ella Lemelbaum Institute for Immuno-Oncology, Tel Hashomer, Israel; 8 Sheba Medical Center, Pathology, Tel Hashomer, Israel; 9 Ontario Institute for Cancer Research, Transnational Research Initiative in Pancreatic Cancer, Toronto, Canada; 10 University Health Network, Surgery, Toronto, Canada.

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies. Germline-BRCA1/2 mutation carriers are the most well defined DNA damage repair deficient subgroup and present up to ~15% of PDAC either de novo1, or 120-day-old NOD-SCID mice display susceptibility to DNA-damaging agents and PARP inhibition (PARPi). However, durable responses are limited and resistance evolves. Immuno therapy strategies have not shown significant clinical benefit in PDAC. DDR-deficient PDAC features increased mutational burden, which may confer sensitivity to checkpoint inhibition. Compared to the general PDAC population, BRCA1/BRCA2-mutated cases have higher incidence of PD-L1 and PD-L1 expression, respectively. We hypothesize that immunomodulating strategies may have therapeutic value in BRACamut PDAC, due to the higher, neo-antigen-encoding mutational burden.

We have developed unique patient-derived xenograft (PDX) models from metastatic PDAC patients (n = 45). These models resemble the morphologic and genomic characteristics of primary PDAC (Golan; Oncotarget; 2017). 13/42 PDX models were established from germline BRCA-mutated patients obtained at distinct time points: before treatment and at progression. Correlation between disease course at tissue acquisition and response to PARPi/ platinum was demonstrated in PDXs in-vivo (Golan; UC, 2018). 46 models were classified as "unstable"/"SDBR" by whole genome sequencing. Hematopoietic-engrafted PDAC PDX model: 3–4 week old NSG mice underwent sub-lethal irradiation and transplanted with CD34+ stem cells purified from umbilical–cord blood. Human cell engraftment (hCD45) was detected from week 12 onwards. On week 18, cryopreserved tumor chunks obtained at distinct time points; before treatment and at progression. Metastatic tissue and MYCN-overexpressing high-grade serous ovarian cancer SRA737, a novel Chk1 inhibitor, shows efficacy in CCNE1-amplified and MYCN-overexpressing preclinical ovarian cancer patient-derived xenograft models

**291 (PB-052) Poster**

**SRA737, a novel Chk1 inhibitor, shows efficacy in CCNE1-amplified and MYCN-overexpressing preclinical ovarian cancer patient-derived xenograft models**

G. H. Yn,2,3, H. Barker1, C. Vanderberg2, O. Kondrashova3, E. Kyran1, E. Liesche1, O. McNally1, A. Hamilton2,3, V. Heong1, R. Hansen2, S. Millinovic4, B. Strosus3, M. Hedrick2, C. Hassig3, D. Bowtell2, W. Matthew1, G. Scott1,3, AOCs. 1 Walter and Eliza Hall Institute for Medical Research, Stem Cells and Cancer, Melbourne, Australia; 2 Royal Women Hospital, Oncology, Melbourne, Australia; 3 Peter MacCallum Cancer Centre, Oncology, Melbourne, Australia; 4 Sierra Oncology, Sierra, Vancouver, Canada.

**Background:** Oncogene-driven high-grade serous ovarian cancers (HGSOC) with CCNE1 or MYCN pathway activation exhibit defective cell cycle checkpoint control and repressible stress (RS). The DNA damage response effector kinase, Chk1, modulates the cellular response to RS and has been shown to be upregulated in these subtypes of HGSOC. SRA737 is a novel, potent, highly selective and orally available Chk1 inhibitor that is currently under clinical investigation in high-RS cancers including HGSOC. We explored the efficacy of single agent SRA737 at three doses compared to olaparib, a PARP inhibitor recently approved for HGSOC, in our preclinical HGSOC models.

**Methods:** Two HGSOC patient derived xenografts (PDX) with defined clinicopathologic and molecular characteristics hypothesized to have high intrinsic RS were selected for in vivo drug testing. PDX #111 was generated from a HGSOC with 59-fold amplification of CCNE1 while PDX #29 has a 9-fold amplification of CCNE1 and over-expression of the MYCN pathway. Both HGSOCs were refractory to standard platinum-based therapy and were WT for BRCA1/2 and related gene mutations. Upon reaching treatment size (180–300 mm3), mice bearing PDAC tumors were randomized to daily treatment for 21 days with SRA737 (100 mg/kg, 50 mg/kg, or 25 mg/kg), olaparib (100 mg/kg) or vehicle. Tumor volume was monitored until the experimental endpoint of 700 mm3, or 120 days post completion of therapy was reached. Short term harvest (STH) experiments were also performed following a single dose of SRA737 of 100 mg/kg or 50 mg/kg. Tumors were collected at 12 hours following treatment to determine on-target drug effects.

**Results:** Treatment of PDX #111 with SRA737 at 100 mg/kg resulted in significant stabilization of disease, leading to prolonged median time to harvest (TTH) of 78 days for SRA737 vs. 43 days for vehicle (p value <0.001). As expected, olaparib treatment was less effective and inferior than SRA737 at 100 mg/kg (TTH = 46 days; p value <0.001) given the absence of BRCA1/2 mutations in this model. Impressively, in PDX #29, SRA737 at 100 mg/kg resulted in tumor regression leading to improved median TTH and outperforming olaparib (median TTH of 81 days for SRA737 100 mg/kg, 46 days for olaparib and 39 days for vehicle; p value <0.05 for both). Analysis of cell cycle and RS biomarkers in the STH samples is underway.

**Conclusion:** Chk1 inhibition by SRA737 shows promising efficacy in CCNE1-amplified and MYCN-overexpressing preclinical PDX models of HGSOC, where other targeted therapies such as PARP inhibitors show limited activity. These in vivo data support the ongoing monotherapy clinical trial of SRA737, which includes the prospective enrolment of patients with these HGSOC subtypes.

**Conflict of interest:** Corporate-sponsored Research: Sierra Oncology, Inc provided financial support for this project. Dr. Ryan Hansen, Dr. Snezana Miluninovic, Mr. Bryan Strosus, Mr. Michael Hedrick and Dr. Christian Hassig are employees of Sierra Oncology, Inc.

**292 (PB-053) Poster**

**Precision cut cancer tissue slices derived from cancer patients as a tool for the investigation of immune-modulatory compounds**

K. Bernoth1, M. Pillen1, M. Petersen1, O. Timm2, J. Krüger2, H. Juht2, K. David3, N. Grabinski1. 1 Indivumed GmbH, Research Services, Hamburg, Germany; 2 Indivumed GmbH, Chief Executive Officer, Hamburg, Germany; 3 Indivumed GmbH, Head of Research and Development, Hamburg, Germany.

**Background:** The goal of personalized medicine is to provide individual patients with the most appropriate treatment. This approach strongly depends on extensive characterization of individual tumors and their sensitivity to therapeutics. We previously have shown that our testing platform based on precision cut cancer tissue slices (PCCTS) is applicable to analyze individual responses of patients to defined compounds. In the context of immunotherapy, investigation on the abundance and activation of immune cells within individual tumors and the testing of immune-modulating compounds such as immune checkpoint modulators or bispecific antibodies gained in importance.
Material and Methods: Solid tumors were freshly collected and cut using a Krumdieck tissue slicer. PCCSTS were cultured in 24-well plates with and without treatment. Patient blood was collected prior to surgery and patient derived, viable PBMCs were isolated. After 24 h, the PCCTS were used for preparation of single cell suspension using the Miltenyi GentleMacs. Subsequently, Flow cytometry was performed on the CyFlow space instrument. In addition, proteins isolated from fresh frozen PCCTS were used for protein expression analysis by Simple Western Size (SWS). Supernatants were analyzed for cytokines by MSD technology.

Results: The PCCTS platform enabled the examination of effects of immune-modulatory compounds in a fully human, patient derived model as well as the immunological interaction with autologous, viable PBMCs. After treatment, the PCCTS were used within the drug testing platform, exhibited distinct populations of immune cells analyzed by flow cytometry using a multiplex panel i.e. for Tregs (CD127, CD25, FoxP3). In the individual cases, different numbers of Tregs were identified within the immune cell populations depending on treatment. For the analysis of cytokines in supernatants, the pro-inflammatory panel of MSD had been used. Treated PCCTS showed increasing release of cytokines such as IFN-γ, TNF-α and IL-2 compared to untreated controls. Overall viability of the PCCTS was influenced by the co-cultivation of PCCTS with autologous PBMCs. Moreover, modulation of downstream signaling of therapeutic targets, including Ras/MAPK and PI3K/AKT pathways were observed by quantifying these targets using SWS technology.

Conclusions: Inducement’s PCCTS platform represents a unique opportunity to test immune-modulatory compounds in a fully human, patient derived model that is close to the in vivo situation. The PCCTS system continues to evolve and further underlines the value of tumor microenvironment. Furthermore, this platform enables the investigation of effects and immunological interaction after addition of patient-derived PBMCs. In summary, Inducement’s PCCTS drug profiling platform is a useful tool for the preclinical investigation of immune-modulatory compounds, such as bispecific antibodies.

No conflict of interest

294 (PB-055) Poster Characterization of tumor infiltrating lymphocytes in a panel of patient-derived xenografts propagated in different humanized mouse models

E. Oswald1, D. Lenhard1, S. Schmitt2, A. Edinger1, A. Loeth1, A. L. Peile2, A. Behrens1, V. Knaut1, J. Schueler1, 1Charles River Discovery Services, Freiburg, Germany

1: The field of cancer immunology is rapidly moving towards innovative therapeutic strategies. As a consequence, the need for robust and predictive preclinical platforms arises. The current project characterizes a panel of >20 patient-derived xenografts (PDX) in different humanized mouse models. This approach elucidates the advantages of PDX models in the presence of human immune cells. New insights into the immune cell infiltration in the different tumor models and entities will help to identify the optimal pre-clinical model for specific therapies in the immune-oncology field.

2: A total of different PDX models were implanted subcutaneously (s.c.) into both flanks of 1-2 immune-deficient or humanized mice of different age, respectively. For immune cell substitution, T cells were expanded and T cells or fresh PBMCs were injected i.v. into tumor bearing mice. Body weights and tumor volumes were determined twice weekly. At termination immune cell infiltration was confirmed in peripheral blood, spleen, bone marrow and tumors by flow cytometry. Human immune cell subsets in spleen, liver and tumor tissue were assayed via Immunohistochemistry.

3: In the present study we compared tumor growth of our PDX model in the presence or absence of immune cells. The existence of immune cells, irrespective of mouse or human, had an impact on tumor growth behavior. In most models, the tumor growth was delayed in the presence of immune cells. Of note, a subset of models showed tumor growth inhibition after >20 days of human immune cell transfer. To clarify the role of different immune cell subtypes, we correlated immune cell infiltration in hematopoietic organs and tumor tissue with tumor take rate. The latter correlated negatively with the percentage of human and mouse immune cells in the tumor as well as peripheral blood. In line with this observation, cytokines indicating activation of the immune cells were upregulated in tumor-bearing immune-deficient mice, substituted with human PBMCs or expanded T cells. Of note, mice injected with human PBMCs showed the onset of GVHD earlier in the course of the experiment as mice engrafted with expanded T cells.

4: In conclusion, our study validates the PDX-based humanized mouse model more in detail. The different players within a tumor have significant impact on tumor growth behavior and biology. The design of future preclinical studies must take those characteristics into account by adapting the read-outs and the necessary control groups. In summary, the platform enables the interaction of tumor cells with human immune cells in a tumor microenvironment. This preclinical PDX based in vivo platform provides an innovative tool to support the development of new drugs targeting the host immune response.

No conflict of interest

295 (PB-056) Poster Identification of the predictive biomarker signatures for TAK-931, a CD77 inhibitor, in a preclinical phase 2-like study


Poster Session (Thursday, 15 November 2018)
Background: TAK-931, a highly specific CDCC7 kinase inhibitor, is an investigational drug under clinical development in Ph1/2 studies for treatment of advanced cancers. To identify potential predictive biomarkers for patient stratification, we conducted a preclinical phase 2-like study in various patient-derived xenograft (PDX) models including colon (n = 40), pancreas (n = 24), lung (n = 23), and ovary (n = 3).

Material and Methods: Genomic and transcriptomic profiles of these human tumors were generated via next-generation sequencing. Antitumor efficacy of TAK-931 was evaluated in these PDX models. Tumor growth inhibition and mouse survival were measured upon TAK-931 treatment. A correlative study was conducted to identify tumor genetic mutations associated with the antitumor efficacy of TAK-931.

Results: From the unbiased association analysis, specific oncogenic gene mutations were found to be significantly correlated with response to TAK-931. For example, TP53 mutations showed significant correlation in all tumor models analyzed (p < 0.0009). To identify which cancer indications are enriched with these biomarker signatures, we performed a genome-wide association analysis using the patient genomic data in The Cancer Genome Atlas (TCGA). Specific tumor types were found enriched with these biomarkers in >50% patients, which guides the cancer indication selection for clinical investigation of TAK-931.

Conclusions: The identification of predictive genomic biomarkers in preclinical PDX followed by in silico interrogation of public TCGA database may allow enrichment for patients who would most likely benefit from TAK-931 treatment and allow for an expedited path to early clinical proof of concept.

Conflict of interest: Other Substantive Relationships: Hyunjin Shin, Erik Koenig, Jie Yu, Mengkun Zhang, Karuppiah Kannan, Eric Lightcap, Akihiro Ohashi, and Huifeng Niu disclose that they are employees of Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited. Tadahiro Nambu is an employee of Takeda Pharmaceutical Co., Ltd.

296 (PB-057) Poster Novel prostate cancer models for immuno-oncology and immunotherapy research

R. Barbosa Marques1, M. van Gelder1, P. van Duijn1, C. de Ridder1, J. Trapman2, R. Debets1, W. van Weerden1, S. Postel-Vinay2, J. Trapman2, R. Debets3, Rotterdam, Netherlands; ErasmusMC, JNI, Medical Oncology, Rotterdam, Netherlands; ErasmusMC, JNI, Pathology, Rotterdam, Netherlands; ErasmusMC, JNI, Medical Oncology, Rotterdam, Netherlands; ErasmusMC, JNI, Pathology, Rotterdam, Netherlands; ErasmusMC, JNI, Pathology, Rotterdam, Netherlands.

Background: Immunotherapy is emerging as a promising treatment option in oncology. So far, clinical trials have shown significant responses in a subset of prostate cancer patients. To improve success rates, current research focuses on combination therapies, novel immune targets, as well as potential biomarkers that can help in patient selection. However, appropriate in vivo models that represent anti-tumor immune responses in prostate cancer are scarce.

Material and method: To investigate the mechanisms of tumor growth and test prostate cancer targeted therapies in the context of a functional immune system we established two novel animal model systems. The syngeneic Pten knockout mouse model of prostate cancer (MuCaP) consists of syngeneic tumor lines, injected subcutaneously in immunocompetent FVB male mice. Tumor take and growth was followed over time with calipers. Tumors and blood were collected at sacrifice. CD3+ positive cells were counted by immunohistochemistry and T-cell profile was assessed by qPCR.

To develop a humanized prostate cancer model, human immune reconstituted mice (HuNOG, Taconic, Denmark) were inoculated subcutaneously with PC339 and PC346C patient-derived xenograft (PDX) lines. Androgen deprivation was performed through surgical castration, tumor growth was monitored by calipers and blood was sampled at various time points for immune cell profiling. Blood and tumor immunoprofiles were analyzed by flow cytometry and qPCR.

Results: Two of the syngeneic MuCaP tumor lines showed aggressive tumor growth, while the two other tumor lines hardly grew and could be considered as indolent. There was a clear association between tumor aggressiveness and involvement of cytotoxic T-cells: aggressive tumors showed low T-cell infiltration, whereas indolent tumors showed increased T-cell infiltration with a characteristic gene signature of effector cytotoxic T-cells (CD8+/PD1+/IFNγ+). To establish a humanized model for the testing of immunomodulatory therapies, we transplanted human immune reconstituted HuNOG mice with prostate cancer PDX lines, which resulted in >90% take rate and consistent tumor growth, comparable to that observed in athymic nude mice. Flow cytometry of peripheral blood revealed significant reconstitution with human immune cells (>80% CD45+ cells), including CD20+ B-cells, CD4+ and CD8+ T-cells. QPCR analysis showed expression of these markers in the tumor tissue, which were increased after castration, indicative of tumor infiltration by the human immune cells.

Conclusions: Here we present two novel in vivo models that allow evaluation of novel immune-targeted therapies in prostate cancer.

No conflict of interest

297 (PB-058) Poster DNA repair deficiency sensitizes lung cancer cells to NAD+ biosynthesis blockade

M. Touat1, T. Sourisseau1, N. Djarvitt2, R.M. Chabanon2, M. Garrido2, D. Morei2, D. Krastev2, L. Bigot1, J. Adam1, J. Frankum3, A. Sarasin3, K. Olausen3, L. Frisoulet4, F. Bouillaut5, G. Pierron5, A. Ashworth6, A. Lombez7, C. Lord7, J.C. Soria8, S. Postel-Vinay1, G. Roussy9, Insère10, Villejuif, France, J. Trapman2, Rotterdam, Netherlands; ErasmusMC, JNI, Pathology, Rotterdam, Netherlands; J. Trapman2, Rotterdam, Netherlands; ErasmusMC, JNI, Pathology, Rotterdam, Netherlands; ErasmusMC, JNI, Pathology, Rotterdam, Netherlands; ErasmusMC, JNI, Pathology, Rotterdam, Netherlands.

Background: Synthetic lethality is a potent mechanism-based approach exploiting specific genetic vulnerabilities of cancer cells. ERCC1, a key protein of the nucleotide excision repair pathway, is frequently deficient in non-small cell lung cancers (NSCLC), thereby representing an attractive target for synthetic lethal approaches in this disease.

Material and Methods: Using large-scale proteomic (SILAC) and metabolomic (LC/MS-MS and LC-QTOF) profiling, we compared in-house generated isogenic models of ERCC1-proficient versus ERCC1-deficient NSCLC. Significant hits were validated using complementary assays including western blotting, gene expression profiling and immunohistochemistry (IHC) on tumor series. Sensitivity to selective nicotinamide phosphoribosyltransferase (NAMPT) inhibitors (FFR66, GNE-617) was assessed both in vitro and in vivo. Mitochondrial structure and function were characterized using electron microscopy, high-resolution respirometry, and respiratory chain spectrophotometric assays. Effects of acute and long-term ERCC1 silencing on DNA repair capabilities and NAMPT expression were evaluated using ERCC1 siRNA and shRNA silencing.

Results: We found marked metabolic rewilding of ERCC1-deficient populations, including decreased NAD+ levels, reduced expression of the NAD+ biosynthetic enzyme NAMPT and of the mitochondrial respiratory chain cytochrome c oxidase subunits COX411, COX5B, and COX6C. NAMPT protein decrease, a potentially targetable node of ERCC1-deficient NSCLC, was validated by IHC in an independent set of ERCC1-deficient human NSCLC. Exposure of ERCC1-deficient cells to NAMPT inhibitors showed profound and selective sensitivity in vitro and in vivo, which was rescued by the addition of nicotinamide mononucleotide (the direct product of NAMPT enzymatic activity) or by re-introducing the functional ERCC1 isoform.

Further metabolomic characterization showed significant mitochondrial defects in ERCC1-deficient populations, including abnormal mitochondrial structure, decreased respiratory capacity with mitochondrial respiratory chain components IV deficiency, and reduced ATP production. In the TCA cycle and NAD+ biosynthesis pathway. In functional studies evaluating the consequences of a gradual loss of ERCC1 over time, acute ERCC1 defect lead to increased ADP-ribosylation activity and NAMPT levels, whereas chronic ERCC1 defect resulted in decreased NAD+ levels, NAMPT expression and ADP-ribosylation capacities. These findings suggest a model for ERCC1-deficient NSCLC sensitivity to NAMPT inhibitors in which NAD+ is the central sensor of fitness.

Conclusions: This study opens novel therapeutic opportunities that exploit a yet undescribed nuclear – mitochondrial synthetic lethal relationship in NSCLC cells. Our findings provide preclinical rationale for the clinical evaluation of novel NAMPT inhibitors in patients with ERCC1-deficient NSCLC.

No conflict of interest

299 (PB-060) Poster Density-dependent regulation of cell growth by KIF5B-RET fusion gene

M. Lee1, J.Y. Shin1, J.O. Kim1, M.Y. Kim1, S.R. Kim2, J.H. Kang2, Cancer Research Institute, College of Medicine, The Catholic University of Korea, Laboratory of Medical Oncology, Seoul, South Korea; Seoul St. Mary’s Hospital, College of Medicine, The Catholic University of Korea 222 Banpadoaero Seochogu, Division of Medical Oncology, Department of Internal Medicine, Seoul, South Korea.

Background: RET rearrangement is detected with 1–2% frequency in lung adenocarcinoma. The KIF5B-RET fusion gene has poor response to
RET tyrosine kinase inhibitors. Hippo and TGF-β pathways are involved in signaling processes which is related to cancer cell density. They also affect the regulation of cell growth and proliferation and tumor suppression. We investigated the role of TGF-β on cell growth and proliferation in KIF5B-RET transfected cells.

Methods: We evaluated anti-proliferative activity of TGF-β/smad inhibitor in HEK293T cells transfected with KIF5B-RET fusion gene and empty vector using CCK8 assay and colony formation assay. We investigated protein expressions of signal molecules of hippoc and TGF-β pathway with cell density using western blot the signal molecules were LATS1, pYAP, pSmad2/3, and smad4. The expression of Smad2/3 and smad4 proteins was higher than that of empty vector cells, and increased in both total and nuclear proteins with high cell density. The expressions of hippoc signal molecules were mostly expressed in nuclear proteins. With low cell density, LATS1 was highly expressed in cytoplasm and nuclear protein. YAP/TAZ expression was observed in cytoplasmic protein with low cell density and in nuclear protein with high cell density.

The anti-proliferative activity of TGF-β/smad inhibitor was similar between empty vector cells (81.5 ± 1.2%) and KIF5B-RET transfected cells (88.0 ± 3.2%). The colony numbers of empty vector cells was increased between 2 days to 7 days of incubation with TGF-β/smad inhibitor (145.3 ± 0.4 vs. 162.8 ± 0.3 colonies/well, p < 0.05). However, in KIF5B-RET transfected cells, no more increase of colony numbers was observed in the period of 7 days of treatment of TGF-β/smad inhibitor (137.2 ± 0.7 colonies/well).

Conclusion: In KIF5B-RET transfected cells, TGF-β expression was increased, decreased expressions of smad2/3 and smad4 in nucleus were consistently observed as cell density increased. With high cell density, YAP/TAZ affecting cell growth and proliferation migrated from cytoplasm into the nucleus. A TGF-β/smad inhibitor significantly delayed the cell growth compared to empty vector cells.

No conflict of interest

300 (PB-061) Poster
Preclinical models of patient-derived xenografts on humanized mice for translational immuno-oncology research

M. Stecklum1, A. Wulf-Goldenberg1, M. Paterka1, B. Brzezicha1, I. Fichtner1,2, J. Hoffmann1,2,3
1Pharmatest Services, Oncology, Turku, Finland; 2Bayer AG, Berlin, Germany; 3ARC-NET University of Verona, Polotolino G.B. Rossi, Verona, Italy; 4QMUL, Barts Cancer Institute, Centre for Stem Cells in Cancer & Ageing, London, United Kingdom; 5Philips University Marburg, Center for Tumor Biology and Immunology, Marburg, Germany; 6DKFZ, University Hospital Essen, West German Cancer Center, Essen, Germany; 7Bayer AG, Oncology Research, Berlin, Germany; 8University of Miami, Sylvester Pancreatic Research Institute and Department of Surgery, Coral Gables, USA; 9EPO, Experimental Pharmacology & Oncology Berlin-Buch GmbH, Building H82, Berlin, Germany

Background: Pancreatic cancer (PC) remains a lethal disease with only 3–8% of patients surviving 5 years after diagnosis of the tumor (WHO, 2012). Within the EU project “CAM-Pac” a comprehensive panel of thirty patient-derived PC xenografts (PDX) was established and used for the efficacy screening of new therapeutic options. Within this study, responders to the MPS-1 inhibitor BAY1161909, the Super Enhancer disrupting agent Minnelide and the MEK inhibitor Trametinib were identified and analyzed for potential biomarkers.

Methods: Patient tumors were collected during surgery and circulating tumorigenic cancer stem cells were isolated from the peripheral blood using VAR2CSA-coated magnetic beads. Both were transplanted subcutaneously into NOD/SCID/Il2γ−/− mice and propagated in NMRI:nu/nu mice after engraftment. These were morphologically and molecularly characterized by histopathological revision and with NGS panels, designed based on pathway aggregated genes identified with the International Cancer consortium (described by Bailey et al., Nature 531, 2016). Standardized drugs were applied using clinically relevant dosages and schedule. MPS-1 inhibitor BAY1161909 was given in monotherapy and in combination with Abraxane. Minnelide (MTD) was applied second line after three cycles of chemotherapy (Gemcitabine, Abraxane, Gemcitabine) and Trametinib was tested as monotherapy.

Results: All PDX correlated with histopathological and molecular characteristics of patient tumours. BAY1161909 monotherapy showed more than anti-tumor efficacy with an average tumor growth inhibition of 30% (p > 0.05). However, tumor relapse after the end of chemotherapy was delayed in mice treated with the combination of BAY1161909 and Abraxane compared to Abraxane alone. 14 out of 28 PDX models tested to date were identified as responders (tumor growth inhibition >50%) to Minnelide and 5 out of 16 to Trametinib. While Minnelide induced tumor growth inhibition above 80% in 32% of the models, Trametinib achieved the same efficacy in only 6% of the tested PDX models.

Conclusion: The described PDX panel clearly reflects clinical situation of pancreatic cancer due to their histologic growth and detection of inherent and acquired treatment resistance as well as recurrent disease. In a few cases, the tested drugs induced complete remissions. We are currently analyzing the molecular data to determine response markers. Our approach may offer personalized treatment options for PC patients.

No conflict of interest

302 (PB-063) Poster
Systemic and local syngeneic bone metastasis models for immuno-oncology drug development

T. Kahkonen1, M.I. Suominen1, J. Mäki-Jouppila2, J.M. Halleen1, A. Scholz2, J. Bernoulli3, 1Pharmatest Services, Oncology, Turku, Finland; 2Bayer AG, Berlin, Germany

Bone metastases count 30–70% of metastases in the most common cancers including breast, lung and bladder cancer, and multiple myeloma. Despite recent progress in cancer treatment bone metastases remain incurable.
However, novel therapies including immunotherapies have potential to cure bone disease. The aim of this study was to establish novel syngeneic models with a focus on bone metastasis that could be used in preclinical efficacy studies.

Syngeneic models were established for breast (4T1-GFP), bladder (MBT-2) and lung cancer (KLN-205), and multiple myeloma (STG1M1). The cells were inoculated into systemic circulation (4T1 intracardially or STG1M1 into the tail vein) or into the bone marrow (MBT-2 and KLN-205 intratibially). In the 4T1 model, tumor growth was followed by GFP imaging ex vivo and in the STG1M1 model by measuring serum paraprotein and TRACP5b (marker of bone-resorbing osteoclasts) levels during the study. Tumor-induced bone changes were followed by X-ray imaging. At sacrifice, hind limbs were collected and analyzed by histology. The effects of standard-of-care (SOC) compounds were assessed in the 4T1 (cyclophosphamide, 100 mg/kg or zoledronic acid, 0.1 mg/kg) and STG1M1 (bortezomib, 1 mg/kg) models. Effect of anti-PD-1 (200 μg/dose) was evaluated in the MBT-2 model.

In the 4T1 model, osteolytic bone lesions were formed within 13 days. The lesions were imaged by X-ray, and tumor burden by GFP. About 50% of the mice had metastases in lungs, ovaries, kidneys and adrenal glands based on GFP imaging. Cyclophosphamide decreased tumor burden and the area of osteolytic bone lesions. Zoledronic acid decreased osteolytic lesion area but had no effect on tumor burden. Osteolytic lesions were observed in the STG1M1 model, and the study was ended at day 35. Soft tissue metastases were observed in ovaries, kidneys and adrenal glands in about 30% of the mice. Bortezomib decreased serum paraprotein and TRACP5b levels compared to the control group. In both 4T1 and STG1M1 models, cachexia and hind-limb paralysis were occasionally observed. In the intratibial MBT-2 and KLN-205 models, large osteolytic lesions were observed within 28 days from cancer cell inoculation, and in the KLN-205 model also lung metastases were observed. Anti-PD-1 treatment decreased osteolytic tumor area in the MBT-2 model. Histological tumors were confirmed in the bone marrow of hind limbs in all models.

A high incidence of bone metastases was achieved in all models. The use of systemic models allows studying the effects of test compounds in prevention or treatment of metastasis. Intratibial models can be used when the primary interest is in cancer-induced changes in bone. Mimicking the clinical situation, none of the SOC compounds could prevent tumor growth completely, and therefore combination therapies are warranted for better overall efficacy.

No conflict of interest

304 (PB-065) Poster HuPharm: a standardized interactive data analysis and reporting platform for preclinical cancer pharmacology

B. Mao1, J. Li1, S. Guo1, H. Li1, 1Crown Bioscience Inc., Systems Biology, Taicang, China; 2Crown Bioscience Inc., Global Scientific Research and Innovations, San Diego, USA

Background: One of the essential components of any anti-cancer drug development is preclinical cancer pharmacology study that is commonly consisted of design, planning, execution, data analysis and reporting. The reliability of the scientific conclusions based on proper design/data integrity is the foundation to the decision to advance agent, or not, to next step human clinical trials. In addition, a standard data analysis platform not only saves labor and reduces errors, but also helps to achieve objective interpretations and comparisons for data generated from different laboratories and/or study directors. This report aims at developing a web platform (HuPharmTM) that automates statistical pipelines and report generation for standard preclinical anti-cancer pharmacology, with intuitive and interactive interface that enables cancer pharmacologists, often non-statisticians, effectively handling data validation, exploratory data analysis and statistical analysis.

Material and Methods: We used RStudio’s Shiny package to build an interactive web app (HuPharmTM), and we used Knitr and Rmarkdown packages to generate a highly informative study report. Parametric and non-parametric statistics is used to analyze pharmacology data from two or more groups by a fully automated workflow.

Results: Based upon user inputs, HuPharmTM can dynamically generate a series of tables and figures to facilitate preclinical data analysis, such as tumor volume summary statistic table and plots, tumor growth curves, survival curves, group comparison plots, all pairwise group comparison tables and post hoc analysis results table. Data, tables and figures can be downloaded in multiple formats. In addition, HuPharmTM can generate an all-in-one study report including all figures and tables.

Conclusions: HuPharmTM is a convenient tool to aid researchers in the field of preclinical tumor trials for study design and data analysis. It likely adds rigor and objectivity into preclinical cancer pharmacology studies, while saving time and labor.

No conflict of interest

305 (PB-066) Poster Evaluation of categorical response methods in predicting drug responses in transplanted tumor models

X. Jiang1, H. Li1, S. Guo1, 1Crown Bioscience Inc., Systems Biology, Taicang, China; 2Crown Bioscience Inc., Global Scientific Research and Innovations, San Diego, USA

Background: Transplanted tumor mouse models are widely used in preclinical oncology studies to evaluate efficacy of therapeutics. Recently, prediction drug accuracy using single mouse per group or per study has been systematically assessed, but only on patient-derived xenograft models (PDXs). Systematic research of the same on cell line derived xenografts (CDXs) and syngeneic tumor models (homografts) are lacking.

Material and Methods: We evaluated three methods that all classify drug response into several categories for their accuracy in predicting drug response among one or multiple mice for all three transplanted tumor models (PDX, CDX and syngeneic) using tumor volume data for >30,000 mice. These methods classified the drug responses into three, four, or six categories. We collected studies with eight or more mice and assigned each mouse a categorical response. A most frequent response was designated as the “Majority Response.” We then counted the number of mice whose response agreed with or deviated from the Majority Response. The response concordance/deviation profiles for all studies were then used to quantify the prediction accuracy of those methods. To investigate how the addition of mice improves prediction accuracy, we randomly sampled n (n = 1 to 7) mice from a study and obtained the median response by ranking the n responses, which was then compared to the Majority Response. The full analysis was applied to PDXs, CDXs, and syngeneic models to obtain and compare their response and mouse number patterns.

Results: By examining the very large efficacy datasets we have accumulated over the decade, we found that when the Majority Response is the two ends of efficacy spectrum, being complete response (CR) and progressive disease (PD), a single mouse response agrees more often with the Majority Response than responses in between (e.g., stable disease (SD), or partial response PR) for all three methods. Such agreement varies by tumor model platforms, where syngeneic models exhibit larger variations in their drug response. Further, increasing mouse numbers improves prediction accuracy but such accuracy varies greatly among response categories. Quantitative results are presented in graphs and tables to illustrate the above observations.

Conclusions: Large preclinical anti-cancer pharmacology datasets can be used to derive the technical parameters that help to guide study design and data analysis for better assessing drug efficacy.

No conflict of interest

306 (PB-067) Poster Cathepsins K and X as possible biomarkers in glioblastoma

B. Breznik1, A. Porčnik2, M. Koprivnikar Krajnc1, J. Kos3, C.J.F. Van Noorden1, T.T. Lah1, 1National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Ljubljana, Slovenia; 2University Medical Centre Ljubljana, Department of Neurosurgery, Ljubljana, Slovenia; Faculty of Pharmacy, University of Ljubljana, Department of Pharmaceutical Biology, Ljubljana, Slovenia

Background: Glioblastoma, the most malignant brain tumor, is characterized by the single cell invasion of cancer cells into brain parenchyma, preventing complete tumor resection by surgery. Cysteine proteases cathepsins B, S and L, which are involved in malignant progression, have been found to be overexpressed in glioblastoma. Here, we focused on cathepsins K and X, which have been poorly investigated in glioblastoma progression, but have an important role in other types of cancers.

Material and Methods: Protein expression of cathepsins K and X in glioblastoma samples was localized and semi-quantified using immunohistochemistry. Kaplan-Maier survival analysis was performed to evaluate the predictive value of cathepsin K and X expression on the protein levels. On the other hand, gene expression of cathepsins K and X were correlated with glioma grade, glioblastoma subtype and overall survival of glioblastoma patients using publically-available transcriptomic datasets.

Results: With respect to localization, we have shown that cathepsin K and X proteins were clustered in CD133+, SDF-1α- and CD68-positive glioblastoma stem cell niche regions around arteries and gene expression of cathepsins K and X correlated with gene expression of niche markers. Glioblastoma stem cell niches are the regions within GBM tissue, where the therapy-resistant and highly-malignant glioblastoma stem cells are protected from therapy and immune system. Protein expression of cathepsins K and X...
was highly and heterogeneously expressed in the glioblastoma samples. Gene expression was highest in glioblastomas with BRCA2 mutations carriers. CD9 expression was particularly in the mesenchymal glioblastoma subtype. High CD9 expression and mRNA expression but not protein expression correlated with poor patient survival. However, cathepsin K expression at mRNA level and glioblastoma patient survival were not found.

Conclusions: Presence of both cathepsins in glioblastoma stem cell niche regions indicates specific roles in glioblastoma stem cell regulation. Cathepsins K and X are highly expressed in glioblastoma tissue and cathepsin X gene expression level has predictive value for patients with glioblastoma. The results have to be confirmed in further prospective studies.

No conflict of interest

307 (PB-058) Poster Transmembrane protein CD9 as a biomarker in different subtypes of glioblastoma
M. Novak1, B. Breznik1, B. Majc1, N. Podgeraja1, M. Nikic-Zakej2, A. Portnik1, J. Mikar2, T.T. Laih1, N1 National Institute of Biology, Department of Genetic Toxicology and Cancer Biology, Ljubljana, Slovenia; 2Institute of Oncology Ljubljana, Department of Experimental Oncology, Ljubljana, Slovenia; 3University Clinical Centre Ljubljana, Department of Neurosurgery, Ljubljana, Slovenia; 4Institute of Pathology, Faculty of Medicine, Ljubljana, Slovenia

Background: The tetraspanin CD9 has been shown to be involved in various cellular activities, including tumor cell invasion, apoptosis and radiosensitivity to chemotherapy. Based on public REBRAND database for brain tumors, CD9 high expression was linked with shorter patient survival. Glioblastoma (GBM) consists of heterogeneous cell types including a subset of stem cell-like cells (GSC) presumably sustaining tumor growth. Silencing of CD9 in glioblastoma cell lines led to decreased cell proliferation, survival, invasion and self-renewal ability. It was enriched across a larger set of GSCs, but not in the normal brain counterparts, thus CD9 may be evaluated as a GSC-biomarker.

Material and Methods: Primary GBM and GSC cell lines were established from a set of patient GBM tumors of different subtypes, from a joint bio-bank of TRANS-GLIOMA project partners (Slovenia-Italy) called GLIOBANK. Quantitative extraction kit was used to isolate mRNA and proteins from GBM tumors, GBM and GSC cell lines. GBM subtypes (proneural (PN), mesenchymal (MES), classical and neural subtype), were defined using 12 fingerprint signature genes (COL1A2, COL1A1, TGFBI, THBS1, DAB2, S100A4, P2RX7, STMN4, SOX10, ERBB3, ACSBG1 and KCN1) using RT-qPCR according to Behnan (2017) and Breznik (2017). GSC cells were irradiated (IR), using Glnay 225 X-ray system. Lentiviral shRNA method was used to silenced CD9. Clonogenic assay and immunohistochemistry were done according to the standard protocol.

Results: CD9 is differently expressed in a cohort of tumor tissue samples of different subtypes and in GBM and GSC cell lines. It is up-regulated in GSC cell lines isolated from tumor tissue samples and in GBM MES subtype. When CD9 was silenced in GSC cells, the one that were irradiated with lower doses had bigger effect on plating efficiency. When using higher irradiation doses, CD9 seemed to act as a tumor suppressor.

Conclusions: Although molecular mechanism of CD9 activity were proposed (Shih, 2017), its role in GSC radiosensitivity remains elusive. Screening for CD9 expression in cohort of patient tumor samples and cell lines, shows different expression pattern in different GBM subtypes. Data provides an opportunity to explore/identify CD9 as a novel, precision biomarker/therapeutics for GBM.

No conflict of interest

308 (PB-069) Poster Exploiting the spectrum of BRCA-associated Pancreatic Ductal Adenocarcinoma
M. Raajtes1, C. Stossel1, D. Atlas1, S. Halperin1, Y. Glick-Gorman1, J.M. Wilson2, R.E. Denroche1, I. Lungu3, S. Gaillingerg, T. Golan1, N1 Sheba Medical Center, Oncology Institute, Ramat Gan, Israel; 2Ontario Institute for Cancer Research, PanCurex, Translational Research Initiative in Pancreatic Cancer, Toronto, Canada; 3Ontario Institute for Cancer Research, Department of Surgery, University Health Network, Toronto, Canada

Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies. BRCA-association is one of the most studied PDAC subtypes, as it is a most studied PDAC subtype. A portion of these patients is highly susceptible to DNA damaging therapies (DDRT) including PARP inhibition (PARPi), however, responses are heterogeneous and clinical resistance evolves. This differential response among germline (GBM) BRCA mutator tumors is suggested to reflect of additional features underlying the nature of response/resistance to treatment. In the whole genome sequencing (WGS) analysis on patient-derived xenografts (PDX) established from BRCA-associated PDAC patients, we have shown correlation between genomic subtype and response to treatment in pre-clinical model and patients’ clinical scenario. Recent studies in ovarian carcinoma patients have suggested that the absence of locus-specific loss of heterozygosity (LOH) may be a biomarker of primary resistance to DDR/PARPi.

The objective of this study was to identify the established genomic classification of BRCA-associated PDAC tumors and to investigate additional genomic features predictive of resistance.

Methods: We have performed an analysis on clinical data of PDAC patients with GL/somatic mutations in BRCA1/2 genes diagnosed at Sheba (Israel) and OICR (Canada) in conjunction with comprehensive WGS analysis of corresponding tumor/PDX samples. We analyzed both primary resected tumors along with PDXs established from primary/metastatic lesions and performed classification into genomic subtypes. Additionally, loss of heterozygosity (LOH) was tested.

Results: Overall, 63 cases of BRCA-associated PDAC were identified in both institutions (July 2008 – Feb 2018). Sixty-two were carriers of GL BRCA1/2 mutations and one harbored somatic mutation in BRCA2. Stage at diagnosis was I/II (n = 16) and III/IV (n = 47); a recurrence of disease was observed in the majority (10/16) of patients with early stage. More than 30% are alive with disease and responding to platinum/PARPi for >12 months, three patients demonstrate a complete response >3 years. A subset of patients (~25%) demonstrated resistance and limited response and died <9 months from diagnosis/progression.

Preliminary WGS data analysis of 5 PDXs and 13 primary resected tumors showed strong correlation between genomic subtype and overall survival. LOH status showed correlation with unstable genomes in 9/12 cases. Additionally, 4/8 stage I/II patients who did not recur, display the unstable genome subtype.

Conclusion: Analysis of 18 BRCA-associated PDAC cases analyzed by WGS presented here, demonstrated correlation between unstable genomic subtype and response to treatment with DDR agents/PARPi. Genomic data to be extracted from WGS and LOH analyses of additional samples will provide more insight into understanding the spectrum of response to treatment.

No conflict of interest

309 (PB-070) Poster The in vivo screen: a format allowing the identification of sensitive PDX models
N. Caushaj1, T. Metz2, J. Schmollinger1, A.L. Peille1, S. Gorynia1, N1 Charles River Discovery Research Services, GmbH, Freiburg, Germany

Background: Patient-derived tumor xenografts (PDXs) represent the gold standard for preclinical efficacy testing of anti-cancer agents. Two main assay formats are commonly used for in vivo efficacy testing: the standard model with group sizes of 6 to 12 mice and the single mouse trial (SMT) format with one mouse per model per treatment. The former approach is typically used for tests with a small number of tumor models whereas the latter is used for large numbers of PDX models. While the standard PDX format can demonstrate statistically significant efficacy of a given treatment in a pre-selected PDX model, the single mouse trial allows determination of response rates at the population level. The disadvantages of both approaches are that the former, because of the required resources, is not suitable for testing a larger number of models while the latter is not fully reliable when it comes to the sensitivity of individual tumor models. We developed an alternative third test format, the in vivo screen, with a group size of three tumor-bearing mice. This group size is still small enough to allow the screening of a large number of models but at the same time increases the confidence in the results obtained for individual models.

Material and Methods: For in vivo screen experiments immunocompromised nude (NMRI nu/nu) bearing a subcutaneous tumor of 50–250 mm³ were used. Per treatment arm three animals were dosed with either the vehicle only or an investigative compound. Tumor volumes were determined twice weekly by caliper measurements. Tumor models used for the in vivo screen were either chosen randomly or pre-selected, e.g. based on specific molecular characteristics.

Results: Our results suggest that three animals per treatment arm are sufficient to accurately identify drug sensitive models. Compared with the single mouse trial, which focuses on a population response, the in vivo screen serves as a rapid and reliable tool to identify responsive models within a large PDX-associated collection. The data show that with a heterogeneously growing model the risk of one tumor exhibiting aberrant growth is comparatively high. Using three tumor-bearing animals per group reduces the impact of aberrant tumor growth and minimizes the risk of false positive or negative results.

No conflict of interest
Conclusions: Our findings demonstrate that the in vivo screen is a cost- and time-effective approach to identify sensitive models within large tumour panels. The identified sensitive models can be used for follow-up experiments using the standard in vivo format and the data generated can also be used for biomarker identification.

No conflict of interest

310 (PB-071) Poster Development of a disseminated AML Mice model to evaluate the therapeutic activity of immune checkpoint inhibitors using bioluminescence imaging

V. Mahajan1, T. Bu1, J. Belle1, L. Usher1, E. Rainbolt1, S. Kolder1, D. Harris1

1Charles River Laboratories, Discovery Services, Morrisville, USA

Acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL) are among the most common leukemia subtypes diagnosed in adults. Treatment options include chemotherapy, stem cell transplantation and more recently the use of checkpoint inhibitors such as anti-PD-1 and anti-CTLA-4 to enhance antitumor responses. In this study we developed a disseminated AML disease model using LD17, C1498 tumor cells to evaluate the therapeutic response to immune checkpoint inhibitors, PD-1, PD-L1 and CTLA-4 and to examine the effect of treatment on infiltration of immune cell populations in selected tissues. C1498 cells were transduced with a lentivirus vector construct containing luciferase and GFP reporter genes. Upon establishment of a clonal population with stable reporter expression, C1498-luc3-gfp expressing cells were implanted intravenously into syngeneic albino C57BL/6 mice and growth of the leukemic tumor cells was monitored via in vivo bioluminescence imaging (BLI) using IVIS Spectrum CT1. In vehicle-treated animals tumor growth was progressive and most animals reached endpoint within 30 days. Luciferase expression was widespread with metastatic development in peripheral organs and tissues. An immune response to therapy directed against the PD-1/PD-L1 axis was observed, with anti-PD-L1 showing greater overall efficacy than anti-PD-1. However, combination of anti-PD-1 and not anti-PDL-1 with anti-CTLA-4 further improved the therapeutic efficacy as measured by prolonged survival and reduced tumor growth. To understand how these immunotherapeutic function in suppressing tumor growth we are analyzing the composition and phenotype of tumors and immune cell infiltrates by flow cytometry, histology, and mRNA transcriptome array analysis. In summary, comprehensive immunological characterization of the C1498 model revealed the importance of PD/L-PD-L1 pathway in immune evasion and provides a rationale for therapeutic intervention with checkpoint blockade.

No conflict of interest

311 (PB-072) Poster Predicting PARPi sensitivity in patient derived ex vivo 3D tumour cultures

S. Basten1, J. Overkamp1, B. Herpers1, K. Yan1, L. Price2, W. Vader2

1OcellO, OcellO BV, Leiden, Netherlands; 2VitroScan, Predictive Diagnostics, Leiden, Netherlands

Background: Poly(ADP-ribose) polymerase (PARP) inhibitors are a new class of anticancer drugs designed to target BRCA1/2-associated hereditary cancers and sporadic tumors with defects in homologous recombination (HR). Several PARP inhibitors (PARPi) have been approved as maintenance therapy for women with platinum-sensitive epithelial ovarian cancer. However, a significant proportion of patients do not respond to PARPi therapy and the treatment comes at high cost. To date it is not possible to predict which patients will respond to treatment. Tools to improve stratification for PARPi treatment in the clinic would be of great value.

We have initiated clinical trials to validate our drug response platform for treatment response prediction for cancer patients, including ovarian cancer. Our technology, which is based on image analysis of 3D tumour cultures, accommodates accurate evaluation of drug sensitivity with small amounts of heterogeneous tumour material (tumour, ascites). Based on the results from these trials we are developing diagnostics to predict drug responses for cancer patients.

Methods: 3D cultures embedded in a protein-rich hydrogel are generated from tumour biopsies, and exposed to standard-of-care therapies, targeted therapies and drug combinations. An automated high content screening platform measures cell and tissue morphology, and reports responses such as tumour cell killing, growth arrest and local invasion. Per tumour type and drug, a time-effective approach to identify sensitive models is used for the response. We correlate clinical response on standard of care drugs (e.g. carboplatin, paclitaxel, olaparib, niraparib) with drug response of patients’ tumour cultures, and associated genetic defects (BRCA1/2, HR). Our x-omics approach has an integrated database and biobank for testing novel molecules to benchmark these against standard of care drugs.

Results: We present first results of drug sensitivity in patient derived ex vivo 3D tumour cultures of fresh and cryopreserved tumour material. Standard-of-care therapies were tested and results are compared with clinical response. Differentiated drug responses are identified for treatment schedules including platinum-based drugs, taxanes, PARPi’s.

Conclusion: Our technology enables drug sensitivity testing in ex vivo 3D cultures from patients. This allows evaluation of patient-specific treatment responses to novel and standard-of-care drugs. Ongoing trials will reveal the correlation of our in vitro test with treatment responses and relevant diagnostics parameters in the clinic.

Conflict of interest: Ownership: Leo Price is a shareholder of OcellO BV. VitroScan B.V. Board of Directors: Willemijn Vader is a director of VitroScan. Leo Price is a director of VitroScan and OcellO.

312 (PB-073) Poster Genomic characterization of Chinese kidney cancer revealed novel prognostic difference across populations

Q. Zhao1, J. Xue2, W. Qian2, T. Liu3, B. Fan3, J. Cai3, Y. Li1, J. Liu1, Y. Yang1, H. Li5, S. Guo5, N. Zhang4, 1Beijing Cancer Hospital, Department of Urology, Beijing, China; 2Crown Bioscience Inc., Systems Biology, Taicang, China; 3Daqing Oilfield General Hospital, Department of Urology, Daqing, China; 4Crown Bioscience Inc., Genomics and Biomarker, Beijing, China; 5Crown Bioscience Inc., Global Scientific Research and Innovations, San Diego, USA

Background: Kidney cancer causes increasing mortality worldwide and in the Chinese patient population. Large scale omics collections like the Cancer Atlas Genome (TCGA) have been released and analyzed for hundreds of kidney cancer patients, mostly North American origins, but the data focusing on cases in the Chinese kidney cancer (CKC) patient population have yet to be reported for comparison.

Material and Methods: We performed transcriptomic profiling and genomic analysis of 65 tumor tissues and 12 matched normal tissues from CKC patients, of which clear cell renal cell carcinoma (ccRCC) is the vast majority (84.6%), followed by papillary (7.7%), chromophobe (3.1%) and medullary (3.1%) RCC.

Results: Transcriptional sequence revealed that PBRM1 mutants with a frequency of 11% in the Chinese ccRCC patients, much lower than that in the TCGA Caucasians (34%), which is confirmed by RT-PCR of the targeted sequencing. We identified 34 gene fusion events including 6 recurrent ones, many are associated with apoptosis, cancer suppression and metastasis. We classified the Chinese ccRCC patients into three classes by gene expression. Class 1 shows significantly elevated gene expression in the VEGF pathway, while Class 3 is comparably depleted. Class 2 is characterized by increased expression of extracellular matrix organization genes and is strongly associated with high-grade tumors. We applied the expression signature to TCGA ccRCC patients and found that it better distinguished tumor prognosis than reported ones[1]. Class 2 showed the worst survival and Class 3 is a rare subtype in RCC in the TCGA cohort. Computational analysis on the immune microenvironment of CKC identified immune-active tumors with remarkably elevated CD8 positive T cells, thus may benefit from immunotherapies. We also found that resting B cells and activation of NK cells are important factors affecting tumor suppression.

Conclusions: CKC patients have distinct genomic profiles that can help cancer prognosis and treatment.

No conflict of interest

313 (PB-074) Poster Orthotopic homograft tumors (syngeneic) display distinct tumor infiltrate immune cells patterns from that of subcutaneous counterparts

X. An1, X. Tu1, M. Zipeto2, X. Feng1, C. Jiang1, H. Li1, 1Crown Bioscience Inc., Scientific Research Innovation, Taicang, China; 2Crown Bioscience Inc., Scientific Operation, San Diego, USA

Background: Subcutaneous homograft, including syngeneic cell derived mouse tumors are the most commonly used preclinical pharmacology models for immunology research.1 The subcutaneous tumors are readily transplanted and monitored. On the other hand, more difficult orthotopically implanted tumors may be more biologically and pharmacologically relevant to true human cancer for growth in more relevant environments. It is widely accepted that tumor microenvironment (TME), including tumor infiltrate immune cells (TILs) play significant role in tumor biology as well as pharmacology, particularly in response to I/O treatment, Abstracts, 30th EORTC-NCI-AACR Symposium Poster Session (Thursday, 15 November 2018)
which maybe better maintained in orthotopic model as compared to subcutaneous models, leading to different properties between the two types of transplantations.

Materials and Methods: We compared histopathology (HE staining) of tumor and immunohistochemistry (IH) staining of TILs of both syngeneic cancer cell line derived tumors model and homograft of primary mouse tumors model (MuPrime™, most derived from tumorigenic GEMM) between subcutaneous and orthotopic transplants (pancreatic models were used in this study), in order to assess whether there are difference in TILs between the two transplantations.

Results: Our examinations documented several distinct features of TILs. First, TILs, including T cells and macrophages, are highly concentrated along the edge of the tumors, in contrast to that the TILs are significantly more infiltrated into the tumors in orthotopic tumors (e.g. pancreatic models were used in this study). Second, in subcutaneous tumors, the I/O treatments, e.g. anti-PD1, anti-CTLA4 antibody or the combination resulted in further infiltration of TILs from tumor margin into tumor center. Third, overall the larger tumors have fewer TILs than smaller ones, regardless of transplantation type.

Conclusions: In general, orthotopic homograft mouse tumors demonstrated distinct TIL pattern from those of subcutaneous counterparts, which may contribute to different tumor biology and pharmacology (I/O).

No conflict of interest

314 (PB-075) Poster Image-based analysis of the myeloid cell landscape in the 3D co-culture with tumor cells G. Goverse1, K. Yan1, L. Guelen1, P. Vink1, L. Price1, L. Daszkiewicz1, 1OcellO, Immuno-Oncology, Leiden, Netherlands; 2Aduro Biotech, Inc., Drug Discovery, Oss, Netherlands

Background: The myeloid cell compartment plays an important role in anti-tumor immune responses and represents a heterogeneous population with both cancer-promoting and cancer-restraining actions. Unleashing the full potential of cancer immunotherapies requires an understanding of the cellular mechanisms that govern these opposite actions. To date, high throughput image-based platforms have shown promise in dissecting the interactions between different cellular players in the tumor microenvironment are lacking. Previously we have shown that our 3D image-based co-culture system allows assessing efficacy of immune-modulators to enhance PD1BM infiltration and tumoroid killing. Our main goal was to improve this model by incorporating a more complete human immune system. To do that we first generated diverse myeloid populations in a 3D environment and then used our image-based platform to describe the different subsets. The image analysis software was trained on a set of features that reproducibly allowed discrimination between undifferentiated monocytes, M1 and M2 macrophages and dendritic cells. The different myeloid subsets were next co-cultured with tumor cells to analyze the complex cellular interplay of the TME.

Material and Methods: Different myeloid populations were generated in 3D from monocytes derived from healthy donors PBMCs. Polarized M1 and M2 macrophages and undifferentiated monocytes were then co-cultured in 3D with SKBR3 tumor cells or 3D tumoroids derived from this cell line. The cellular interactions were visualized using high-content microscopy and quantified with multiparametric morphometric analysis with OMiner™ software.

Results: 3D image analysis enabled the discrimination of immune-tumor cell interactions and revealed the effect of myeloid cells on tumor growth in co-culture. Our approach also enables the analysis of how tumor-driven mechanisms regulate myeloid cell differentiation and contribute to the immunosuppressive microenvironment. These results provide a means to elucidate the bi-directional interplay between tumor and immune cells and allows for analysis of functional reprogramming of the suppressive population towards a M1 phenotype induced by drug candidates.

Conclusions: The 3D assay presented here enables visualization and measurement of effects of immunotherapies on cells that engage in a more physiologically relevant spatial setting than when culturing them in traditional 2D cultures. Using morphological measurements different myeloid cell subsets can be distinguished, which offers a very attractive alternative for complex and labor-intensive phenotyping based on markers expression and induced Wnt activity.

Background: The myeloid compartment presents a heterogeneous population with both cancer-promoting and cancer-restraining actions. Understanding the cellular mechanisms that govern these opposite actions is essential for the development of more effective cancer immunotherapies. To date, high throughput image-based platforms have shown promise in dissecting the interactions between different cellular players in the tumor microenvironment. Previously we have shown that our 3D image-based co-culture system allows assessing efficacy of immune modulators to enhance PD1BM infiltration and tumoroid killing. Our main goal was to improve this model by incorporating a more complete human immune system. To do that we first generated diverse myeloid populations in a 3D environment and then used our image-based platform to describe the different subsets.

Material and Methods: Different myeloid populations were generated in 3D from monocytes derived from healthy donors PBMCs. Polarized M1 and M2 macrophages and undifferentiated monocytes were then co-cultured in 3D with SKBR3 tumor cells or 3D tumoroids derived from this cell line. The cellular interactions were visualized using high-content microscopy and quantified with multiparametric morphometric analysis with OMiner™ software.

Results: 3D image analysis enabled the discrimination of immune-tumor cell interactions and revealed the effect of myeloid cells on tumor growth in co-culture. Our approach also enables the analysis of how tumor-driven mechanisms regulate myeloid cell differentiation and contribute to the immunosuppressive microenvironment. These results provide a means to elucidate the bi-directional interplay between tumor and immune cells and allows for analysis of functional reprogramming of the suppressive population towards a M1 phenotype induced by drug candidates.

Conclusions: The 3D assay presented here enables visualization and measurement of effects of immunotherapies on cells that engage in a more physiologically relevant spatial setting than when culturing them in traditional 2D cultures. Using morphological measurements different myeloid cell subsets can be distinguished, which offers a very attractive alternative for complex and labor-intensive phenotyping based on markers expression and induced Wnt activity. The ultimate goal is to develop a highly sophisticated platform for testing cancer immunotherapies that combines the complexity of the TME and the robustness of a high throughput screening platform.

Conflict of interest: Corporate-sponsored Research: Gera Goverse, Kuan Yan, Leo Price, Lidia Daszkiewicz are full time employees of OcellO, B.V. Lars Guelen, Paul Vink are full time employees of Aduro Biotech, Inc. Both companies co-sponsored this research.

Poster Session (Thursday, 15 November 2018) Abstracts, 30th EORTC-NCI-AACR Symposium
Results: From anatomical coronal MR images necrotic area within tumor was clearly visible. Also, increased diffusion coefficient values were seen over tumor progression, most likely originating from dominant necrotic core. In DCE-MRI dynamic gadolinium enhancing experiment different perfusion rate was seen in total, outer area and core part of the tumor. Rapid tumor growth often leads to inadequate angiogenesis resulting to hypoxic and even necrotic areas in tumor mass, which was visualized and quantified using corresponding tracers for metabolism and hypoxia in PET imaging. The images showed clearly both the metabolically active and hypoxic regions within the tumor. Standard uptake values (SUV) of hypoxia and metabolic activity tracers were analysed. These values can be used to evaluate treatment response to tumor growth.

Conclusions: In this study various changes related to tumor progression were studied using MRI and PET. In MRI necrotic areas within tumor, diffusion, perfusion rate in different parts of the tumor as well metabolites indicating tumor status were followed. From the PET data hypoxia and metabolic profiling was assessed. As a summary, in vivo imaging studies in animal models provides a powerful and translational research tool for comprehensive evaluation of neoplastic disease progression and treatment efficacy.

No conflict of interest

317 (PB-078)

Poster Anti-podoplanin cancer-specific antibody is advantageous for antitumor activities and a prognostic marker of oral cancer

Y. Kato1, M. Kaneko1, S. Yamada1, Y. Sawá2, 1Tohoku University Graduate School of Medicine, Department of Antibody Drug Development, Sendai, Japan; 2Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Department of Oral Function & Anatomy, Okayama, Japan

Background: Podoplanin/PDPN, a ligand of CLEC-2, is involved in platelet aggregation and cancer metastasis. The physiological function of PDPN has been reported to be very important in many normal tissues such as type I alveolar cells of lung, podocytes of kidney, and lymphatic endothelial cells. In this study, we aimed to produce cancer-specific anti-PDPN monoclonal antibodies (mAbs). Furthermore, we investigated whether the reactivity of cancer-specific anti-PDPN mAbs might be a prognostic marker of oral cancer.

Material and Methods: We immunized mice with LN229/PDPN cells (PDPN-transfected glioblastoma cell line), and selected mAbs, which react with PDPN-expressing cancer cells such as LN319 (glioblastoma cell line) and PC-10 (lung squamous cell carcinoma cell line) and do not react with PDPN-expressing normal cells such as primary lymphatic endothelial cells (LECs) and HEK-293T (renal epithelial cell line) in flow cytometry. We further investigated the reactivity of anti-PDPN mAbs using immunohistochemistry (IHC) against oral cancers. The binding epitope of anti-PDPN mAbs was analyzed using alanine scanning method. Antibody-dependent cellular cytotoxicity (ADCC) and anti-tumor activities were examined against oral cancer cells. Finally, we compared the reactivity of well-known anti-PDPN mAbs, D2-40 and a newly established cancer-specific anti-PDPN mAb using IHC about 50 oral cancer patients. The association between the reactivity of anti-PDPN mAbs and clinical/pathological features were analyzed.

Results: We established a cancer-specific anti-PDPN mAb (clone: LpMab-23). LpMab-23 reacted with PDPN-expressing cancer cell lines whereas it weakly recognized PDPN-expressing normal cells in flow cytometry. LpMab-23 reacted only with PDPN-expressing cancer cells, not with LECs in oral cancer tissues using IHC although LECs were detected by D2-40 in all oral cancer tissues. Furthermore, D2-40 and LpMab-23 showed different reactions to cancer cells in oral cancer tissues. The epitope mapping of anti-PDPN mAbs revealed that LpMab-23 recognized a cancer-specific glycoprotein which is recognized by Th55/Ser56, in contrast, the epitope of D2-40 was independent of glycans. LpMab-23 revealed high ADCC and anti-tumor activities against oral cancers. The Kaplan-Meier curves of the five-year metastasis-free survival rate (mMFS) were significantly lower in LpMab-23-positive patients than in the patients with LpMab-23-negative ones.

Conclusions: A cancer-specific anti-PDPN mAb LpMab-23 was successfully established. LpMab-23 is advantageous in antitumor activities. LpMab-23-positive cases could be a useful predictor of poor prognosis for oral cancer.

No conflict of interest

318 (PB-079)

Poster The metabolic landscape browser: a novel tool for understanding pan-cancer metabolic processes through variance in gene expression data

Y. Leeuwenburgh1,2, A. Bhattacharya1, C. Urzúa3, M. Walvoort1, H. Jalving1, 1University Medical Center Groningen, Department of Medical Oncology, Groningen, Netherlands; 2University of Groningen, Stratingh Institute for Chemistry, Department of Chemical Biology, Groningen, Netherlands

Background: Altered metabolism is a hallmark of cancer. To study metabolic processes in (cancer) cells we have developed an open-access web-based tool based on patient-derived tumor and healthy tissue samples as well as cell line data.

Materials and Methods: A total of 34,494 samples were obtained from the Gene Expression Omnibus (GEO), The Cancer Genome Atlas (TCGA), Genomics of Drugs Sensitivity in Cancer (GDSC) and the Cancer Cell Line Encyclopedia (CCLE), representing 108 tissue (sub)types. This included 28,200 patient-derived tumor samples. We applied independent component analysis (ICA), which is a computational method to separate multivariate signals (gene expression profiles) into additive subcomponents, so-called transcriptional components (TCs). To characterize the biology of these TCs, gene set enrichment analysis (GSEA) was applied, utilizing 671 gene sets describing metabolic processes from 5 different gene set databases. Subsequently, the TCs were filtered based on high enrichments for gene sets related to metabolic processes. In addition, ICA also provided the "activity" of these metabolic TCs for each individual sample present in our data set.

Results: ICA on 34,494 samples resulted in 132, 151, 136 and 137 metabolic TCs for GEO, TCGA, CCLE and GDSC, respectively. These metabolic TCs were used to define the metabolic landscape in 88 different tumor (sub)types. The tool will be accessible through an open access, web-based portal that will be made available soon. To demonstrate the potential of this tool, we used these metabolic TCs to find genes previously not known to be involved in metabolism that coregulate with known metabolic genes. Furthermore, based on the activity of metabolic TCs in each individual sample, we were able to identify metabolic patterns specific for certain types of tumor. Known metabolic processes, such as melanin metabolism in melanoma and heme metabolism in haematopoietic cells could readily be identified. In addition, we were able to identify transcription factor activities that showed high correlation with several metabolic TCs, such as the well-known metabolism-associated HIF-1, NRF-2 and CHOP/ATF4 transcription factors. Furthermore, we assessed the association of these metabolic TCs with drug sensitivity (defined by IC50 values) utilizing the CCLE and GDSC cell line compendia.

Conclusions: Our metabolic landscape in a large set of tumor, healthy tissue and cell line samples provides researchers with a comprehensive resource to gain more insight into metabolic processes relevant in different cancer types. This might lead to new therapeutic strategies aimed at modulating specific metabolic pathways relevant for tumor behavior or treatment response.

Supported by grants from the Young Academy Groningen, NWO-VENI (grant 916-16029) and Bas Mulder award of Alpe d’HuZes/Dutch Cancer Society (grant RUG 2013-5960).

No conflict of interest

319 (PB-080)

Poster Radiotherapy in combination with immunotherapy in a variety of syngeneic mouse models of cancer

A. Wulf-Goldenberg1, M. Stecklum1, M. Paterka1, I. Fichtner1, J. Hoffmann1, 1EPO, Experimental Pharmacology & Oncology Berlin-Buch GmbH, Immuno-Oncology, Berlin, Germany

Background: The recent clinical success of immune checkpoint modulators has stimulated immune-oncology research leading to the identification of new tumor immunology targets. However both, target validation and drug development need highly characterized preclinical immune oncology models for identification of clinically relevant biomarkers and defining rational combination strategies. Tumor expression of PD-L1 has recently been approved as first predictive biomarker for immunotherapy with the PD1/PD-L1 antibody inhibitors.

Results: We screened our syngeneic mouse models for PD-L1 expression to identify such a correlation. PD-L1 positive and negative murine tumor models were transplanted on C57Bl/6 or Balb/c mice. Most of them showed no difference in tumor growth comparing PD-L1 body treated with untreated mice (= fully immune resistant). However, P388 and some others showed a delayed growth (= partly immune resistant), whereas no tumor model where fully growth inhibited (= immune sensitive).
Furthermore, we were interested, whether the antitumoral treatment effect of checkpoint inhibitors can be increased by combination with radiation. For the evaluation of local tumor radiation in combination with checkpoint inhibitor we optimized multiple parameters, including radiation dose and frequency, drug treatment sequence and duration.

We tested a panel of syngeneic tumor models towards their response towards the combination with local tumor radiation and PD-L1 antibodies. The studies were accompanied by measurement of tumor infiltrating immune cells.

Increased efficacy of the combination compared to single treatment was observed in several models as P388. PD-L1 treatment of P388 bearing mice resulted in 19% tumor growth inhibition, radiation alone in 40% tumor growth inhibition, but the combination of checkpoint inhibitor and radiation resulted in 71% tumor growth inhibition. However, frequently models as Lewis lung showed only a slight tumor growth delay. Treatment resistance seemed to correlate with a low percentage of immune cells in the tumor (cold tumors). The evaluation of the PD-L1 expression in the tumors revealed, that radiation can induce PD-L1 expression.

Conclusion: Our results are demonstrating, that syngeneic tumor models in mice can be used to evaluate strategies for the combination of immunotherapies with radiation.

No conflict of interest

320 (PB-081) Poster

TLR2 inhibition reprograms the tumor microenvironment and delays progression of recurrent ovarian cancer in vivo

A. Alvero1, M. Pitruzzello1, B. Keogh2, P. Mcguirk2, M. Reilly2, G. Mor1. 1Yale University, Ob-Gyn, New Haven, Connecticut, USA; 2Opsona Therapeutics Ltd, In Vivo Pharmacology, Dublin, Ireland

Background: Primary ovarian cancer is highly responsive to standard of care comprised of combination chemotherapy and surgery. In contrast, recurrent ovarian cancer presents as an extremely chemoresistant and widely metastatic disease thus limiting the value of chemotherapy and surgery. More than 80% of patients that initially respond to 1st-line treatment experience recurrent ovarian cancer and eventually succumb to the disease.

We showed in previous studies the importance of the TLR2 signaling pathway during ovarian cancer recurrence. TLR2 signaling is able to induce PD-L1 expression, which can induce PD-L1 expression.

Materials and Methods: mCherry-positive human ovarian cancer cells were injected into peritoneally (i.p.) in athymic nude mice. The growth of i.p. tumors were measured using the In Vivo FX system. Mice received four doses of Paclitaxel (12 mg/kg) or vehicle control to treat primary disease. Afterwards, mice were re-randomized to receive maintenance therapy with either saline (n = 7) or OPN-305 (n = 11). Tumor burden was quantified using mCherry fluorescence area. Protein levels were determined by western blot analysis.

A decrease in the number of CD11b+/Gr-1+ myeloid derived suppressor cells (MDSC) was observed. A significant decrease in the number of MDSC was observed in mice treated with OPN-305.

Conflict of interest: Other Substantive Relationships: Brian Keogh, Peter Mcguirk, and Mary Reilly are employed by Opsona Therapeutics.
most prominent cytokine. Signs of a deteriorating general condition, such as red skin, reduced food consumption, and body weight loss, were also noted in a dose-dependent manner. Histopathological findings such as decreased lymphocytes in the thymus and increased immune cell infiltration in multiple tissues were also seen at the highest dose. From these results, we determined the highest dose 0.1 μg/kg as the NOAEL, and calculated a starting dose of 3.2 ng/kg, considering body surface area and species difference. On the other hand, the starting dose from the MABEL approach was calculated to be 4.9 ng/kg, at which Cmax after 4 hours IV infusion is predicted to be close to the EC10 value of the cytotoxic assay. By combining the NOAEL and MABEL approaches, we selected 3.0 ng/kg as a FIH dose for the Phase 1 clinical trial (NCT02748837).

Conflict of interest: Ownership: Chugai Pharmaceutical Co., Ltd.

324 (PB-085) Poster
Cross-talk between macrophages and thyroid cells in early and late thyroid tumor stages: in vitro studies

G. Mauro1, M. Mazzoni1, M. Erreni1, P. Romeo1, M.C. Anania1, R. Avigni1, M.G. Rizzetti1, M.G. Borrello1, P. Allavena2, A. Greco1. 1City University, National Institute for Cellular Biotechnology, Dublin 9, Ireland; 2Pancreatic cancer survival rate is poor, with a 5 year survival rate of 7%. Pancreatic cancer is one of the only solid tumours to have minimal improvement in patient outcome. In Ireland pancreatic cancer surgical resection is limited to two hospitals, Cork University Hospital and St Vincent’s University Hospital (SVUH), Dublin. In collaboration with SVUH, we have established Ireland’s only pancreatic cancer patient derived xenograft (PDx) bio-bank program, including the establishment of primary cell lines when possible. Chemotherapy options for pancreatic adenocarcinomas (PDAC) patients are usually Gemcitabine/Abraxane or FOLFIRINOX, depending on EOCG status.

Methods: Tumour material was obtained from candidate patients following surgical resection with curative intent. After initial macroscopic pathological confirmation, material surplus to diagnostic sampling was cold transferred and implanted sub-cutaneous into CB17/ICr-Pkd1-/- mice, with an approximate mean transfer time of 80 minutes from surgery to implantation. A biobank was created of patient tumours, PDx tumours, and cryopreserved tumour lines. Following the passage in vivo, using a cold digestion with hyaluronidase collagenase, primary cell lines were derived.

Primary culture cells and established cell lines were examined in a 2D and 3D in vitro model for the response to standard of care chemotherapeutic agents.

Results: To date, 20 PDAC tumours have been expanded as patient derived xenograft (PDx) models, with a take rate of 71%. These PDx tumours have the same three-dimensional architecture, and are phenotypically to primary cell lines from pancreatic cancer patient derived xenografts. 1Dublin City University, National Institute for Cellular Biotechnology, Dublin 9, Ireland; 2St Vincent’s University Hospital, Department of Pathology and Laboratory Medicine, Dublin 4, Ireland; 3University at Buffalo, Department of Pharmaceutical Sciences, Buffalo, USA; 4St Vincent’s University Hospital, Department of Surgery, Dublin 4, Ireland; 5St Vincent’s University Hospital, Department Of Medical Oncology, Dublin 4, Ireland

Background: Pancreatic cancer survival rate is poor, with a 5 year survival rate of 7% in the Republic of Ireland. Over the last 40 years, pancreatic cancer is one of the only solid tumours to have minimal improvement in patient outcome. In Ireland pancreatic cancer surgical resection is limited to two hospitals, Cork University Hospital and St Vincent’s University Hospital (SVUH), Dublin. In collaboration with SVUH, we have established Ireland’s only pancreatic cancer patient derived xenograft (PDx) bio-bank program, including the establishment of primary cell lines when possible. Chemotherapy options for pancreatic adenocarcinomas (PDAC) patients are usually Gemcitabine/Abraxane or FOLFIRINOX, depending on EOCG status.

Methods: Tumour material was obtained from candidate patients following surgical resection with curative intent. After initial macroscopic pathological confirmation, material surplus to diagnostic sampling was cold transferred and implanted sub-cutaneous into CB17/ICr-Pkd1-/- mice, with an approximate mean transfer time of 80 minutes from surgery to implantation. A biobank was created of patient tumours, PDx tumours, and cryopreserved tumour lines. Following the passage in vivo, using a cold digestion with hyaluronidase collagenase, primary cell lines were derived.

Primary culture cells and established cell lines were examined in a 2D and 3D in vitro model for the response to standard of care chemotherapeutic agents.

Results: To date, 20 PDAC tumours have been expanded as patient derived xenograft (PDx) models, with a take rate of 71%. These PDx tumours have the same three-dimensional architecture, and are phenotypically to primary cell lines from pancreatic cancer patient derived xenografts. 1Dublin City University, National Institute for Cellular Biotechnology, Dublin 9, Ireland; 2St Vincent’s University Hospital, Department of Pathology and Laboratory Medicine, Dublin 4, Ireland; 3University at Buffalo, Department of Pharmaceutical Sciences, Buffalo, USA; 4St Vincent’s University Hospital, Department of Surgery, Dublin 4, Ireland; 5St Vincent’s University Hospital, Department Of Medical Oncology, Dublin 4, Ireland

Background: Pancreatic cancer survival rate is poor, with a 5 year survival rate of 7% in the Republic of Ireland. Over the last 40 years, pancreatic cancer is one of the only solid tumours to have minimal improvement in patient outcome. In Ireland pancreatic cancer surgical resection is limited to two hospitals, Cork University Hospital and St Vincent’s University Hospital (SVUH), Dublin. In collaboration with SVUH, we have established Ireland’s only pancreatic cancer patient derived xenograft (PDx) bio-bank program, including the establishment of primary cell lines when possible. Chemotherapy options for pancreatic adenocarcinomas (PDAC) patients are usually Gemcitabine/Abraxane or FOLFIRINOX, depending on EOCG status.

Methods: Tumour material was obtained from candidate patients following surgical resection with curative intent. After initial macroscopic pathological confirmation, material surplus to diagnostic sampling was cold transferred and implanted sub-cutaneous into CB17/ICr-Pkd1-/- mice, with an approximate mean transfer time of 80 minutes from surgery to implantation. A biobank was created of patient tumours, PDx tumours, and cryopreserved tumour lines. Following the passage in vivo, using a cold digestion with hyaluronidase collagenase, primary cell lines were derived.

Primary culture cells and established cell lines were examined in a 2D and 3D in vitro model for the response to standard of care chemotherapeutic agents.

Results: To date, 20 PDAC tumours have been expanded as patient derived xenograft (PDx) models, with a take rate of 71%. These PDx tumours have the same three-dimensional architecture, and are phenotypically to primary cell lines from pancreatic cancer patient derived xenografts. 1Dublin City University, National Institute for Cellular Biotechnology, Dublin 9, Ireland; 2St Vincent’s University Hospital, Department of Pathology and Laboratory Medicine, Dublin 4, Ireland; 3University at Buffalo, Department of Pharmaceutical Sciences, Buffalo, USA; 4St Vincent’s University Hospital, Department of Surgery, Dublin 4, Ireland; 5St Vincent’s University Hospital, Department Of Medical Oncology, Dublin 4, Ireland

Background: Pancreatic cancer survival rate is poor, with a 5 year survival rate of 7% in the Republic of Ireland. Over the last 40 years, pancreatic cancer is one of the only solid tumours to have minimal improvement in patient outcome. In Ireland pancreatic cancer surgical resection is limited to two hospitals, Cork University Hospital and St Vincent’s University Hospital (SVUH), Dublin. In collaboration with SVUH, we have established Ireland’s only pancreatic cancer patient derived xenograft (PDx) bio-bank program, including the establishment of primary cell lines when possible. Chemotherapy options for pancreatic adenocarcinomas (PDAC) patients are usually Gemcitabine/Abraxane or FOLFIRINOX, depending on EOCG status.

Methods: Tumour material was obtained from candidate patients following surgical resection with curative intent. After initial macroscopic pathological confirmation, material surplus to diagnostic sampling was cold transferred and implanted sub-cutaneous into CB17/ICr-Pkd1-/- mice, with an approximate mean transfer time of 80 minutes from surgery to implantation. A biobank was created of patient tumours, PDx tumours, and cryopreserved tumour lines. Following the passage in vivo, using a cold digestion with hyaluronidase collagenase, primary cell lines were derived.

Primary culture cells and established cell lines were examined in a 2D and 3D in vitro model for the response to standard of care chemotherapeutic agents.

Results: To date, 20 PDAC tumours have been expanded as patient derived xenograft (PDx) models, with a take rate of 71%. These PDx tumours have the same three-dimensional architecture, and are phenotypically to primary cell lines from pancreatic cancer patient derived xenografts. 1Dublin City University, National Institute for Cellular Biotechnology, Dublin 9, Ireland; 2St Vincent’s University Hospital, Department of Pathology and Laboratory Medicine, Dublin 4, Ireland; 3University at Buffalo, Department of Pharmaceutical Sciences, Buffalo, USA; 4St Vincent’s University Hospital, Department of Surgery, Dublin 4, Ireland; 5St Vincent’s University Hospital, Department Of Medical Oncology, Dublin 4, Ireland
Background: Prostate cancer is the most common cancer in men and in the UK and the second most common cancer worldwide and the fourth most common cancer overall, with more than 1 million new cases diagnosed each year. Understanding the biology of prostate cancer has improved and metastatic forms of the human disease has hampered research. Patient cell lines and as a result an increase in the generation of PDX have provided a breadth of new models for different cancer types. Here we report that cell lines and as a result an increase in the generation of PDX have provided unique and clinically relevant models for preclinical drug evaluation for prostate cancer.

No conflict of interest

327 (PB-088) Poster Evaluation of anti-androgen therapy in a panel of prostate patient-derived xenograft models

J. Davies1, N. Papadopoulou1, L. Wainwright1, A. Oakeden1, C. Roberts1, J. Wrigley1, J. King1, W. Qian2, L. Zhang2, B. Fan3, Y. Yin3, A. Collins3, J. Cai4, D. Ouyang4, J. Kumar1, 1Crown Bioscience UK Ltd., Operations, Loughborough, Leicestershire, United Kingdom; 2Crown Bioscience Inc, Loughborough, Leicestershire, United Kingdom; 3Crown Bioscience Inc., Pharmacology, Beijing, China; 4University of York, York, United Kingdom

Material and Methods: Primary prostate cancer samples obtained from patients in the UK undergoing radical prostatectomy were collected with ethical consent, disaggregated and established subcutaneously in Rag2−/−g-cfo−/− mice (Jackson Laboratory) to generate PDX models. Subcutaneous tumor growth was evaluated 3 times a week by electronic callipers and volume estimated using the formula 0.5 × (L × W)2. Once established, tumours were expanded in NSG mice (Jackson Laboratory) and tissue collected at termination for DNA sequencing and immunohistochemistry (IHC) for prostate markers. PDX were tested for sensitivity to hormone therapy (anti-AR) androgen receptor antagonist (Bicalutamide) and abiraterone acetate. Histologically the structure of the original patient tumours was maintained and confocal microscopy was used to evaluate the impact of immunotherapies on tumour growth and tumour invasiveness. Orthotopic models were established by lentiviral transduction. Orthotopic models were used to evaluate the impact of immunotherapies on tumour growth and tumour invasion.

Results: A panel of prostate PDX models have been established and expanded. Two models were developed from patients diagnosed with castrate resistant prostate cancer (CRPC) and two models from patients who showed hormone sensitivity. Histologically the structure of the original patient sample was retained by the PDX models. In addition, these models showed high KLK3 expression levels by RNA sequencing and IHC staining as well as androgen receptor expression. One of the CRPC models showed a TMPRSS-2:ETS fusion, partial response to docetaxel (p < 0.0001). Two way ANOVA in vivo when compared to vehicle group and poor response to abiraterone and enzalutamide, whereas the second CRPC model showed no response to any of the agents tested.

Conclusions: We have established and characterised a panel of prostate PDX models which provide unique and clinically relevant models for preclinical drug evaluation for prostate cancer.

No conflict of interest

328 (PB-089) Poster Functional protein and pathway profiling of patient-derived tumor model for drug testing and precision medicine applications using Reverse Phase Protein Arrays (RPPA)

B. Gierke1, M. Bodenhöfer2, C. Schmees2, M. Pawlik1, 1NMI Protein Profiling, Reutlingen, Germany; 2NMI, Tumor Biology, Reutlingen, Germany

Background: Molecular profiling of patient tumors today uses high throughput genome and transcriptome technologies, yet proteins and especially their functions, on which drugs can act, are poorly predicted. Proteomic immunoassay approaches like Reverse Phase Protein Arrays (RPPA) have demonstrated that signaling pathway profiling of up to hundreds of samples and proteins in parallel can add valuable information (i) providing multiple marker patterns from small amounts of tissue specimens, and (ii) providing biological answers based on a functional protein level: Are pathways active downstream of a driver mutation? Will drug treatment be effective? What are the underlying mechanisms? Can we identify predictive marker proteins? We combine RPPA with cell line and patient-derived model systems as efficient “close-to-in-vivo” in vitro systems to generate phenotype/pathway signature information to be used for drug testing and personalized medicine applications.

Material and Methods: Protein lysates were prepared and analyzed from various tumor model systems treated with different chemotherapeutic and targeted drugs and compounds. Sample sources included patient-derived XenoCult cell models, homo and hetero xenograft models (Insphero) and 3D microtumour cultures derived from fresh patient tumor material, treated at different doses and times. Protein lysates were analyzed via RPPA applying sensitive fluorescence immunoassays with up-front validated antibodies against 100+ focused protein markers (total, phospho forms) of key oncology pathways (e.g. MAPK, PI3K/Akt/mTOR).

Results: Phenotype/pathway signatures were generated from the RPPA data. Changes upon treatment of the individual pathways – their activities and associate protein markers – are used for correlations to mechanisms of drug action e.g. efficacy/resistance, and for treatment response prediction. Treatment responses of 3D tumor models were benchmarked with conventional 2D cell culture models as controls.

Conclusion: Multiplex protein and pathway profiling using RPPA can provide meaningful biological information from functional protein and pathway signatures generated from of up to hundreds of proteins and can be measured in parallel and from minute amounts of starting material. Leveraging several advanced patient-derived tumor models, our results demonstrate the power of phenotype profiling for gaining conclusive drug mode-of-action information, identification of early response treatment markers and development of precision therapies.

No conflict of interest

Poster Session (Thursday, 15 November 2018) Abstracts, 30th EORTC-NCI-AACR Symposium
Results: The success rate of tumour transplantation into different organs ranged from 60 to 100% as confirmed by both in-life imaging and ex vivo imaging at termination. For example, Hepa 1−6 appeared to grow within the parenchyma of the liver whereas 4T1 metastasized to the lungs and bone from the mammary fat pad, which was easily quantified by BLI. Real-time quantification of tumour size could be correlated with biomarkers and end stage assessment, for example, treatment with Sorafenib and check point inhibitors was correlated with alpha-fetoprotein (AFP) and end stage tumour burden, showing significant response in the orthotopic setting (p < 0.001, Two way ANOVA). Differences in TIL infiltration as detected by FACS and IHC were observed as well as stromal and blood capillary infiltration when compared to the subcutaneous site.

Conclusions: Bioluminescent syngeneic models enables clinically relevant assessment of standard of care agents, immunotherapies and combination which is distinct from the subcutaneous setting. The orthotopic microenvironment influences the tumour growth and TIL infiltration which requires characterisation by both FACS and IHC. These models also enable the assessment of disease progression and modelling the metastatic environment.

No conflict of interest

331 (PB-092) Poster
Identification of predictive biomarkers in novel patient-derived xenograft (PDX) models of peritoneal metastasis from colorectal cancer

E. Pachmayr1, B. Brzezicha2, B. Bux2, B. Rau2, U. Stein1, W. Waltham2, 1ECRC, Charité and Max-Delbrück-Center Berlin, Translational Oncology of Solid Tumors, Berlin, Germany; 2Epo GmbH Berlin, Experimental Pharmacology, Berlin, Germany; Charité, Surgical Clinic, Berlin, Germany; Charité, Universitätmedizin Berlin, Campus Berlin-Buch, Translational Oncology of Solid Tumors, Berlin, Germany

Background: One terminal stage of colorectal cancer (CRC) is represented by the peritoneal metastasis (PM) with only limited therapy options. To improve therapeutic outcome, identification of clinically relevant prognostic and predictive markers is essential. In this context patient derived xenograft (PDX) models represent a useful platform for molecular as well as response analyses. Access to in vivo models of PM will improve the evaluation of chemosensitivity as well as identification of novel biomarkers and therapeutic targets. Here we generated the first patient PDX models of PM from CRC to test chemotherapy response and to analyze predictive biomarkers, such as the Wnt signaling target S100A4.

Material and Methods: For PDX establishment surgical tumor specimens of 48 patients were transplanted subcutaneously onto NOG mice and later transferred to NMRI nude mice for further passages. Of those, 23 PDX have stably engrafted. Thirteen PDX models thereof were characterized regarding histology (immunohistochemistry, IHC), chemosensitivity (in vivo drug treatment) and biomarker expression (IHC, real-time RT-PCR) in more detail. Chemosensitivity of PDX models was tested towards a panel of different chemotherapeutic treatments and/or schedules. In particular, our work, we hypothesize that we can target the NF-kappaB pathway to achieve disease control while reducing toxicity is metronomic chemotherapy with limited treatment options and very poor prognosis following progression after standard chemotherapeutic regimes. One of the emerging strategy to achieve disease control while reducing toxicity is metronomic chemotherapy (mCHT), which inhibits the tumor growth. In the recent clinical study, Victor-2, we evaluated a new metronomic combination (mCHT) of Capecitabine (CAPE) and Vinorelbine (VNR) in breast cancer patients showing a disease control rate with a median Progression-Free Survival (PFS) of 4.7 months in 28 TNBC patients.

In the in vitro study, named Victor-0, we examined the effect of metronomic (mCHT) vs standard (STD) schedule of administration of different combinations of 5-Fluorouracil (5FU), the active metabolite of CAPE, and VNR in TNBC cell lines MDA-MB-231 and BT-474.

Methods: TNBC cell lines MDA-MB-231 and BT-474 were exposed to different concentration of VNR alone or with 5-FU for 4 and 96 h. To simulate the metronomic dosing schedule, we replaced the drug-enriched medium every 24 h, while to simulate the conventional administration protocol cells were exposed to VNR alone or with 5-FU for 4 h, then the medium was changed and replaced with fresh medium without drug every 24 h. The IC50 was calculated by non-linear regression fit of the mean values of data obtained in triplicate experiments. Cell viability/cytotoxicity assays was evaluated by MTT assay and senescence by S-b-gal staining. Cell cycle was analyzed by FACS of PI-stained cells. Modulation of apoptotic/autophagic markers was assessed by western blot analysis and immunofluorescence.

Results: A significant anti-proliferative activity was observed in cells treated with mCHT vs STD administration of 5FU or VNR alone. Combination of the two drugs showed an additive inhibitor effect on cell growth in both cell lines. After exposure of cells to 5FU and VNR under mCHT vs conventional schedule of administration we observed a different modulation of chemoresistance factor Bcl-2, the pro-apoptotic protein Bax, cleaved effector caspase-3 and expression of LC3A/B autophagy protein indicating that autophagy and cellular senescence contribute more than apoptosis to the growth suppressive effect triggered by metronomic therapy.

In conclusion, our data give novel insights and help to understand which molecular mechanism involved in the cell death of TNBC are triggered by the different chemotherapeutic treatments and/or schedules. In particular, our data indicate that the efficacy of the metronomic schedule is due to the combined triggering of autophagy and cellular senescence in TNBC cells.

No conflict of interest

333 (PB-094) Poster
Eliminating tumor-initiating cells in an ovarian cancer relapse model

I. Ozaki1, C. House1, K. Mazan-Mamczarz2, M. Lal-Nag2, C. Thomas2, C. Annunziata1, 1National Cancer Institute, Women’s Malignancies Branch, Bethesda, USA; 2National Institutes of Health, National Center for Advancing Translational Sciences, Rockville, USA

Background: Tumor-initiating cells (TICs) are a subpopulation of cells that have been found to be chemotherapy resistant, and contribute to cancer relapse. Ovarian cancer has an extremely high rate of relapse, with over 70% of advanced stage cases relapsing within 2 years. Based on our previously published work, we hypothesize that we can target the NF-kappaB pathway to eliminate TIC populations.

Methods: We performed both siRNA screen and high throughput drug screen to identify pathways critical to ovarian cancer cell growth in spheroid conditions. NF-kB reporter assay was used to measure changes in NF-kappaB signaling by RNAi or drug exposure. CD133 expression and ALDH activity assay measured changes in the frequencies following treatment. Spheroid formation and spheroid viability assays measured the effects of pathway interuption on ovarian cancer cell biology. We investigated two mouse models – survival and relapse – to pinpoint the role of these signaling events in the course of ovarian cancer pathogenesis.

Results: Our siRNA screen showed that NF-kappaB genes NFXF1, RAC2, CDC42, BCL2L1, and SERPINB3 were essential for TICs. Using a GFP NF-kappaB reporter, cells grown in TIC conditions had higher NF-kappaB signaling than cells grown in adherent conditions. Our drug screen identified a cohort of drugs toxic to TICs: bardoxolone methyl, salinomycin, disulfiram, and elesclomol. Addition of the drugs to cells grown in TIC conditions...
Ovarian cancer tumor initiating cells depend on NF-kappaB signaling. This pathway may become an important target to prevent ovarian cancer relapse.

**No conflict of interest**

**Materials and Methods:** To effectively recapitulate and therapeutically interrogate the heterogeneity of human cancer, a dozen European cancer centres and university hospitals involved in translational oncology joined forces in 2013 to start EurOPDX, an academic research consortium that now gathers 18 institutions throughout Europe and in the US (www.europdx.eu). The goal of the Consortium is to maximize exploitation of PDXs and other patient-derived models for cancer research by: (i) integrating institutional collections into a multicentre repository now reaching more than 1500 models for 30+ different cancer types; (ii) defining common standards to improve the quality and reproducibility of oncology preclinical data; (iii) sharing models within and outside the consortium to perform collaborative precision oncology “xenopatient” trials. Building on its first successes, EurOPDX is now teaming up with other key academic and SME partners in a four-year project to build the “EurOPDX Distributed Infrastructure for Research on patient-derived Xenografts” (EDIREX project funded under EU’s H2020 research and innovation programme, grant no. 731105).

**Results:** This new cutting-edge European infrastructure will offer access to PDX resources for academic and industrial cancer researchers through 6 state-of-the-art installations or “nodes.” We will present the specific objectives of the project, including our work towards standardisation and optimisation of biobanking, quality control and data tracking, and the performance of in vivo drug efficacy experiments. Access to the resource, including the distribution of cryopreserved samples from established models, the structured biobanking of user-developed models and the performance of drug efficacy studies, will be offered through a grant application system to open in October 2018. Selection of the models by users will be made possible thanks to the newly-developed EurOPDX Data Portal, of which a prototype will be presented.

**No conflict of interest**

**Background:** Tumor microenvironment plays a significant role to tumor survival, proliferation and metastasis. It consists of both cellular components and ECM. This study examines the role of cellular components in lung cancer concerning the expression of several markers including hypoxia markers and cytokines, as well as markers usually found in immune cells, using a 3D multi-culture model.

**Materials and Methods:** The lung cancer cell line CORL105 was cultured alone and with endothelial cell line HUVEC, in co-culture, in hanging drop; monocytes isolated from healthy donor were added to the mono and co-culture, in order to determine their effect on CD163, CD206 and CD64 marker expression. Moreover, the expression of HIF-1α, CXCR4 and CXCL12 was examined both in mono, co and tri-cultures. Marker expression was measured via flow cytometry.

**Results:** CORL105 spheroids are larger than the ones consisting of both cancer cells and endothelial cells, or cancer cells, endothelial and monocytes. Moreover, spheroids that include endothelial cells are formed faster than the mono-culture ones. Marker HIF-1α expression is higher in mono-culture spheroids, but tri-culture spheroids exhibit higher CD14, CD163 and CD206 expression than the spheroids consisting of CORL105 and monocytes. CXCR4 and HIF-1α co-express higher in tri-cultures in contrast to mono and co-cultures, whereas HIF-1α and CXCL12 exhibit similar expression pattern in co and tri-culture spheroids. Concerning CXCR4 and CXCL12 simultaneous expression, it seems to be higher in tri-culture spheroids.

**Discussion:** Clearly, cancer manipulates its environment and affects the expression levels of cytokines and markers that are involved in macrophage polarization. The results above indicate that immune cells could be involved in higher cytokine production, aided by hypoxia. Nevertheless, in order to establish their actual role in lung cancer more studies are required.

**No conflict of interest**
Targeting checkpoint kinases for the more effective treatment of radioresistant aggressive breast cancers

B. Chandler¹, S. Nyati², C. Ritter³, L. Moubaddber¹, E. Olsen¹, A. Michmerhuizen¹, A. Pesch¹, K. Wilder-Romans¹, L. Pierce¹, A. Chinniyan², C. Speers¹, ¹University of Michigan, Department of Radiation Oncology, Ann Arbor, USA; ²University of Michigan, Department of Pathology, Michigan Center for Translational Pathology, Ann Arbor, USA

Background: Increased rates of locoregional recurrence leading to poorer clinical outcomes have been observed in triple-negative breast cancer (TNBC) despite the use of radiation therapy (RT), therefore approaches that result in radiosensitization in TNBC are critically needed. Our previous work identified a group of cell cycle kinases overexpressed in TNBC. Here we described the impact of elevated expression of two of these identified kinases, MELK and TTK, on radiation response and patient outcomes in TNBC.

Methods: TCGA breast cancer datasets were used to determine MELK and TTK expression in the intrinsic subtypes of BC. Clonogenic survival assays were used to determine the radiosensitization in cell lines after pharmacologic MELK or TTK inhibition. DNA damage was quantified using gH2AX staining in TNBC cell lines. NHEJ and HR stable reporter systems were used. Kaplan-Meier analysis was used to determine the impact of MELK or TTK expression on locoregional recurrence (LRR) and overall survival (OS). A Cox proportional hazards model was constructed to identify potential factors of LRR-free survival in univariate (UVA) and multivariable analyses (MVA).

Results: Our radiosensitizer screen nominated two cell cycle checkpoint kinases, MELK and TTK, as the kinases most highly expressed in radioresistant breast tumors. MELK and TTK expression is significantly elevated in breast cancer tissue compared to normal breast tissue (Q-VAL: 8.7 E – 291) and are most highly expressed in basal-like tumors compared to other subtypes (N = 2,900 samples) and TNBC cell lines (N ≥ 51, p < 0.0001). MELK and TTK expression is significantly correlated with intrinsic radiosensitivity in a panel of 23 BC cell lines (R = 0.58–0.65, p-value = <0.001). Genomic (siRNA knockdown) or pharmacologic inhibition of MELK and TTK increased radiosensitivity in vitro in 4 different TNBC cell lines (ER+ 1.38–1.72). Mechanistically, MELK inhibition led to impaired NHEJ repair after RT, and TTK inhibition inhibited HR repair. MELK or TTK inhibition in vitro or knockdown significantly radiosensitizedTNBC xenografts and PDOxs and markedly delayed tumor doubling time and growth (median tumor doubling 7.95 (RT alone) vs. 29.1 days (combination), 8.5 (RT alone) vs. 24.5 days (combination); p-value <0.0001). Clinically, patients treated with breast-conserving surgery and RT with lower than median MELK or TTK expression had worse local-recurrence free (LRF) survival and overall survival (HR for local recurrence 1.7–3.1 as continuous variable, p-value <0.001) compared to patients with higher than median expression. In MVA only MELK expression, TTK expression, and grade were associated with worse LRF survival in 3 independent datasets.

Conclusion: Our results support the rationale for clinical development of cell cycle checkpoint kinases inhibitors (MELK and TTK) as a novel radiosensitizing strategy in TNBC.

No conflict of interest
We have recently reported the discovery of YAP-TEAD interaction inhibitors that disrupt the YAP-TEAD complex and block proliferation of tumor cells. These compounds represent promising new treatment modalities for mesothelioma where the Hippo pathway is highly deregulated and YAP mainly localized in the nucleus (AACR 2016).

The aim of this study was to investigate the potential of our YAP-TEAD interaction inhibitors to alleviate the resistance to standard of care agents of mesothelioma and NSCLC cell lines. The effect of our compounds alone and in combination with Pemetrexed, a folate antimetabolite drug were investigated in H2292 and A549, mesothelioma and NSCLC cancer cells respectively. To assess the efficacy of Pemetrexed+/-Inventiva compounds, the cell lines in 2D or in 3D cultures were exposed to single agents and combinations in a dose response for 4 days or 15 days. ATP CellTiterGlo or EDU incorporation assays were used to measure cell growth.

We found a synergistic effect between the YAP-TEAD interaction inhibitors and Pemetrexed, leading to a drastic inhibition of cancer cell proliferation and an increase of cytotoxicity. This suggests that YAP-TEAD interaction inhibitors used in combination with existing chemotherapeutics could be used to attenuate multidrug resistance and re-sensitize chemo-resistant cancer cells.

No conflict of interest

343 (PB-006) Poster
Mechanisms of tumor cell invasion in a pancreatic neuroendocrine tumor before and after the anti-angiogenic treatment
J. Pinto1, J. Zuazo1, Á. Martínez-López1, O. Casanovas1. 1Catalan Institute of Oncology, IDIBELL, Program Against Cancer Therapeutic Resistance, Barcelona, Spain

Introduction: Local invasion is a key cellular biological event in the metastatic cascade. In response to a changing microenvironment, cancer cells may act using two main strategies of invasion: single cell invasion and collective invasion. Results from our group demonstrated an irreversible increase in the incidence of invasive tumors during anti-angiogenic treatment in the RIP1-TAG2 mouse model. Determining how tumor cells initiate and sustain this behavior may help improve the development of new intervention modalities.

Materials and Methods: The RIP1-Tag2 is a transgenic mouse model of pancreatic islet cell tumorigenesis (PNETs). In order to study invasion mechanisms in these tumors, they were molecularly characterized regarding their invasion capacity before and after the anti-angiogenic treatment (sunitinib). Furthermore, using jTC4 cells from RIP1-Tag2 we developed a three-dimensional (3D) tumor spheroid model to the evaluate invasion pattern in 3D culture in vitro.

Results: Deeply studying tumor invasion in RIP1-Tag2 model, we observed that it does not follow a classical epithelial-mesenchymal transition, but it rather involves mechanisms of collective tumor cell invasion. In these tumors, molecular signatures were identified implicated in cell-cell adhesion, specifically on cadherin and claudin families. We also found a correlation between the expression of these targets and a high tumor invasion capacity. Moreover, these data were also confirmed in a 3D tumor spheroid model. Finally, E-cadherin and Claudin14 expression were significantly correlated in neuroendocrine primary tumors and metastases from patients.

Conclusions: Using two different approaches, we have verified that members of cadherin and claudin families might be interesting targets implicated in invasiveness in pancreatic neuroendocrine tumors before and after anti-angiogenic treatment. The analysis of clinical samples supports our preclinical data suggesting the integrative action of E-cadherin and Claudin14 in pancreatic neuroendocrine tumors from patients.

This project has the support of a National Council for Scientific and Technological Development (CNPq) from Science Without Borders, Brazil.

No conflict of interest

344 (PB-007) Poster
Development and characterization of CV6-168, a novel and selective dUTPase inhibitor that enhances the antitumour efficacy of TS-targeted therapies
P. Wilson1, K.A. Mulligan1, K.A. Mclaughlin1, V. Prise1, M.L. Wilson2, P.G. Johnstone1, R.D. Ladner1. 1CV6 Therapeutics NI Ltd., Research and Development, Belfast, United Kingdom; 2Queen’s University Belfast, Centre for Cancer Research and Cell Biology, Belfast, United Kingdom

Background: Thymidine synthase (TS) inhibitors are standard-of-care therapies for the majority of high incidence cancers. Deoxoyuridine triphosphate nucleotidohydrolase (dUTPase) is a gatekeeper enzyme that protects cancer cell DNA from the misincorporation of uracil that would otherwise occur during treatment with TS-targeted therapies such as FUDR, capcitabine, S-1, pemetrexed and methotrexate. Uracil misincorporation induced by inhibition of dUTPase causes extensive and irreparable DNA damage during treatment with TS-targeted therapies and strongly enhances cancer cell death. dUTPase is highly-expressed across many cancer types and is associated with resistance to TS-targeted therapies. Here we report the development and characterization of CV6-168, a novel and selective inhibitor of dUTPase that significantly enhances the efficacy of TS-targeted therapies against a broad spectrum of human cancer models.

Materials and Methods: The mode of inhibition of dUTPase by CV6-168 was determined against recombination human dUTPase in a fluorescence-based assay developed in our laboratory. In vitro cell growth inhibition was demonstrated by CellTiter-Glo in 22 human cancer cell lines spanning 13 cancer types. Cell viability was measured by clonogenic cell survival assay in 46 human cancer cell lines spanning 18 cancer types. DNA damage was assessed by flow cytometry by measuring H2A.X and ATM activation.

Results: CV6-168 inhibited dUTPase in a competitive and reversible mode with a K_i of 251 nmol/L. CV6-168 exhibited no intrinsic cytotoxicity as a single agent but significantly enhanced cancer cell death in clonogenic assays when combined with FUDR (5-FU active metabolite) in all cell lines assessed. All cancer cell types tested were sensitive to CV6-168 in the HCT116 5-FU-resistant colon cancer model. CV6-168 significantly enhanced the growth inhibition induced by FUDR in 21 out of 22 cancer cell lines analysed. The mean sensitization factor was 23.7 and median 11.7 (range 1.5 – 119). Mechanistic analysis demonstrated that this enhancement was accompanied with a significant increase in dUTP pool expansion and DNA damage. CV6-168 significantly increased the antitumour activity of 5-FU in both the HCT116 and LoVo colon xenograft cancer models with no evidence of increased toxicity.

Conclusions: CV6-168 is a potent and selective dUTPase inhibitor that significantly enhances the antitumour activity of 5-FU as demonstrated in both in vitro and in vivo models. CV6-168 represents a promising new therapeutic agent with the potential to significantly improve the clinical efficacy of TS-targeted therapies across multiple cancer types. CV6-168 will be evaluated in an upcoming Phase I clinical trial.


345 (PB-008) Poster
Therapeutic resistance of 3D pancreatic cancer tumourspheres involves differential cancer stem cell response
N. Walsh1, S. Nelson1, S. Roche1, F. O’neill1, J. Crown2. 1Dublin City University, National Institute for Cellular Biotechnology, Dublin, Ireland; 2St Vincent’s University Hospital, Department of Medical Oncology, Dublin, Ireland

Background: Pancreatic cancer is a highly lethal disease. Despite improvements in diagnosis, treatment and surgical care, the overall 5-year survival rate is 8%. Therapeutic resistance to chemotherapeutics is 8%. Treatment failure and recurrence in pancreatic cancer. Cancer Stem Cells (CSCs) are a small population of cells responsible for tumour initiation, progression, metastasis and chemo-resistance. CSCs also have been considered as the main cause of cancer recurrence. Therefore, targeting CSCs could be an effective strategy to improve the survival outcomes of pancreatic cancer patients.

Material and Methods: 3D pancreatic cancer tumourspheres were developed in established and primary pancreatic cancer cultures and resistance generated to a range of chemotherapeutic drugs (Gemcitabine, 5-FU, Paclitaxel and Cisplatin). Tumoursphere culture was used to analyse the self-renewal capability of CSCs, utilizing an HCT-116 5-FU-resistant colon cancer model. CSC sub-populations were enriched for using CD133+/CD44+/ESA+ populations from therapeutic resistant tumourspheres.

Results: Not all cell lines formed primary tumourspheres however, therapeutic resistance of 3D tumourspheres was higher compared compared to 2D monolayer. Therapeutic resistance of the 3D tumourspheres was verified by applying a secondary tumoursphere formation with dissociated single-cell suspension from primary tumourspheres. HPVAC resistant tumoursphere exhibited the most robust response to chemotherapy treatment and possessed a high differential CSC content. Profiling of the therapeutic resistant 3D pancreatic cancer tumourspheres revealed increased invasive phenotype, expression of self-renewal related genes and epithelial-mesenchymal transition.

Conclusion: Development of therapeutic resistant 3D pancreatic cancer tumourspheres allowed for the characterisation of the CSC-associated
phenotype in response to chemotherapy which more accurately reflects the cellular architecture and heterogeneity in vivo.

No conflict of interest

346 (PB-009) Poster
AZD3229, a KIT/PDGFRA inhibitor with best in class potential for the treatment of gastrointestinal stromal tumors (GIST)


AstraZeneca, Oncology, IMED Biotech Unit, Boston, USA;1AstraZeneca, Oncology, IMED Biotech Unit, Cambridge, United Kingdom;2AstraZeneca, Oncology, IMED Biotech Unit, Cambridge, United Kingdom;3AstraZeneca, Drug Safety and Metabolism, IMED Biotech Unit, Cambridge, United Kingdom;4AstraZeneca, Drug Safety and Metabolism, IMED Biotech Unit, Boston, USA;5AstraZeneca, Discovery Sciences, IMED Biotech Unit, Cambridge, United Kingdom

Background: GIST is the most common human sarcoma driven by gain of function mutations in either KIT or PDGFRA. Although first line imatinib has revolutionized the treatment of GIST, drug resistance caused by secondary KIT or PDGFRA mutations develops in over 80% of patients. Sunlitinib (2nd line) and regorafenib (3rd line) do not have activity against the full spectrum of KIT/PDGFRA mutations and show an overall median time to tumor progression of only 5–6 months. Potent inhibition of KDR in these agents is associated with significant high-grade hypertension, amongst other significant toxicities. A well tolerated drug that targets the broad spectrum of KIT/PDGFRA mutations is therefore an unmet need for the treatment of GIST patients.

Materials and Methods: AZD3229 cellular potency was assessed in GIST 430 cells (Ex11 del/V654A) and Ba/F3 cell lines driven by primary and secondary/mutations of KIT or PDGFRA and KDR in a 3-day growth assay. AZD3229 was dosed in vivo in xenograft models with primary and secondary/KIT mutations and evaluated for pKIT signaling and tumor growth inhibition (TGI). Change in arterial blood pressure was measured using telemetry in rats dosed with AZD3229.

Results: AZD3229, identified using structure-based drug design, potently inhibits growth of a wide spectrum of mutant KIT and PDGFRA-driven Ba/F3 cell lines (>25 cell lines tested: G50 range: 1–50 nM) with over 50-fold selectivity vs KDR (Table 1). At a dose of 20 mg/kg, AZD3229 causes extensive and durable inhibition of pKIT (90% inhibition for ~12 h) in xenograft models with primary ex11 del mutation and primary/secondary KIT mutations and shows an overall median time to tumor progression of only 5–6 months. Potent inhibition of KDR in these agents is associated with significant high-grade hypertension, amongst other significant toxicities. A well tolerated drug that targets the broad spectrum of KIT/PDGFRA mutations is therefore an unmet need for the treatment of GIST patients.

Conclusions: AZD3229 leads to extensive and durable pKIT inhibition in multiple preclinical models driven by KIT mutations and shows a potential to regressions with a margin to KDR-driven blood pressure changes. AZD3229 therefore has potential as a best in class mutant KIT/PDGFR inhibitor leading to durable responses as a 2nd line therapy in advanced GIST with an ambition to move into 1st line GIST without risk of hypertension.

Conflict of interest: Other Substantive Relationships: We are employees and shareholders of AstraZeneca Pharmaceuticals.

Table 1. Effect of AZD3229 and standard of care agents on growth of Ba/F3 cells with representative KIT and PDGFRA mutations and KDR respectively (G50: μM)

<table>
<thead>
<tr>
<th>KIT</th>
<th>KIT Ex</th>
<th>PDGFRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID</td>
<td>del</td>
<td>V654A</td>
</tr>
<tr>
<td>AZD3229</td>
<td>0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>Imatinib</td>
<td>0.017</td>
<td>0.393</td>
</tr>
<tr>
<td>Sunlitinib</td>
<td>0.004</td>
<td>0.006</td>
</tr>
<tr>
<td>Regorafenib</td>
<td>0.021</td>
<td>0.231</td>
</tr>
</tbody>
</table>

347 (PB-010) Poster
Understanding acquired resistance to CDK9 inhibition in hematologic tumor models

C. Andersen1, T. Proia1, J. Cidado1, S. Criscione1, S. Boiko1, G. Mills4, T. Drew1, K. Katayama1, K. Noguchi1, Y. Sugimoto1.

1Keio University, Faculty of Pharmacy, Division of Chemistry, Tokyo, Japan

Background: Transient inhibition of the transcriptional regulator cyclin-dependent kinase 9 (CDK9) leads to downregulation of short half-life genes, including the pro-survival gene MCL1. Consequently, MCL1-addicted cancers undergo rapid apoptosis upon CDK9 inhibition (CDK9) in vitro and in vivo. This observation has prompted entry of several CDK9i agents into the clinic. Clinically, as with many targeted therapies, emergence of acquired CDK9i resistance is anticipated. However, mechanisms of CDK9i resistance are poorly understood.

Materials and Methods: We generated in vivo models of CDK9 resistance through repeated, intermittent dosing of haematologic cancer xenografts with AZ5576, a potent and selective CDK9 inhibitor. MV411 (leukemia) and MOLP8 (myeloma) cells were implanted subcutaneously in SCID mice and dosed twice weekly with 60 mg/kg AZ5576 until tumors were actively growing through treatment. Resistant tumors were then passaged into naive mice and further characterized by evaluating efficacy of other CDK9i compounds and BI93 micromolecules. Molecular changes between resistant and parental tumors were profiled using RNA sequencing (RNAseq) and reverse phase protein array (RPPA) analysis; hits were confirmed using western blot and PCR analyses.

Results: AZ5576 treatment initially produced robust antitumor effects; 90% tumor growth inhibition (TGI) for MV411 and >100% (regressions) for MOLP8. However, resistant tumors began to emerge after 5–6 weeks of treatment. AZ5576-resistant (5576R) tumors displayed cross-resistance to other CDK9 inhibitors in clinical development including dinaciclib and AZ4573. MOLP8 5576R xenografts were also less responsive to direct MCL1 inhibition relative to parental (66% vs >100%, TGI, respectively). Transcriptomics and proteomics identified alterations in several signaling pathways in MV411 and MOLP8 5576R tumors including the apoptosis pathway. Specifically, 5576R tumors showed decreased MCL1, BIM, and BAK, as well as upregulation of BCL2. Neither model was sensitive to single agent BCL2 inhibition. However, combining BCL2 with CDK9 inhibition in MOLP8 5576R tumors led to disease stabilization (90% TGI). Additional altered signaling pathways in 5576R tumors include AKT and MAPK. However, perturbation of these pathways did not impact CDK9i sensitivity.

Conclusions: Hematologic cancer xenografts develop CDK9i resistance in vivo after extended treatment with AZ5576. AZ5576-resistant tumors have significant changes at the transcriptional and proteomic level including upregulation of BCL2. Combining BCL2 inhibition with CDK9i partially overcomes resistance, leading to tumor stasis in 5576R xenografts. Understanding CDK9i resistance preclinically will identify strategies to overcome and potentially prevent it from developing in patients.

Conflict of interest: Advisory Board: G. Mills is a Scientific Advisory Board member/consultant with AstraZeneca. Corporate-sponsored Research: C. Andersen, T. Proia, J. Cidado, S. Criscione, S. Boiko, G. Mills, and L. Drew are current employees of AstraZeneca. Other Substantive Relationships: P. Janne has served as a consultant for and has received research funding from AstraZeneca.

348 (PB-011) Poster
Heat shock protein 90 inhibitors overcome the resistance to various Fms-like tyrosine kinase 3 inhibitors

K. Katayama1, K. Noguchi1, Y. Sugimoto1.

1Keio University, Faculty of Pharmacy, Division of Chemistry, Tokyo, Japan

Background: Fms-like tyrosine kinase 3 (FLT3) mutation, FLT3-ITD, is a driver oncogene and recognized as a molecular target in acute myeloid leukemia (AML). Three FLT3 inhibitors, midostaurin, quizartinib, and gilteritinib, are currently under consideration for the treatment of FLT3-ITD-positive AML. However, several resistant mutations (N676, F691, D835, and Y842 mutations) in tyrosine kinase domain (TKD) of FLT3-ITD have been reported to confer the resistance to quizartinib and/or midostaurin. This study aimed to explore new effective inhibitors to overcome the resistance to FLT3-ITD plasmids by retroviral infection method. Two quizartinib-resistant cell lines, QR1 and QR2, were generated by exposing FLT3-ITD-positive AML MV4-11 cells to the increasing concentrations of quizartinib for 6 months.

Materials and Methods: Ba/F3-ITD, Ba/F3-ITD+N676K (N676K), Ba/F3-ITD+F691L (F691L), Ba/F3-ITD+D835V (D835V), and Ba/F3-ITD+Y842C (Y842C) cells were established by transfection with the corresponding Ba/F3-ITD plasmids by retroviral infection method. Two quizartinib-resistant cell lines, QR1 and QR2, were generated by exposing FLT3-ITD-positive AML MV4-11 cells to the increasing concentrations of quizartinib for 6 months.
Growth inhibition assays were performed to assess sensitivity to drugs. Protein expression and phosphorylation were examined by immunoblotting, and cell cycle and apoptosis analyses were by flow cytometry.

**Results:** Growth inhibition assays revealed that N676K cells showed resistance to midostaurin, and D835V and Y842C cells were resistant to quizzatinib, as reported previously. Interestingly, F691L cells showed hyper resistance not only to quizzatinib and midostaurin but also to giltertinib. We then screened 50 small molecule inhibitors for overcoming the resistance to FLT3 inhibitors in Ba/F3 transfectants and found that heat shock protein 90 (HSP90) inhibitors, 17-AAG, 17-DMAG, resatinipcin, and thymoepid, suppressed proliferation of the transfectants, accompanied with the down-regulation of STAT5, AKT, and MAPK pathways. 17-AAG induced G1 arrest followed by increasing sub-G1 fraction in all transfectants. The effect of HSP90 inhibitors on D835V cells was superior to the other cell lines. Similar results were obtained in Q1R and Q2R cells that harbor D835H and D835V mutations, respectively, in FLT3-ITD gene. Q1R and Q2R cells showed resistance to quizzatinib but not to giltertinib and midostaurin; however, they were more sensitive to HSP90 inhibitors than MV4-11 cells. HSP90 inhibitors well-downregulated STAT5, AKT, ERK, cyclins, and phosphorylated RB, and induced caspase-dependent apoptosis in Q1R and Q2R cells. Finally, 17-AAG significantly enhanced the sensitivity of Q1R and Q2R cells to daunorubicin.

**Conclusions:** HSP90 inhibitors are effective against FLT3 inhibitors-resistant cells harboring various TKD mutations in FLT3-ITD.

**No conflict of interest**

---

**349 (PB-012) Poster**

Poly (ADP-ribose) polymerase1 deficiency is not correlated with rucaparib and veliparib sensitivity in A2780 cells

Y. Nonomiya1, K. Noguchi1, K. Katayama1, Y. Sugimoto1, 1Faculty of Pharmacy, Keio University, Division of Chemotherapy, Tokyo, Japan

**Background:** Poly (ADP-ribose) polymerase (PARP) inhibitors have high anti-tumor activity against BRCA1/2-mutated ovarian and breast cancer. In Japan, olaparib, a PARP1/2 inhibitor has been approved for the maintenance treatment of platinum-sensitive recurrent ovarian cancer. In this study, we have isolated three olaparib-resistant cells lines (ola-R cl.3, cl.10 and cl.15) from human ovarian cancer A2780 cells, and explored the mechanism for the sensitivity to PARP inhibitors.

**Material and Methods:** Sensitivity to the PARP inhibitors, olaparib, rucaparib and veliparib, was evaluated by the cell growth inhibition assay and the colony formation assays. The expression of PARP1 and PARP2 was confirmed by western blotting. Poly-ADP-ribosylation of cellular proteins was also evaluated by western blotting. PARP inhibitor-induced γH2AX foci were visualized with Immunofluorescence confocal microscopy. The number of γH2AX foci with a diameter of 0.5 μm or more in each cell was counted. At least 100 cells were used in each experiment.

**Results:** Ola-R cl.3, cl.10 and cl.15 cells showed 17–30-fold higher resistance to olaparib than the parental A2780 cells. These resistant cells showed only marginal levels of cross-resistance to other PARP inhibitors, rucaparib and veliparib. In addition, the resistant cells showed 2–5-fold higher cellular sensitivity to other cytotoxic agents, cisplatin and SN-38. The resistant cells did not express the drug efflux transporters, P-GP/ABCB1, BCRP/ABCG2, MRP1/ABCC1, MRP2/ABCC2 and MRP3/ABCC3. The numbers of olaparib-induced γH2AX foci of the resistant cells were significantly lower than that of the parental A2780 cells. However, the numbers of rucaparib- and veliparib-induced γH2AX foci of the resistant cells were similar to those of the parental A2780 cells. PARP1 expression was detected in A2780 cells, but not in the resistant cells. A2780 and the resistant cells expressed similar levels of PARP2. Similar results were obtained in the RT-PCR experiments for PARP1 and PARP2. In the resistant cells, the cellular content of poly-ADP-ribosylated proteins detected by anti-poly-ADP-ribose antibody, was lower than that in the parental A2780 cells. Cell growth inhibition and colony formation assays showed that PARP1 knockdown conferred resistance to olaparib, but not to rucaparib and veliparib.

**Conclusions:** Our results suggest that PARP1 expression is one of the sensitivity factors of olaparib, but not of rucaparib and veliparib in human ovarian cancer A2780 cells.

**No conflict of interest**
difference in the mRNA expression profiles respect to parental cells. Gene ontology analysis indicated that the top of the neighboring coding gene function of differentially expressed mRNAs were involved in cell proliferation and cell death. Consistent with these data, SPRY1 inactivation was associated with: i) decreased phosphorylation of p38; ii) enhanced activation of p53; iii) decreased protein levels of several anti-apoptotic proteins. Furthermore, BRAFI treatment was more effective in SPRY1- clones which also exhibited higher basal ROS levels respect to parental cells.

Conclusions: Altogether, our findings reveal important insights into SPRY1 function in mCRPC, and suggest its potential involvement in response to BRAFI treatment.

No conflict of interest

352 (PB-015) Poster
The synergistic effect of melphalan and XPO1 inhibition in preclinical models of multiple myeloma
J. Turner1, A. Bauer1, J. Dawson1, J. Gomez2, T. Nishihori1, D. Sullivan1, 1H Lee Moffitt Cancer Center, Experimental Therapeutics, Tampa, USA; 2H Lee Moffitt Cancer Center, Hematological Oncology/BMT, Tampa, USA; 3H Lee Moffitt Cancer Center, Clinical Science, Tampa, USA

Background: Multiple myeloma (MM) accounts for approximately 10% of all hematologic malignancies, with 30,770 new cases (16,400 men and 14,370 women) and 12,770 related deaths (6,830 men and 5,940 women) estimated to occur in the USA in 2018. Significant increases in resistance have been seen over the past several years, although MM remains incurable and treated patients ultimately die from progressive disease refractory to anti-myeloma therapy. Melphalan, an alkylating agent that produces DNA interstrand crosslinks, is one of the most widely used and effective drugs for the treatment of MM. However, acquired melphalan resistance is a major obstacle to the improvement of outcomes in MM therapy.

Materials and Methods: In this study, we show that the exportin 1 inhibitors (XPO1i’s) selinexor, eltanexor, and KOS-2464 sensitizes human MM to melphalan. We demonstrate this antitumor effect in both parental and melphalan-resistant human MM cell lines in vitro, in NOD-SCID-g mouse models in vivo, and in patient-derived MM cells ex vivo. Mechanistic studies include g-H2AX and alkaline comet assays for DNA damage and repair and ELISA, PAGE, and intracellular proximity ligation assays for monoubiquitinated FANCD2 activation.

Results: Human RPMI8226 (8226) (P < 0.0003), HS29 (P < 0.0001), and U266 (P < 0.0001) MM cell lines and melphalan-resistant 8226/LR5 (P < 0.0001) and U266/LR6 (P < 0.0001) cell lines were highly sensitized to melphalan by XPO1i treatment. XPO1i/melphalan combination treatment demonstrated a strong synergistic antitumor effect when compared to single-agent melphalan in NOD-SCID-g mice challenged with both parental and melphalan-resistant U266 (P < 0.002) and melphalan-resistant U266/LR6 (P < 0.005) MM tumors, with little toxicity (less than 10% weight loss). The XPO1i/melphalan combination showed a synergistic antitumor effect in mouse xenografts, with significant tumor growth delay and partial tumor regression with a 17-day treatment. Furthermore, U266/LR6 cell lysis was increased in the presence of XPO1i/melphalan compared to melphalan alone and was shown to have reduced rates of UGT2B mediated glucuronidation in vivo. Western blot and immunoblot were performed to identify and characterize key proteins of XPO1i/melphalan synergistic cell kill. Addition of XPO1i decreased monoubiquitinated FANCD2 protein expression and was shown to have reduced rates of UGT2B mediated glucuronidation by HPLC. Sensitivity of LNCaP-RAL3 cells to EPI-045 was confirmed in vitro and in vivo.

Results: Melphalan displayed antitumor activity in LNCaP but not LNCaP-RAL3 xenografts; a function of its impaired ability to inhibit AR transcriptional activity. Strikingly, LNCaP-RAL3 cells retained sensitivity to androgenreceptors and AR knockdown by targetd siRNA, implying growth remains driven by AR signaling. Interrogation of the drug resistance genomics (UGTB family) whose expression and activity was associated with androgen resistance in additional samples. Knockdown of UGT2B isoforms was sufficient to restore sensitivity to melphalan and selinexor in resistant cell lines. Gene expression and metabolomic data using clinical samples, revealed that androgens is glucuronidated in humans. EPI-045 (which is resistant to glucuronidation) significantly inhibited AR mediated transcription and proliferation in LNCaP-RAL3 cells – both in vitro and in vivo.

Conclusions: We have generated a model of acquired androgen resistance, and demonstrated that selective modification of androgen can improve drug sensitivity by reducing its metabolism by glucuronidation. LNCaP-RAL3 cells remain dependent upon AR signalling, and are sensitive to both EPI-045 and androgenreceptors used clinically. This work highlights the potential for combination or sequential therapy following androgen resistance, and will hopefully drive the discovery of additional AR-NTD inhibitors.

Conflict of interest: Ownership: JO, JW, KJ, DW, AHT, NRM, YCY, RJA, MDS have stock equity in ESSA Pharma, Inc. JW, DW, NRM, RJA, MDS have stock equity in ESSA Pharma, Inc. Board of Directors: MDS and RJA are consultants, Directors and Officers of ESSA Pharma Inc.

354 (PB-017) Poster
Downregulation of Cyclin B1 as a potential mechanism of resistance to the cell cycle checkpoint kinase 1 (CHK1) inhibitor, Prexerabot (Prex) J. Nair1, B. Haynes3, T.T. Huang1, J.M. Lee1 1National Cancer Institute, Women’s Malignancies Branch, Bethesda, USA

Background: High grade serous ovarian cancer (HGSOC) is the most lethal gynecologic malignancy in the United States. Most HGSOCs have a loss of G2 cell cycle arrest for survival. We recently showed early clinical activity of CHK1 inhibitor (CHK1) Prex monotherapy in heavily pretreated HGSOC patients (pts). But, most pts ultimately develop resistance to Prex. Alterations of cell cycle is one of the major mechanisms of resistance to DNA repair inhibitors. Thus, we hypothesized that a CHK1-independent delay in G2/M phase might give HGSOC cells sufficient time to repair DNA damage, making cells resistance to Prex treatment.

Methods: We developed Prex-resistant (PrexR) cells line with OVCAR5 and OVCAR8 (both BRCA wild type, platinum-resistant HGSOC cell lines) by culturing them in progressively increasing concentrations of Prex over 3–4 months. Immunofluorescence (IF) for γH2AX staining, cell cycle analysis (IF) for H2AX staining, and immunoblot were performed to identify and characterize key proteins of cell cycle and DNA damage response pathways. Experiments were repeated at least twice. Statistical analysis was done by Student’s t test. Cell growth curves are shown as mean ± SD. Transcriptome profiles of PrexR parental and parental cells were generated using RNA-seq. Ablation pathways were identified through GSEA analysis for Prex-resistance.

Results: Cell growth assays using first TTT and >1000-fold increase in viability to Prex treatment in Prex-R cells compared to parental cells. An increase in γH2AX staining (>3 fold over untreated), indicative of DNA damage, was observed in parental cells when treated with 20 nM Prex overnight, while no changes were seen in PrexR cells. Cell cycle analysis showed a 2-fold increase in G2 population (28–34%) in PrexR relative to parental cells (12–20%). Prex treatment (20 nM overnight) did not affect this G2 delay in PrexR while parental cells showed markedly increased S
Novel RET+ patient derived cell lines reveal unique signaling dynamics and dependencies

1University of Colorado School of Medicine, Department of Medicine, Division of Medical Oncology, Aurora, USA; 2 Moffitt Cancer Center, Department of Drug Discovery, Tampa, USA

Background: Approximately 1% of NSCLCs harbor chromosomal rearrangements of the ret proto-oncogene (RET). RET rearrangements result in the aberrant expression and constitutive, ligand-independent activation of the RET kinase, which promotes cancer cell growth, proliferation and survival. RET is fused to KIF5B in ~70% of RET+ lung cancers, but can have other 5 partners such as CCDC6, NCOA, TRIM33 and TRIM24. In RET+ lung cancer, RET signaling is amplified in 99% of patient samples, while 10% show high levels of RET expression. We generated 5 RET+ lung cell lines to better understand the mechanisms of RET+ lung cancer and to assess the efficacy and signaling dynamics of RET inhibition. To isolate the role of the 5 partners in response to RET inhibitors we have also employed CRISPR/Cas9 technology to model endogenous RET rearrangements in Ba/F3 cells. RNA sequencing was completed for the CUTO22, CUTO32 and LC-2/Ad (commercial CDDC6-RET+) cell lines

Results: We discovered that the CUTO22 and CUTO42 cell lines were sensitive to RET inhibition, while the CUTO32 cells were resistant to multiple RET inhibitors. We determined that phospho-RET (pRET) was successfully inhibited by ponatinib and RDX5-105 in all three cell lines, however, downstream inhibition varied across cell lines. The two non-KIF5B RET+ cell lines, LC-2/Ad and CUTO42 had more dramatic inhibition of pAKT than the KIF5B-RET+ cell lines following RET inhibition. Ba/F3 cells harboring CRISPR-Cas9 generated KIF5B-Ret and Trim24-Ret rearrangements were equally sensitive to multiple RET inhibitors. In order to address possible bypass pathways that may account for resistance to RET inhibitors in the CUTO32 cell line we found that inhibition of Src sensitized the cells to RET inhibition. In order to determine other pathways RET+ cells may be dependent on we performed GSEA analysis of RNAseq data which showed that PI3K signaling, EMT and angiogenesis were enriched in CUTO22 and CUTO32 cells compared to LC-2/Ad. Drug screening data similarly revealed unique vulnerabilities; the CUTO32 cell line was uniquely sensitive to two PLK1 inhibitors, valasertib and ON-01910. CUTO22 and CUTO32 cells both demonstrated sensitivity to several Aurora kinase inhibitors in the drug screen.

Conclusions: These data suggest that lack of response to RET inhibitors, particularly in the case of KIF5B-RET, is not due to poor drug binding. Overall, RET+ lung cancers may have heterogeneous cellular signaling and pathway reliance.


Poster Session (Friday, 16 November 2018) Abstracts, 30th EORTC-NCI-AACR Symposium
28.2 nM vs SKBR3-Par 10.9 ± 3.4 nM). RPRA interrogation of the SKBR3-A cells showed alterations in several pathways, including significant increased levels of p-Src (Y416). SKBR3-A cells were more sensitive to Src inhibition with dasatinib compared to SKBR3-Par cells and the combination of afatinib and dasatinib was highly synergistic in SKBR3-A cells. The median CAC value was 0.09 ± 0.06. Combination treatment did not cause significant induction of G1 cell cycle arrest (p = 0.36), apoptosis (p = 0.3) or autophagy (p = 0.21). However, afatinib plus dasatinib stimulated a significant increase in secreted HMGB1, a marker of necrosis induction.

Conclusion: Src represents a potential therapeutic target to overcome resistance to afatinib in HER2-positive breast cancer. The effect of afatinib and dasatinib is apoptosis-independent and, instead, stimulated necrotic cell death in SKBR3-A cells.


358 (PB-021) Poster

Preliminary results MATCH-R trial, a prospective trial to study acquired resistance of tumors from patients treated with molecular targeted agents or immunotherapy


Background: It is increasingly important to understand the molecular mechanisms underpinning primary or acquired resistance to targeted therapies and immunotherapies as it can point to the use of specific drug combinations that can prevent or delay resistance.

Material and Methods: MATCH-R () is a prospective trial to identify molecular mechanisms of primary or acquired resistance to targeted therapies and immunotherapies in patients with unresectable or metastatic cancer (NCT02517892). Clinical characteristics are recorded, a biopsy tissue is performed (and rebiopsy is required in selected cohorts) and serial blood samples are collected. Targeted NGS, SNP array, WES and RNAseq are performed on the tissue biopsies, and PDX are established (in collaboration with Xentech) for selected drugs/target. Five cohorts are open. Cohort 1 includes patients with drug’s acquired resistance, defined as a PD after a PVR/CR or a SD for at least 6 months. Cohorts 2–5 mandate pre and post drug biopsies, for selected population based on tumor localization (prostate, bladder), drug category (immunotherapy) or presence of a target (ALK, EGFR, etc.).. We report preliminary results of the feasibility of MATCH-R.

Results: From June 29th 2015 and as of June 15th 2018, 309 patients were included. Primary tumors were mainly lung (n = 142), Prostate (n = 86), urothelial (n = 24). Tumor cells content was ≤10% in 28 patients (9%), median was 50% (0 ≤ 50) for all samples, 50% for the liver (n = 50), nodes (n = 46), or lung (n = 96) biopsies, 40% for prostate (n = 54) biopsies. There were no grade 5 toxicities. Analysis failed in 56 (19%) patients, are pending in 10. WES was successfully performed in 236 (75%) patients, RNAseq in 212 (71%), both in 205 (68%). Out of 93 patients treated with a kinase inhibitor, a resistance mechanism was successfully identified in 70 patients (75%); secondary mutations in 41 of the cases, bypass mechanisms in 36% and non genetic mechanisms in 23%. Out of 154 patients grafted, 48 (31%) PDX models were established from 15 lung tumors, 12 prostate tumors, 9 bladder tumors, 3 belliini tumors and 9 other tumor types. 12 PDXs were tested for their drug’s resistance and 11 matched the patient’s tumor resistance to FGFR, EGFR, ALK, MEK, MDM2 or NOTCH1 inhibitors.

Conclusions: MATCH-R successfully delivers large molecular characterizations, that allow to identify resistance mechanisms. MATCH-R generated PDXs models that mimic the patient’s tumor resistance. Our data sharing policy allow partnering with academic or industrial entities. This ongoing trial will be amended to better suit potential future collaboration.

No conflict of interest

360 (PB-023) Poster

Integrated pharmacodynamic analysis identifies two metabolic adaptation pathways to metformin in breast cancer


Background: Epidemiological studies have shown that treatment of type 2 diabetes with metformin is associated with reduced cancer risk and phase 3 trials investigating metformin as a cancer therapy are underway. However, there remains controversy as to the mode of action of metformin in tumours at clinical doses. To investigate this we conducted a clinical study that integrated measurement of markers of systemic metabolism, dynamic FDG-PET-CT, transcriptomics and metabolomics at paired time points to profile the bioactivity of metformin in primary breast cancer.

Materials and Methods: 40 non-diabetic patients were recruited with primary breast cancer to a neoadjuvant window trial. Patients received an escalating dose of metformin to 1500 mg for 2 weeks with pre- and post-metformin dynamic 18F-FDG-PET-CT scans, serum metabolic markers, and tumour biopsies for whole transcriptome RNASeq, tumour metabolomics and immunohistochemistry.

Results: Assessment of tumour FDG kinetics using a classic 2-tissue compartment model with three rate constants showed a 1.3 fold change (FC) post-metformin in the composite 18F-FDG flux constant, K12 (p = 0.041, paired t-test). Mass spectrometry metabolic analysis revealed a decrease in intratumoral levels of propionylcarbamide (FC -0.5, p = 0.039) and acetylcarbamide (FC -0.4, p = 0.046) (Wilcoxon rank test). RNASeq revealed metformin activates multiple mitochondrial metabolic pathways. Two tumour groups were identified with distinct metabolic responses, an OXPHOS transcriptional response group (OTR) for which there is an increase in OXPHOS gene transcription and an FDG response group (FR) with increased 18-FDG uptake. Increase in proliferation, as measured by a validated proliferation signature, suggested patients in the OTR group were resistant to metformin treatment. No correlation existed between metformin’s effects on host metabolism and its tumour specific effects.

Conclusions: This data provides strong evidence that metformin has a direct effect on breast cancer metabolism at clinical doses. We conclude that mitochondrial response to metformin in primary breast cancer is likely to define anti-tumour effect.

No conflict of interest

Friday, 16 November 2018

POSTER SESSION

Epigenetic modulators

361 (PB-024) Poster

Expanding the potential for epigenetic therapies in combination with current standard-of-care therapy for prostate cancer

J. Manley 1, J. Gregg 2, S. Mcadade 3, I. Mills 4, D. Waugh 5, M. LaBonte Wilson 6, Queen’s University Belfast, Centre for Cancer Research and Cell Biology, Belfast, United Kingdom

Background: Prostate cancer (CaP) is the second leading cause of male cancer in the Western world and therefore significant health burden worldwide. First line Standard-of-care (SOC) therapies for organ confined disease are surgery and radiation. Radiation therapy, although highly effective, results in release in over 30% of patients within 3 years and resistance to subsequent hormonal therapies brings about progression to incurable castrate-resistant disease.

Abstracts, 30th EORTC-NCI-AACR Symposium Poster Session (Friday, 16 November 2018)
Epigenetic silencing of cancer-related genes has been shown to be highly influential in CaP disease progression. Epigenetic regulators, including histone deacetylases (HDAC), are commonly mutated and highly expressed in cancer, representing attractive clinical targets. Although five HDAC inhibitors are FDA-approved for myelodysplastic syndromes, pan-HDAC inhibitors (HDACi) have not been effective to date in CaP clinical trials.

As class 1 HDACs are highly expressed in CaP, we hypothesised that class 1 HDAC inhibition by Entinostat is sufficient to induce anti-tumour effects and synergise with the current SOC therapies, radiation and androgen deprivation, demonstrating its potential to be integrated as a novel treatment strategy for CaP.

**Material and Methods:** PC3, LNCaP and C4-2B cell lines were treated with Entinostat as a single agent and in combination with Radiation, Enzalutamide or Abiraterone. Synergistic responses were determined through growth inhibition and colony formation assays with transcriptome and proteome analysis to understand the mechanism of action in vitro and the determination of optimum treatment scheduling for combination therapy. Caspase-Glo 3/7 assay and FACS analysis were performed to measure apoptotic activation.

**Results:** Entinostat was assessed as a single agent across a panel of CaP cell lines and was active with a median IC50 of 1.08 μM (range, 0.586–1.871 μM). A combination of Entinostat with either radiation or androgen deprivation therapy resulted in a synergistic reduction in cell proliferation and survival with increased apoptotic population and caspase-3/7 activation. Molecular analyses showed a decrease in androgen receptor in C4-2B cells treated with Entinostat compared with vehicle control, accompanied by a reduction in expression of AR-regulated gene KLK3 encoding prostate-specific antigen which was further reduced in response to combination with androgen deprivation.

**Conclusions:** HDACi have shown limited success in clinical trials for solid tumours including CaP, however, there is great potential for the use of class-1 specific HDACi Entinostat in combination with current SOC therapies. Determining the mechanism of action of these synergistic effects of HDACi in CaP will improve clinical application and determination of patient stratification through better understanding of predictive biomarkers.

**No conflict of interest**

---

**364 (PB-027) Poster**

**Enzalutamide and decitabine in combination inhibits growth of castration resistant prostate cancer cells with a greater response than enzalutamide alone**

J. Gaddy1, D. Goodrich2, G. Chatta3, K. Wadowsky2. 1University at Buffalo, Internal Medicine, Buffalo, USA; 2Roswell Park Comprehensive Cancer Center, Pharmacology and Therapeutics, Buffalo, USA; 3Roswell Park Comprehensive Cancer Center, Department of Medicine, Buffalo, USA.

**Background:** Metastatic castrate resistant prostate cancer (mCRPC) remains a fatal disease. It has contributed to high mortality and morbidity. Until 2011, limited treatment options were available. Despite the availability of potent AR targeted agents such as enzalutamide emergence of resistance is inevitable. We have demonstrated that in part, enzalutamide-resistance is due to reversible epigenetic changes. Treatment of prostate cancer (PCA) cell lines with inhibitors of EzH2 histone methyltransferase can restore enzalutamide sensitivity. While in clinical development, EzH2 inhibitors are not yet approved. We hypothesized that enzalutamide resistance may be reversible via epigenetic modulation using the DNA hypomethylating agent decitabine, an inhibitor of DNA methyltransferase (DNMT).

**Methods:** We treated human CRPC cell lines with decitabine as a single agent or in combination with enzalutamide. Each line was treated with decitabine at 10 nM or 100 nM when used in combination or as a single agent. Enzalutamide was used at 10 μM. Our endpoint was cell number.

**Results:** Our data show that enzalutamide and decitabine in combination is more effective compared to enzalutamide alone. In 22Rv1 cells, established mCRPC cell line, decitabine with enzalutamide was significantly more effective at reducing cell number compared to decitabine alone, with a decrease of 50.6% vs. 28.7%. In 22Rv1 cells, decitabine in combination with enzalutamide than 100 nM, with a 5.96% greater decrease in cell number. These data are consistent with known clinically effective doses, where lower doses of decitabine have been shown to decrease disease burden while reducing the cytotoxic effects of DNMT inhibition.

**Conclusions:** In human CRPC cell lines, enzalutamide and decitabine are more effective in combination than enzalutamide alone. These data suggest that decitabine can improve the efficacy of enzalutamide and support rationale for pursuing a clinical trial for enzalutamide and decitabine for the treatment of mCRPC.

**No conflict of interest**

---

**365 (PB-028) Poster**

**Aristoyagonine, a naturally occurring alkaloid, reveals anticancer activity by inhibiting the bromodomain-containing protein 4 (Brd4)**

S.U. Choi1, H.K. Lee1, C.O. Lee1, C.H. Park1. 1Korea Research Institute of Chemical Technology, Drug Discovery Division, Daejeon, South Korea

**Background:** Bromodomain-containing protein 4 (Brd4) is known to play a key role in tumorigenesis. It binds acetylated histones to regulate the expression of numerous genes. Because of the importance of brd4 in tumorigenesis, much research has been undertaken to develop brd4 inhibitors with therapeutic potential. As a result, various scaffolds for bromodomain inhibitors have been identified.

**Material and Methods:** To search for novel BRD4 inhibitors with new scaffolds, we performed mid-throughput screening using two different enzyme assays, alpha-screen and ELISA. In addition, we measured the c-Myc level in cancer cells to confirm the BRD4 inhibiting activity of the hit compounds. Then, we tested anticancer activity in vivo by using Tu92 cancer cell xenograft assay.

**Results:** We found several BRD4 inhibitory hit compounds including the aristoyagonine. Aristoyagonine is a natural compound and is reported its isolation from Sarcocapnos plants in 1984, firstly. The 50% inhibitory concentrations (IC50) of the aristoyagonine are 0.8 μM in BRD4 inhibitory alpha-screen assay and 0.75 μM in Tu92 cancer cell cytotoxicity assay. It also reduced the cancer volume about 56% in vivo assay by using the Tu92-xenograft mouse model. In addition, we have tested hERG pach clamp assay, and it revealed the IC50 value about 80 μM. Then, we tested the cytotoxicity of the compound against human gastric cancer cells in comparison with the I-BAT 762, a BRD4 inhibitory standard drug, and we found it revealed comparable cytotoxicity to I-BAT 762 in some cell lines.

**Conclusions:** We found aristoyagonine as a novel BRD4 inhibitor, and it revealed anticancer activity in vivo cancer xenograft system. This is the first report to describe a natural compound as a Brd4 inhibitor, and the structural characteristics of aristoyagonine could be helpful for designing more effective BRD4 inhibitors.

**No conflict of interest**
Background: The role of mutations in genes involved in epigenetic and chromatin remodeling processes has been well studied in tumorigenesis, but their prevalence in metastatic solid tumors is unknown. We aimed at: (i) performing a systematic description of the presence of mutations in genes encoding chromatin remodelers, erasers, writers, readers and histones were assessed by a molecular tumor board. Clinical characteristics and outcome were collected.

Results: Between Dec 2011 and Oct 2016, molecular data from successful WES from 301 tumors were evaluated; 414 mutations in epigenetic genes – 134 (32%) PV and 280 (68%) VUP – were identified in 187 pts (55.5%). Median age of these pts was 59 y/o (range, 4–83); 46% were male. Twenty different tumor types were represented, including non-small cell lung cancer (NSCLC; 35 pts, 21%), breast cancer (20 pts, 12%), small cell lung cancer (NSCLC; 14%, 10%) and HNSCC (17 pts, 10%); 93 tumor samples (56%) presented more than one mutation in epigenetic genes. Most frequent altered genes were: KMT2A (10%), and KMT2D (17%). KMT2C (14%), KMT2A (10%), and ARID1A (10%). These alterations were more frequent in NSCLC 94 (23%), urothelial cancer 59 (14%) and breast cancer 20 (12%) and HNSCC (17 pts, 10%); 93 tumor samples (56%) presented more than one mutation in epigenetic genes. Most frequent altered genes were: KMT2A (10%) and KMT2D (17%). KMT2C (14%), KMT2A (10%) and ARID1A (10%). These alterations were more frequent in NSCLC 94 (23%), urothelial cancer 59 (14%) and breast cancer 20 (12%) and HNSCC (17 pts, 10%). These alterations were more frequent in NSCLC 94 (23%), urothelial cancer 59 (14%) and breast cancer 20 (12%) and HNSCC (17 pts, 10%). These alterations were more frequent in NSCLC 94 (23%), urothelial cancer 59 (14%) and breast cancer 20 (12%) and HNSCC (17 pts, 10%). These alterations were more frequent in NSCLC 94 (23%), urothelial cancer 59 (14%) and breast cancer 20 (12%) and HNSCC (17 pts, 10%).

Conclusions: Mutations in epigenetic genes occur in more than half of the pts with metastatic solid tumors. With the current development of novel epigenetic-modifying drugs and synthetic lethal strategies for epigenetic deficiencies, these might soon represent druggable targets in a selective and targeted fashion. The functional significance of most variants is still unknown; functional studies and correlation with clinical outcome will help deciphering their druggability, role in disease progression and treatment resistance.

Conflict of interest: Other Substantive Relationships: Pr. JC SORIAL is full time employee of Medimmune since sept 2017. All remaining authors have declared no conflicts of interest for this work. Background: PRMT5, an epigenetic modulator is highly expressed in several cancers, including lymphoma and glioblastoma. PRMT5 over-expression is thought to be an important factor in its tumorigenicity due to its repressive function on the expression of tumor suppressor genes. Recently, specific novel role of PRMT5 has been reported in glioblastoma (GBM), where cancer cells with altered splicing are addicted to PRMT5 for their survival. Therefore, PRMT5 inhibition could be a novel and effective approach for GBM and PRMT5 inhibitors with brain penetration could be valuable to treat this cancer. Materials and Methods: Structure based drug design were used to identify novel PRMT5 inhibitors. FlashPlate™ methylation assay was used to assess in vitro potency. Cell based activity of these inhibitors was assessed by measuring the symmetrical dimethylation of known cellular protein SmD3. Long term cell proliferation assays were used to assess the functional effect of PRMT5 inhibition. Orthotopic models were used to assess tumor growth inhibition in vivo. Results: A number of compounds from two different series showed strong in vitro potency against PRMT5, which were comparable to the clinical molecule GSK-3326595. Multiple co-crystal structures have been solved in-house and are extensively used in optimization of these novel scaffolds. Their cell based potency, as measured by proliferation assay in multiple haematological and solid tumor cell lines was comparable to biochemical potency. Jubilant PRMT5i (JPRMT5i), from one of the series showed an in vitro potency of 0.0045 μM and 0.005 μM in inhibiting SmD3 dimethylation, and PRMT5i exhibited an GI₅₀ of 0.029 to 1.5 μM in inhibiting proliferation of GBM cell lines. JPRMT5i showed good in vitro ADME properties in terms of aqueous solubility and metabolic stability and reasonable oral bioavailability and brain exposure in mouse pharmacokinetics studies. In GMB orthotopic model, oral administration of JPRMT5i at 50 mg/kg resulted in statistically significant increase in survival, which was accompanied by strong biomarker modulation. Treatment was tolerated well as observed by body weight changes.

Conclusions: Additional back-up molecules have been identified in both series that show comparable potency with better PK properties, including brain exposure. Few of these NCEs are being tested as standalone therapy and in combination with other standard of care agents in GMB models. Data from these studies will be presented in this meeting. No conflict of interest

Abstracts, 30th EORTC-NCI-AACR Symposium Poster Session (Friday, 16 November 2018)
AR-V7 splice variant or AR over-expression. CPI-1205 cooperates with both enzalutamide or abiraterone resulting in synergistic cell growth inhibition, and enhanced efficacy in mouse xenograft models of PC. Transcriptional approaches revealed that CPI-1205 inhibits cell growth via alteration of lineage and differentiation transcriptional programs independent of the AR pathway. When combined with AR inhibitors this results in changes in lineage specific genes, as well as an enhanced downregulation of the AR pathway. Thus, EZH2 inhibition represents a novel therapeutic approach for mCRPC patients that remain dependent on AR signaling, yet no longer respond to anti-androgens.

Conflict of interest: Ownership: Constellation Pharmaceuticals.

370 (PB-033) Poster Functional annotation of SWI/SNF complex protein Arid1a in pancreatic cancer
S. Gupta1, S. Ferri-Borgogno1, T. Griffiths1. 1The UT M.D. Anderson Cancer Center, Translational Molecular Pathology, Houston, USA

Background: The genetic complexity of pancreatic adenocarcinoma (PDAC), revealed by exome analyses of patient tumors, demands a personalized molecular-targeted approach to treatment. While it has been difficult to successfully target the top four mutant genes, Kras, Tp53, Cdkn2a and Smad4, found in most patient samples; there is a defined subset of patients with deleterious mutations in genes encoding SWI/SNF complex, mainly in AT-rich interactive domain 1A (Arid1a).

Material and Methods: To understand functional role of Arid1a in PDAC development and malignancy, we generated a genetically engineered mouse model (GEMM) of PDAC driven by mutant Kras (G12D) either with (KAC) or without Arid1a-deletion (“KC”). These mice were necropsied at regular time intervals to study the spontaneous disease progression or allowed to get moribund to generate K-M survival curve. Upon necropsy, pancreas and other organs were collected for formalin fixation to allow histochemical analysis. Some live tumors were also collected to derive cell lines, which were further analyzed by various functional assays and transcriptome analyses. Such lines were also subjected to high throughput drug screens targeting mouse kinome and clinically actionable drugs to discover therapeutic vulnerability of Arid1a-deficient PDAC tumors.

Results: Littermate comparison of KAC with KC mice revealed faster disease development in absence of Arid1a expression and shorter survival of these mice. Transcriptome analysis on KAC cells showed enrichment of epithelial-to-mesenchymal transition (EMT) associated genes and other oncogenic pathways.

Conclusions: Based on our observations, we conclude that early loss of Arid1a cooperate with mutant Kras signaling in faster disease progression in a genetically engineered mouse model (GEMM) of PDAC.

No conflict of interest

Friday, 16 November 2018
POSTER SESSION
Molecular Targeted Agents – PART II

371 (PB-034) Poster Combination of the AKT inhibitor ARQ 751 with Immune Checkpoint Inhibitor and Other Therapeutic Agents
Y. Yu1, R.E. Savage1, S. Eathiraj1, T. Hall1, B. Schwartz1, 1ArQule Inc, Clinical Development, Burlington, USA

Background: Dysregulation of the PI3K-AKT signaling pathway is associated with many cancers. There are multiple mechanisms of AKT dysregulation in cancer including activation of receptor tyrosine kinases, gain-of-function mutations of PIK3CA, PTEN deficiency, AKT amplification and activating mutations in AKT such as AKT1-E17K. ARQ 751 is a second-generation AKT inhibitor, which has distinct physico-chemical properties compared to ArQule’s first-generation inhibitor, mirostilbi (ARQ 092). ARQ 751 is currently in a dose-escalation phase 1 clinical study in molecularly defined cancer patients.

Methods: The biochemical IC50 for ARQ 751 against AKT 1/2/3 was determined. The binding of ARQ 751 to both wild-type AKT1 and AKT1E17K was was assessed using intrinsic tryptophan fluorescence quench. In vitro combination studies were performed using an anti-proliferative MTs assay as either single agents or in combination. In vivo efficacy was tested in patient-derived tumors with endometrial cancer cells bearing AKT1E17K mutations. A syngeneic mouse colon CT-26 model was used to test the combined effect of ARQ 751 with an anti-PD-1 antibody.

Results: ARQ 751 inhibited AKT1/2/3 activity with IC50 values of 0.54, 0.79, and 1.3 nM, respectively. In binding studies, ARQ 751 exhibited IC50 values of 1.2 nM for wild-type AKT1 and 8.6 nM for the AKT1E17K mutant. Endometrial PDX models harboring AKT1E17K showed tumor growth inhibition of 68, 78 and 98% at ARQ 751 dose levels of 25, 50 and 75 mg/kg, respectively. The combination of ARQ 751 with either an ER antagonist or aromatase inhibitor exerted a higher anti-proliferative effect in ER+ and PIK3CA mutant endometrial cancer cells compared to single agent. Combination studies were also performed with ARQ 751 and enzalutamide in LNCaP prostate cancer cells (PTEN null). In this study, pathway analysis showed that inhibition of AR by enzalutamide elevated pAKT while inhibition of AKT by ARQ 751 increased AR protein expression, demonstrating that the combination of ARQ 751 and enzalutamide blocked both AR and AKT pathways. Additionally, combination studies with ARQ 751 and ARQ 531 (reversible BTK inhibitor) were performed in 1 CLL and 3 MCL cell lines and showed either synergistic or additive effects. Similar results were obtained when ARQ 751 was combined with the BTK inhibitor ibrutinib. In vivo combination of ARQ 751 with anti-PD-1 antibody in a syngeneic mouse colon CT-26 model exhibited superior anti-tumor activity compared to the single agents.

Conclusions: ARQ 751, a potent, selective, next generation AKT inhibitor, can be combined with multiple therapeutic agents including immune checkpoint inhibitors, ER antagonists, AR antagonists and BTK inhibitors. These data provide the rationale for testing combinations of ARQ 751 with these therapeutic agents clinically.

Conflict of interest: Corporate-sponsored Research: Yi Yu, Ronald E. Savage, Sudhardtshen Eathiraj, Terence Hall, Brian Schwartz are the employees of ArQule, Inc.

372 (PB-035) Poster Coccoquinones, new anthraquinone derivatives, suppress p53-dependent growth of cancer cells
D. Tatsuda1, I. Momose2, M. Amemiya1, K. Suniyoshi3, T. Watanabe1, M. Kawada1, M. Shibasaki1. 1Institute of Microbial Chemistry, Tokyo, Tokyo, Japan; 2Institute of Microbial Chemistry, Numazu, Shizuoka, Japan; 3Meiji Seika Pharma Co., Ltd., Bioscience Laboratories, Kanagawa, Japan

Background: Transcription factor p53 is a negative regulator of cell growth in eukaryotic cells. p53 induces cell cycle arrest or apoptosis in response to damages induced by ultraviolet light, infrared light, and chemical agents. In cancer cells, p53 activity is usually suppressed by binding to Mdm2, a ubiquitin E3 ligase. Mdm2 ubiquinates p53 and induces proteasome degradation of p53. Because p53 activation can induce p53-dependent cell cycle arrest and cell death in cancer cells, activation of the p53-dependent signaling pathway is an attractive target for the development of anti-cancer drugs.

Material and Methods: We established a cell-based assay using human glioblastoma LNZTA3 cells in which p53 expression can be regulated by tetracycline. To search for a new activator of the p53-dependent signaling pathway, we screened microbial metabolites. Human glioblastoma LNZTA3 cells (tetracycline-regulated p53 expression), human gastric cancer MKN45 cells (p53 wild-type), and MKN7 cells (p53 mutant-type) were incubated with the metabolites for 3 days, and cell viability was measured by MTT assay.

Results: As a result of screening, we identified coccoquinones A and B, novel anthraquinone derivatives, from Staphylotrichum coccosporum PF1460. Staphylotrichum coccosporum PF1460 was isolated from a soil sample collected in Ishigaki Island, Okinawa prefecture, Japan. The 28S rrNA-D1/D2 and ITS-5.8S rrRNA sequences of strain PF1460 were identical to those of Staphylotrichum coccosporum NBRC33272 (100%). Coccoquinones isolated as a red amorphous solid. The UV spectrum of coccoquinones was similar to that of quinofuracins. The structures of coccoquinones were closely related to versiconolone and paecilocinone. Coccoquinones induced p53-dependent growth suppression and the accumulation of p53, p21, and PUMA; and promoted the degradation of PARP in p53-expressing LNZTA3 cells.

Conclusions: These data suggest that coccoquinones activate p53 and induce cell death in vitro.

No conflict of interest
In vitro and in vivo activity of a HER2-targeted thorium-227 conjugate (HER2-TTC) in HER2 low expressing and T-DM1/trastuzumab resistant preclinical mouse models

J. Karlsson1, V. Cruciani1, D. Grant1, C. Ellingsen1, D. Petros1, A. Kristian1, U. Hagemanns2, C. Schatz2, R. Bjelke1, O. Ryan1, D. Mumberg3, A. Cuthbertson1, 1Bayer AS, Thrombolytic Ceramic Research, Oslo, Norway; 2Bayer AG, Oncology, Berlin, Germany

HER2 is overexpressed in several cancers and is a highly validated target for the treatment of breast and gastric cancer also serving as a prognostic and predictive biomarker. Several HER2-targeting antibodies are approved or are in late phase clinical development. Current HER2-targeted therapies are effective in treating cancers overexpressing HER2, but find utility amongst a limited population of patients with breast or gastric cancer (~20%). During treatment, many patients become resistant or are simply not eligible for these therapies, due to low expression levels of the target (~55%). Therefore there exists a high unmet medical need for new drugs with alternative mechanisms of action targeting HER2.

Targeted alpha therapy (TAT) has become an established modality in the treatment of metastatic castration-resistant prostate cancer following the approval of radium-223 dichloride. TATs are highly cytotoxic due to the high linear energy transfer of the alpha-particle emitting radionuclide which induces complex DNA double-strand breaks in the targeted tumor cell. Thorium-227 has a half-life of 18.7 days, decaying to radium-223 via alpha- particle emission. We describe herein an antibody conjugate capable of delivering the payload thorium-227 (227Th) to cancer cells expressing the human epidermal growth factor receptor 2 (HER2).

The HER2 targeted thorium-227 conjugate (HER2-TTC) comprises a humanized HER2 targeting IgG1 antibody covalently linked via an amide bond to a chelator moiety (3,2-HOPO), enabling room temperature radiolabeling with the alpha particle emitter 227Th. The preclinical activity of HER2-TTC was characterized in low HER2 expressing as well as in T-DM1/trastuzumab resistant models.

HER2-TTC was prepared at high radiochemical yield and purity. Binding of HER2-TTC to recombinant HER2 was shown to be comparable to trastuzumab. In vitro, pM cytotoxicity and specificity of HER2-TTC was shown on HER2 positive cancer cell lines. In vivo, specific tumor accumulation and anti-tumor activity of HER2-TTC was observed following i.v. injection of 125–500 kBq/kg a protein dose of 0.18 mg/kg in mice bearing and gastric cancer patient derived xenograft models. These models were chosen as they were known to be resistant to T-DM1/trastuzumab and had a range of HER2 expression as evidenced from IHC (1+ to 3+).

The results support the development of a HalT-TTC for the treatment of T-DM1/trastuzumab resistant disease and offer potential for the population of patients with low HER2 expression.

No conflict of interest

374 (PB-037) Poster

Development of PROTACs for targeted degradation of MALT1

D. Jones1, W. Gibbs1, M. Ambler1, C. Crompton1, B. Saxty1, M. Newman1, K. Ansell1, 1LifeArc, Biology, Stevenage, United Kingdom; 2LifeArc, Chemistry, Stevenage, United Kingdom

Background: Proteolysis Targeting Chimeras (PROTACs) are heterobifunctional molecules that facilitate degradation of targeted proteins in the cell via the ubiquitin proteasome system. PROTACs are comprised of a ligand for the protein of interest, and a ligand for an E3 ligase (typically VHL or Cereblon) that work catalytically to degrade their targets and in so doing eliminate scaffolding protein of interest.

Results: We have synthesised multiple PROTACs that are effective at knocking-down MALT1, affecting both its catalytic and scaffold functions.

Conflict of interest: Other Substantive Relationships: All authors are employees of LifeArc.

375 (PB-038) Poster

Role of MALT1 in cell cycle control: scaffold vs protease activity

D. Jones1, W. Gibbs1, M. Newman1, A. Levy1, M. Ambler1, K. Ansell1, B. Saxty1, 1LifeArc, Biology, Stevenage, United Kingdom; 2LifeArc, Chemistry, Stevenage, United Kingdom

Introduction: The paracaspase MALT1 is a cysteine-dependent, arginine-directed protease that is essential for the initiation of adaptive immune responses. Oncogenic, constitutive activation of MALT1 has been associated with the formation of diffuse large B-cell lymphomas of the activated B-cell subtype (ABC DLBCL), a highly aggressive human B-cell lymphoma characterized by poor patient outcome.

As a scaffold protein, MALT1 promotes TRAF6-dependent IKK activation, which triggers a rapid but transient NFκB activation. MALT1 also has a protease activity and cleaves a number of substrates, including ReIB, CyD and A20. This has been reported to result in a variety of cell responses including cytokine production and proliferation.

Results: A high throughput screening programme performed at LifeArc identified compounds which biochemically inhibit MALT1 protease activity. We also developed a set of MALT1-targeting PROTAC molecules (see accompanying poster) which efficiently knock down MALT1 in a dose and time related manner. Differential effects of inhibition and knock-down were observed on NFκB signalling in Jurkat cells.

MALT1 is constitutively active in the B-cell lymphoma cell line OCI-Ly3. In this cell line, enzymatic activity of MALT1 appeared key for the observed reduction in cell proliferation following compound exposure. Overt cell kill was not observed however. The mechanism by which MALT1 inhibitors mediate a cell cycle arrest was investigated and the AP-1 family of transcriptional regulators were implicated in this activity, with up-regulation of the cyclin dependent kinase inhibitor p21.

Conclusions: These approaches to elucidate the role of MALT1 in specific cell backgrounds is driving new combination approaches for development of an oncology therapeutic, while the PROTAC knock-down offers an alternative modality with different impact on downstream signalling pathways.

Conflict of interest: Other Substantive Relationships: All authors are employees of LifeArc.

376 (PB-039) Poster

Integrated pharmacokinetic-pharmacodynamic (PK-PD) and exposure-response (E-R) analyses to support the first-in-human (FIH) Phase I/II study of FGF401

Y. Gu1, M. Wilbaux2, S. Yang3, D. Demanse4, A. Jullion4, C. Meille2, R. Siddani1, D. Graus Ponts5, A. Myers2, 1Novartis, Pharmacokinetic Sciences, Shanghai, China; 2Novartis, Pharmacometrics, Basel, Switzerland; 3Novartis, Pharmacokinetics, East Hanover, USA; 4Novartis, Biostatistics, Basel, Switzerland; 5Novartis, Oncology Translational Research, Basel, Switzerland; 6Novartis, Translational Clinical Oncology, Shanghai, China

Background: The fibroblast growth factor factor 19 (FGF19)-fibroblast growth factor factor receptor 4 (FGFR4)–b Klotho (KLB) signaling pathway regulates bile acid synthesis and is also a key driver in certain forms of hepatocellular carcinoma (HCC), a deadly disease with limited treatment options. FGF401, a highly potent and selective FGFR4 inhibitor, is being evaluated in a Phase I FIH I/II study (NCT02325739) in patients with HCC or solid tumors characterized by FGF4R/KLB expression. The aim of this work was to utilize modeling approaches to establish the exposure-response (E-R) relationships of FGF401 with various efficacy, safety and biomarker endpoints, and to characterize the selected dose in this trial.

Material and Methods: All data were extracted from the ongoing study. Concentration-time profiles in man were compared to the preclinical IC50 for pFGFR4 inhibition (~26 ng/mL) to evaluate the target coverage. Primary PK parameters and dose proportionality were assessed using non-compartmental analysis. A population (pop) PK model was developed to further characterize FGF401 exposures and derive PK metrics. To investigate exposure-efficacy relationship, a popPKPD model was developed to describe the tumor growth inhibition. In addition, time to progression (TTP) was analyzed by Kaplan-Meier method, and stratified by PK metrics quartiles. To evaluate exposure-safety relationship, discrete longitudinal analyses were performed on the occurrence of common adverse events related to FGF4R pathway inhibition (AST1 and diarhoea). A popPKPD model was also developed to describe the evolution of ALT over time. To evaluate the E-R of PD biomarkers, circulating FGF19 and 7-alpha-hydroxy-4- chol ester-3-1 (C4) were analyzed with popPKPD models. Appropriate covariates were evaluated in all modeling analyses.
Results: FGF401 shows favorable PK in man. Exposure increases almost dose-proportionally. Of the evaluated PK metrics, C trough >28 ng/mL was associated with a significant prolonged TTP, consistent with the preclinical observation that C trough >IC90 drives the antitumor efficacy. The majority of individuals maintained the efficacy-required C trough at >120 mg QD. Simulations of the popPKPD model growth inhibition model also favored the dose of 120 mg QD. No relationship between FGF401 exposure and AST or diareea was identified within the observed dose range. The popPKPD ALT model also showed consistent findings. Both FGF19 and C4 popPKPD models indicate that the maximal PD effect reached at clinical tested dose range.

Conclusions: This integrated analysis, covering PK, efficacy, safety and biomarkers, provides insights into the E-R relationships of FGF401, and demonstrates the utility of this methodology in early clinical development. These data support the FGF401 120 mg QD dose regimen.

Conflict of interest: Corporate-sponsored Research: All the authors are employees of Novartis. NCT02325739 is a Novartis-sponsored clinical trial.

378 (PB-041) Poster Leveraging the pentarin platform to selectively deliver PI3 K inhibitors to solid tumors leading to superior efficacy in preclinical models S. Pierro1, B. Moreau1, J. Quin1, A. Cirello1, T. Cipriani1, G. Sharma1, K. Kirksiluckale1, B. White1, K. Whalen1, M. Bilodeau1, R. Wooster1 1Tarveda Therapeutics, Biology, Watertown, USA HSP90 is a chaperone protein that is overexpressed and activated in cancer cells, small molecule ligands of HSP90 are retained in tumors for as much as 20 times longer than in blood or normal tissue and have been used to image tumors in patients. Tarveda Therapeutics has leveraged this preferential accumulation of HSP90-targeting ligands in tumors to selectively accumulate and release anti-cancer payloads, such as phosphoinositide 3-kinase (PI3K) inhibitors.

The PI3K pathway is an intracellular signaling pathway that regulates cell survival, proliferation, and differentiation. In cancer, there is frequent activation of the PI3K pathway and its critical role in cell growth and survival has made it an attractive target for pharmacologic intervention. To date, while PI3K inhibitors are approved for the treatment of leukemia and lymphoma, only allosteric mTOR inhibitors, such as everolimus and temsirolimus, are approved for clinical use for the treatment of solid tumors with PI3K/AKT/mTOR pathway activation. In addition, there is increasing evidence that PI3K pathway inhibitors are insufficient at achieving deep inhibition of the pathway at doses that can be tolerated by patients. We hypothesize, by leveraging the preferential accumulation of HSP90-targeting ligands in tumors, and by masking of the PI3K inhibitor active site though conjugation to HSP90 targeting ligands, HSP90-PI3K conjugates could selectively accumulate and be retained in tumors leading to deep pathway inhibition over time with an overall decrease in toxicity.

To test this hypothesis, we have generated HSP90-PI3K conjugates and evaluated them in preclinical models of human cancer. We demonstrated superior tumor accumulation, in comparison to the PI3K inhibitor alone, with multiple conjugates in multiple xenograft models. Human tumor xenograft studies in animal models resulted in greater efficacy than that achieved with the PI3K inhibitor alone. In addition, since a known and potentially dose limiting side effect of inhibitors targeting these pathways is hyperglycemia, glucose levels were monitored post PI3K inhibitor and HSP90-PI3K conjugate dosing. Selective delivery of our HSP90-PI3K conjugate was able to mitigate the increase in glucose levels observed post dosing with the PI3K inhibitor alone, demonstrating that selective delivery may be able to increase the therapeutic window in comparison to PI3K inhibitors alone. These data demonstrate that by leveraging the preferential accumulation of HSP90-targeting ligands in tumors, we can selectively deliver PI3K inhibitors to achieve deep pathway inhibition leading to efficacy in multiple tumor models without hyperglycemia induction in mice.

No conflict of interest

Table 1 (abstract 379 PB-042): In the two groups were followed up for 1 and 3 months objective response

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>CR</th>
<th>PR</th>
<th>SD</th>
<th>PD</th>
<th>ORR(%)</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month</td>
<td>TACE-apatinib</td>
<td>0</td>
<td>18</td>
<td>6</td>
<td>3</td>
<td>18/27 (66.7)</td>
<td>5.236</td>
</tr>
<tr>
<td>TACE alone</td>
<td>0</td>
<td>21</td>
<td>14</td>
<td>18</td>
<td>21/53 (39.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>TACE-apatinib</td>
<td>0</td>
<td>11</td>
<td>3</td>
<td>10</td>
<td>11/24 (45.8)</td>
<td>5.395</td>
</tr>
<tr>
<td>TACE alone</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>23</td>
<td>6/34 (17.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; TACE, transarterial chemoembolization.

Poster Session (Friday, 16 November 2018) Abstracts, 30th EORTC-NCI-AACR Symposium
CAIX and hTERT) were assessed with immunochemistry. HPV was detected and typed. Polymorphism for the p53 gene codon 72 was analyzed.

Results: In the locally advanced cancer sample an increase in the expression of IGF-IRx, IGF-IRβ, hTERT and decrease in the expression of CAIX, GLUT1 and Survivin was observed, whereas in high-grade intraepithelial lesion sample was observed an increase in the expression of CAIX, GLUT1, hTERT and decreased IGF-IRx, IGF-IRβ. A HPV co-infection with the same variants and a p53 comparable “Arligine/Proline” genotype was reported both on 1986’s and 2002’s samples.

Conclusions: The variations of protein expressions suggest that these biomarkers not contribute in the same proportion at each stage of cancer development. CAIX, GLUT1, Survivin and hTERT seem to play a key role in the evolution from high-grade intraepithelial lesion to cervical carcinoma. IGF-IRβ might also play a key role in cancer appearance and its local and distant promotion. The persistence of HPV infection, all the more for co-infection, suggests its clinical impact in tumoral transformation and the heterogeneous genotype containing Proline might explain the surprisingly long time (more than 16 years) between diagnosis of pre-cancerous lesions and of cervical cancer without any treatment, with a probably resistant to HPV-induced mutation p53 genotype. Thus, the biological and molecular profiling might be valuable biomarkers, reflecting the future or current disease aggressiveness. If the present hypotheses are validated by further studies, such molecular profiling could guide the selection of the most appropriate targeted molecular compound or personalized therapy, as previously suggested in trials evaluating antiviral drugs in HPV-mediated cervical cancer treatments.

No conflict of interest

References


381 (PB-044) Poster Antitumor activity of novel STAT3 inhibitor YHO-1701 in combination with alecitinib, an ALK inhibitor, in non-small cell lung cancer model with EML4-ALK fusion gene


Background: Signal transducer and activator of transcription (STAT) 3 plays a critical role in regulating cell growth, metastasis, and survival. STAT3 signaling is constitutively activated in various types of cancers and hematological malignancies. YHO-1701 is an orally available STAT3 inhibitor. The objective of this study was to assess the efficacy of YHO-1701 either alone or in combination with molecular targeted agents on cell proliferation in vitro and tumor growth in a mouse xenograft model.

Methods: The anti-proliferative effects of YHO-1701 and/or relevant molecular targeted agents were evaluated in a panel of 18 human leukemias, melanomas, and cancers of the lung, breast, colon, and thyroid by using WST-8 dye-based assay after 48 hours of administration. The median-effect method was used to analyze the combined drug effects. Immunoblot analysis was carried out to investigate change in levels of the key target molecules in each signal transduction pathway. The antitumor efficacy of YHO-1701 in combination with alecitinib, an ALK inhibitor, was explored in an EML4-ALK fusion gene-positive NCI-H2228 xenograft mouse model. The test compounds were administered orally once a day for 5 consecutive weekdays followed by a 2-day rest at the weekend for 3 weeks.

Results: Additive or synergistic effects were observed in two thirds of the total number of combination treatments given. Of particular interest was the synergistic effect observed when YHO-1701 was combined with osimertinib (EGFR inhibitor), crizotinib, alecitinib, ceritinib (ALK inhibitors), imatinib, or dasatinib (BCL-ABL inhibitors) over a wide range of drug concentration ratios. The results further revealed a close correlation between this synergistic effect and the cellular levels of the target molecules of YHO-1701, and each combination drug. In the NCI-H2228 xenograft mouse models, the tumor growth inhibitory rate of the YHO-1701/alecitinib combination increased to 91.2%, whereas that of YHO-1701 or alecitinib alone was 24.1% and 30.9%, respectively. The combination therapy was well tolerated in tumor-bearing nude mice.

Conclusions: The present study suggests that the combination of YHO-1701 and alecitinib is worthy of further clinical investigation. In addition to ALK inhibitors, EGFR and BCR-ABL inhibitors also appear to be good candidates for combination with YHO-1701.

No conflict of interest

382 (PB-045) Poster Sphingosine kinase 2 (SK2) targeting in the treatment of multiple myeloma: preclinical and phase I studies of opaganib, an SK2 inhibitor, in multiple myeloma

Y. Kang1, S. Fan1, P. Sundaramoorthy1, C. Gasparetto1, G. Long1, A. Garrett1, E. Sellar1, J. McIntyre1, L. Maines2, V. Katz Ben-Yar2, C. Smith1, T. Plass1, 1Duke University, Medicine, Durham, USA; 2Apogee Biotechnology Corporation, Biotech, Hummelstown, USA; 3RedHill Biopharma, Biopharma, Tel Aviv, Israel

Background: Multiple myeloma (MM) remains an incurable disease; novel therapeutic agents not sharing similar mechanisms of action with existing drugs are needed. Sphingosine kinase 2 (SK2) is overexpressed in MM cell lines and primary MM samples. Inhibition of SK2 by RNA interference or treatment with opaganib induces apoptosis in MM cell lines and inhibits proliferation of primary human MM cells. Moreover, opaganib effectively inhibited myeloma tumor growth in vivo in mouse xenograft models. Opaganib reduces the expression of Mcl-1 and c-Myc by inducing their proteasome degradation and exhibits synergistic anti-myeloma activity when combining with ABT-199 (venetoclax), a specific Bcl-2 inhibitor, in MM cells without a (11;14) translocation in vitro and in mouse xenograft models. In addition SK2 regulates mitophagy (i.e., autophagy of mitochondria) in myeloma cells. Furthermore, when VKMYC myeloma cells were injected into WT recipients, SK2-/- knockout mice, or SK2-/-/c-Myc recipient mice developed myeloma, whereas both WT and SK2-/- recipient mice developed myeloma, indicating a critical role of SK2 in the bone marrow microenvironment regulating myeloma pathogenesis.

Nine patients were enrolled in the clinical trial and were treated in continuous 28-day cycles at the doses shown: 3 patients at 250 mg bid; 4 patients at 500 mg bid, and 2 patients at 750 mg bid. Average lines of prior treatment were 5. PK, PD and correlative studies were performed. No dose-limiting toxicities were observed. The peak plasma concentrations (Cmax) of opaganib in patients receiving 500 mg bid were in the range expected to have therapeutic activity. Two patients had stable disease for about 4 months.

Conclusions: SK2 is an innovative molecular target for anti-myeloma therapy. Opaganib single agent or in combination has potential for treatment of relapsed/refractory MM patients previously treated with immunomodulatory agents and proteasome inhibitors.

Conflict of interest: Ownership: Dr. Charles Smith is the President and CEO of Apogee Biotechnology Corporation. Advisory Board: None. Board of Directors: None. Corporate-sponsored Research: RedHill Biopharma owns the right to opaganib and is the IND holder and one of the sponsors of the clinical study. Other Substantive Relationships: Dr. Terry Plass and Ms. Veer Katz are employees of RedHill Biopharma, Ltd. The study was supported by NCI R01 CA197979 and NCI R44 CA195767 sub-awarded by Apogee Biotechnology Corporation.

383 (PB-046) Poster TAS0728, a covalent binding, selective inhibitor of HER2, shows antitumor activity in HER2+ tumor models resistant to established anti-HER2 therapy

H. Ito1, Y. Fujoka1, K. Ito1, K. Oguchi1, H. Osawa1, K. Funabashi1, T. Shimamura1, F. Nakagawa1, R. Kawabata1, H. Nagase1, J. Uchida1, S. Okubo1, K. Matsuo1, T. Utsugi1, Y. Iwasawa1, 1Taiho Pharmaceutical Co., Ltd, Discovery and Preclinical Research Division, Tsukuba, Ibaraki, Japan

Background: HER2 is a promising therapeutic target in various cancers. HER2-targeting antibodies (trastuzumab, pertuzumab) and a HER2-directed
antibody-drug conjugate (trastuzumab emtansine: T-DM1) are approved for the treatment of HER2 overexpressed breast cancer. However, there is still a medical need for patients refractory to anti-HER2 therapies. TAS0728 is a HER2-selective, covalent binding kinase inhibitor that has high kinase specificity excluding EGFR. In the present study, the antitumor activity of TAS0728 was evaluated in tumor models including NCI-N87 xenograft, tumor model refractory for T-DM1, and a patient-derived xenograft (PDX) model resistant to anti-HER2 therapies and other chemotherapies.

Material and Methods: Mixture with recombinant human HER2 cytoplasmic domain. TAS0728 or DS2 for 3 days was evaluated using the liquid chromatography-mass spectrometry (LC-MS) to test for covalent adduct formation. In the MCF10A cells, TAS0728 had a potent inhibitory activity for phosphorylation of various HER2 mutations as well as wild type HER2 at 30–300 nM. In contrast, a higher concentration of TAS0728 (3,000 nM) was required for EGFR wild type inhibition. In vivo, TAS0728 induced tumor regression in the T-DM1 refractory NCI-N87 xenograft model, while T-DM1 was not effective. Moreover, in breast cancer PDX model refractory against anti-HER2 therapies, TAS0728 exerted the potent antitumor effect.

Conclusion: TAS0728 is a covalent binding, selective inhibitor of HER2 kinase with efficacy in HER2+ tumor models resistant to anti-HER2 therapy. These results provide a rationale for patients refractory to established anti-HER2 therapy.

No conflict of interest

384 (PB-047) Poster Comparison of pharmacokinetics and safety of reference bevacizumab (EU and US bevacizumab) and its biosimilar candidate CT-P16

J.G. Shin1, J.L. Ghim1, J.L. Kim1, S.H. Cho2, M.S. Nam2, S. Han3, T. Park3, S. Kim3, J. Bae4. Inje University Busan Paik Hospital, Department of Pharmacology and Clinical Pharmacology, Busan, South Korea. 1School of Medicine (KAIST), Department of Clinical Pharmacology, Incheon, South Korea. 2The Catholic University of Korea Seoul St.Mary's Hospital, Department of Clinical Pharmacology & Therapeutics, Seoul, South Korea; 3CELLTRION Inc., Clinical Development, Incheon, South Korea

Background: CT-P16 is on the development as a proposed biosimilar product of bevacizumab, a recombinant humanized monoclonal antibody that binds selectively to vascular endothelial growth factor-A. We conducted a phase I study to compare similarity in pharmacokinetic (PK), safety and immunogenicity between CT-P16 and 2 reference products, EU-approved bevacizumab (EU-B) and US-approved bevacizumab (US-B).

Materials and Methods: In this double blind, randomized, three-arm, parallel-group, single-dose study, a total of 144 healthy male subjects were enrolled and 141 subjects received a single dose (5 mg/kg) of CT-P16 (n = 46), EU-B (n = 47) or US-B (n = 48) by intravenous infusion over 90 minutes. The safety and immunogenicity were monitored throughout the study.

Results: The 90% CI for the ratio of geometric means of all 3 comparisons (CT-P16/EU-B, CT-P16-US-B, and EU-B/US-B) for primary PK endpoints were entirely contained within the predefined bioequivalence margin of 80 to 125%, indicating bioequivalence of all 3 study drugs (Table 1). The additional PK parameters including %AUCmax, T1/2, T1/2, and CL were similar among 3 groups. From 141 subjects, infusion-related reactions were reported for 2 (4.3%), 2 (4.3%) and 4 (8.3%) subjects in CT-P16, EU-B, and US-B groups, respectively, and all cases were Grade 1 in intensity. After study drug administration, no proteinuria, hypertension, anemia, neutropenia or thrombocytopenia with higher than Grade 2 was reported. Post baseline anti-drug antibody positive results were reported from 2 (4.3%), 2 (4.3%) and 3 (6.3%) in CT-P16, EU-B, and US-B, respectively, however no subject reported neutralizing antibody positive result.

Conclusions: The bioequivalence of CT-P16 was demonstrated with the reference products, EU-B and US-B, as measured by primary PK endpoints (%AUCmax, T1/2, and CL) in healthy male subjects. The overall safety and immunogenicity profile of CT-P16 were comparable to those of reference products.

Conflict of interest: Other Substantive Relationships: SunYoung Yu, Taehong Park, Sinhye Kim, and Jihun Bae are employees of CELLTRION, Inc. Other authors have no potential conflict of interest to disclose.

385 (PB-048) Poster Hypermolarization-activated cyclic nucleotide-gated channel (HCN) blocker, Ivabradine, a novel potential targeted treatment for triple-negative breast cancer

U.S. Khoo1, C. K. Mo2, H. Tsoi1, E. P. Man1, K.M. Chau1, T.K. Lee2, C. Gong1, 1The University of Hong Kong, Pathology, Hong Kong, Hong Kong. 2The Hong Kong Polytechnic University, Department of Applied Biology & Chemical Technology, Hong Kong, Hong Kong.

Background: Triple-negative breast cancer patients who cannot benefit from targeted therapies, receive chemotherapeutic agents with attendant side effects. Ivabradine is an FDA approved Hypermolarization-activated cyclic nucleotide-gated channels (HCN 1–4) targeting therapeutic agent, used clinically to treat chronic angina. We found HCN2 and HCN3 overexpressed in breast cancer compared with low expression in normal breast, both from TCGA data, cell line samples and clinical tissue samples. Treatment with Ivabradine significantly suppressed proliferation of breast cancer cells. We hypothesized that Ivabradine may be used to preferentially target breast cancer.

Materials and Methods: In-vitro functional assays using breast cancer cell-lines were used to characterize the mechanism of action of HCN channel inhibition on breast cancer cells. In-vivo mouse models using both breast cancer xenograft and patient derived tumor xenograft (PDTX) were also used to demonstrate the efficacy of Ivabradine treatment.

Results: Subcellular fractionation experiments confirmed HCN2 and HCN3 were largely localized to the cell membrane. MCF-10A, MDA-MB-231, MDA-MB-453 and BT-474 cell-lines were treated with 200 μM Ivabradine, 40 nM Paclitaxel or 625 nM Doxorubicin. All 3 drugs caused reduced cell viability and proliferation, and induced apoptosis of breast cancer cells. In contrast, Ivabradine did not significantly alter cell viability, proliferation nor apoptosis of the normal breast epithelial cell line MCF-10A. Mechanistically, we found that Ivabradine treatment did not deplete intracellular calcium ion concentration, induce ER stress, caspase-mediated apoptosis, autophagy and cell senescence. Breast cancer xenografts MDA-MB-231 and MDA-MB-453 treated with Ivabradine showed decreased tumor growth compared with control group. Knockdown of HCN2 and HCN3 showed similar results as that with Ivabradine treatment in vitro as well as for in vivo xenografts. Ivabradine treatment also effectively reduced tumor growth in 6 out of 8 PDTX models established so far of triple negative breast cancer, with no discernable side effects on the treated mice.

Conclusions: Targeting HCN channels using Ivabradine can be an effective novel targeted therapy for triple negative breast cancer. Being a clinically approved drug, it can more readily pave the way for clinical trials. There is also potential to test the application of antibodies against HCN2 and HCN3 on PDTX and develop humanized antibodies for use as anti-cancer drugs.

No conflict of interest

Table 1 (abstract 384 PB-047): Statistical Analysis (ANCOVA) of PK Parameters: % Ratio (Test/Reference) [90% CI]

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>CT-P16/EU-B</th>
<th>CT-P16/US-B</th>
<th>EU-B/US-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUCmax (h·μg/mL)</td>
<td>97.69 [93.14, 102.46]</td>
<td>94.04 [90.68, 98.61]</td>
<td></td>
</tr>
<tr>
<td>AUClast (h·μg/mL)</td>
<td>98.42 [93.99, 103.06]</td>
<td>94.34 [90.14, 98.74]</td>
<td></td>
</tr>
<tr>
<td>Cmax (μg/mL)</td>
<td>104.02 [99.24, 109.03]</td>
<td>101.01 [96.39, 105.85]</td>
<td></td>
</tr>
</tbody>
</table>

Poster Session (Friday, 16 November 2018) Abstracts, 30th EORTC-NCI-AACR Symposium
so, several small molecules targeting Wnt signaling pathway have been developed. However, there is still no effective therapeutic compound. Recent years, synthetic lethality is reported as a promising strategy for tumor treatment because of targeting respective gene mutation, and expected less side-effect for normal tissue. So, we searched for the compound which exerted synthetic lethality in β-catenin mutated tumors, in other words, induced cell death selectively in β-catenin mutated tumor cells.

Materials and Methods: We established the synthetic lethal screening assay system. Briefly, we treated the test samples to β-catenin mutated HCT116 cells and β-catenin wild type A375 cells, and determined cell death. In this assay, we screened more than 10,000 compounds from synthetic compound library.

Results: We found DS2280164 (DS23), which is provided by Daiichi-Sankyo Pharmaceutical Company, exhibited selective cell death against β-catenin mutated HCT116 cells. In order to confirm synthetic lethality with mutated β-catenin, we used β-catenin isogenic HCT116 cells. As mentioned above, DS23 induced cell death in β-catenin hetero-mutated parent HCT116 CTNNB1 WT/Δ45 cells and wild type allele knockout CTNNB1 Δ45-cells. However, DS23 failed to induce cell death in mutant allele deficient HCT116 CTNNB1 WT/Δ-cells. Further analysis showed that DS23 induced reactive oxygen species (ROS) production, results in cell death.

Conclusions: It is suggested that DS23 exerts synthetic lethality in β-catenin mutated tumor cells. One of the cause of DS23-induced cell death might be ROS production.

No conflict of interest

388 (PB-051) Poster Evaluation of antitumor activity of lenvatinib plus anti-PD-1 antibody combination in the hepatocellular carcinoma Hepa1-6 mouse syngeneic tumor model


Background: Lenvatinib (LEN) is a multitargeted tyrosine kinase inhibitor that selectively inhibits vascular endothelial growth factor receptor (VEGFR) 1–3, fibroblast growth factor receptor (FGFR) 1–4, platelet derived growth factor receptor (PDGFR) α, RET and KIT. In a phase 3 clinical trial in unresectable hepatocellular carcinoma (HCC) (REFLECT study), LEN showed statistical non-inferiority of overall survival compared to sorafenib (SOR). Clinical study of LEN combined with an anti-PD-1 antibody (Ab) in HCC is in progress. Here, we investigated the effects of LEN on cancer immunity and evaluated antitumor activity with combination of anti-PD-1 Ab using the HCC Hepa1-6 mouse syngeneic tumor model.

Material and Methods: Immune cell populations in the Hepa1-6 syngeneic tumor model were analyzed by flow cytometry (FCM). Antitumor activities of LEN (10 mg/kg, p.o., q.d.) and SOR (30 mg/kg, p.o., q.d.) were tested in immunocompetent and immunodeficient mice bearing Hepa1-6 HCC tumors. CD8+ T cell depletion by anti-mouse CD8 antibody (clone 2G3, 200 μg/animal, twice weekly) was performed to assess contribution of CD8+ T cells to antitumor activity of LEN and SOR treatments. Antitumor activities of LEN combined with anti-PD-1 Ab (200 μg/head, twice weekly) were evaluated in immunocompetent mice.

Single-cell RNA sequencing (scRNA) analysis was conducted to investigate effects of LEN, anti-PD-1 Ab, and their combination on immune cell populations in tumor tissues.

Results: FCM analysis of immune cell populations in Hepa1-6 tumors showed high infiltration of activated CD8+ cytotoxic T cells and indicated that Hepa1-6 tumor is a T cell-inflamed tumor. Although both LEN and SOR showed equivalent antitumor activities in immunodeficient mice, LEN showed greater antitumor activity compared to SOR in immunocompetent mice. With CD8+ T cell depletion, antitumor activity of LEN was attenuated,

386 (PB-049) Poster Quantifying the effects of hepatic impairment on abemaciclib exposure to support dosing recommendations

S.W. van Beek1, P.K. Turner2, J.S. Van Der Walt3, Eli Lilly and Company Limited, PKPD, Global PK/PD & Pharmacometrics, Windlesham, United Kingdom;4Eli Lilly and Company, Global PK/PD & Pharmacometrics, Indianapolis, USA

Background: Abemaciclib and its metabolites (M2, M18 and M20), which are formed in the liver, inhibit CDK4&6. The abemaciclib half-life doubles in severe hepatic impairment (HI), and the dose is adjusted from twice to once daily. The best measure of the active moiety is the potency-adjusted unbound area under the curve (AUC) of abemaciclib plus its metabolites. We assessed the adequacy of dose adjustment regimens on the predicted steady-state exposure.

Material and Methods: Weight, liver volume estimates and pharmacokinetic (PK) data after a single dose of 200 mg abemaciclib in healthy subjects (HS, n = 10), and subjects with mild (n = 9), moderate (n = 10) and severe (n = 6) HI based on Child-Pugh classification (NCT02387814) was analysed. A mechanistic population PK model (developed from 12 clinical trials in cancer patients and HS) was used to estimate changes in drug extraction and CYP3A4-mediated biotransformation. To evaluate dose adjustments, steady-state concentrations after once daily (OD) or twice daily (BD) doses of 50, 100, 150 and 200 mg in subjects with mild, moderate or severe HI were simulated. The abemaciclib, metabolites and active moiety AUC was compared to HS using ANOVA.

Results: Hepatic blood flow decreased 30% (mild), 44% (moderate), and 76% (severe). Metabolism of abemaciclib to M2 decreased 15% (mild), 23% (moderate) and 78% (severe). The fraction of M2 metabolized to M18 decreased 30% (mild), 50% (moderate) and 70% (severe). These changes resulted in increased abemaciclib and decreased M2, M20 and M18 exposure (Table 1). The active moiety increased 1.8 (95% CI 1.24–2.42) fold in severe HI, but was not different for mild and moderate HI from HS. At steady-state these changes resulted in a significant increase in active moiety exposure in severe HI (2.55 fold, 95% CI 1.25–3.85) but no difference was found for mild and moderate HI. A dose of 200 mg OD in severe HI provided similar exposure as 200 mg BD in HS (1.28 fold, 95% CI 0.00–2.58). Reduction to 100 mg BD maintained trough concentrations above 200 ng/mL (efficacy threshold in xenograft models) in severe HI.

Conclusions: Changes in liver blood-flow and reduced metabolism increased the total active moiety of abemaciclib in severe HI. At steady state, the recommended dose adjustment for severe HI to 200 mg OD would result in exposure of the active moiety comparable to healthy individuals and maintain abemaciclib blood concentrations above the clinical threshold.

Conflict of interest: Corporate-sponsored Research: Clinical trial NCT02387814 was sponsored by Eli Lilly and Company. PKT and JSW are employees of Eli Lilly and Company.

387 (PB-050) Poster Studies of compound exerting synthetic lethality in β-catenin mutated tumor cells

H. Ikeda1, E. Tashiro1, M. Imoto1, Keio University, Biosciences & Informatics, Kanagawa, Japan

Background: Wnt signaling pathway is known as a proliferation signaling which has crucial roles in the regulation of diverse processes, including embryonic development. β-Catenin is one of the major component of Wnt signaling pathway and plays a main role in this pathway. Mutation of the β-catenin gene, CTNNB1, would cause stabilization and nuclear accumulation of β-catenin protein, results in aberrant downstream transcriptional activity. β-Catenin is mutated in a wide variety of tumors, including 10% of all sporadic colon carcinomas and 20% of hepatocellular carcinomas.

Table 1 (abstract 386 PB-049): Fold change of AUC of HI groups compared to HS

<table>
<thead>
<tr>
<th>Child-Pugh group (n)</th>
<th>Abemaciclib</th>
<th>M2</th>
<th>M20</th>
<th>M18</th>
<th>Total active species</th>
<th>Adjusted Total active moiety</th>
<th>Adjusted Total active moiety*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild (9)</td>
<td>1.06</td>
<td>0.75</td>
<td>0.70</td>
<td>0.46</td>
<td>0.86</td>
<td>1.14</td>
<td>1.06</td>
</tr>
<tr>
<td>Moderate (10)</td>
<td>1.23</td>
<td>0.61*</td>
<td>0.51*</td>
<td>0.47</td>
<td>0.84</td>
<td>0.89</td>
<td>0.90</td>
</tr>
<tr>
<td>Severe (6)</td>
<td>2.02*</td>
<td>0.52*</td>
<td>0.31*</td>
<td>0.12</td>
<td>1.10</td>
<td>1.83*</td>
<td>2.55*</td>
</tr>
</tbody>
</table>

*p-value < 0.05, Dunnet’s many-to-one test.

AUCMST

Abstracts, 30th EORTC-NCI-AACR Symposium Poster Session (Friday, 16 November 2018)
HCC. Lenvatinib (LEN) is a multi-targeted tyrosine kinase inhibitor that inhibits also tumor growth and reduces tumor volume in GIST xenograft mouse models characterized by hypervascularization and aggressive tumor growth.

**Material and Methods:** Proliferation and tube formation of human umbilical vascular endothelial cells (HUVEC) were assessed in vitro. VEGF was exogenously overexpressed in HCC cell lines SNU-398 (SNU-398/VEGF) and SK-HEP-1 (SK-HEP-1/VEGF), with high VEGF expression confirmed by ELISA. Xenograft tumor models of these transfectants were established with anti-VEGF-deficient mice. Tumor microvessels were analyzed by immunohistochemical staining for endothelial cell marker CD31.

**Results:** LEN inhibited VEGF-induced HUVEC proliferation with IC50 values of 4.6 (for VEGF121) or 4.4 nM (for VEGF165), and IC50 values of SOR were 250 or 320 nM, respectively. The inhibitory activity of LEN at 10 nM against tube formation in VEGF plus FGF-induced tube formation assay was more potent than that of sorafenib at the same concentration (LEN, 98% inhibition; SOR, 22% inhibition). Ectopic VEGF overexpression in SNU-398 and SK-HEP-1 cell lines (with low VEGF-expression) led to hypervascularity and aggressive tumor growth of both transfectant xenografts. Both LEN (10 mg/kg) and SOR (30 mg/kg) inhibited tumor growth of the mock transfected SNU-398 model. However, only LEN showed clear antitumor activity in the hypervascular SNU-398/VEGF transfectant model. In the hypervascular SK-HEP-1/VEGF model, LEN led to nearly complete tumor stasis while SOR resulted in moderate inhibition of tumor growth rates. In all these models, LEN overexpressing antitumor and angiogenic activity of LEN was significantly more potent than that of SOR.

**Conclusion:** LEN has potent inhibitory activity against VEGF signaling pathways, and this activity underlies robust antitumor and antivascular activities of LEN in VEGF-overexpressing hypervascular HCC models.

**Conflict of interest:** Corporate-sponsored Research: This research was funded by Eisai Co., Ltd. Other Substantive Relationships: All authors are employees of Eisai Co., Ltd.

**Background:** There is unmet need for biomarkers to guide Tx selections for mBCa. Phenotypic heterogeneity is a major problem in mBCa pathological subtypes. A range of CTC phenotypic heterogeneity was used to assess the intra-patient CTC heterogeneity. 138 CTCs from 165 pts, 85 HR+, 19 Her2+, 8 were studied by the Epic Sciences platform. Following methods with greater than 85% of variance taken into account. Shannon Index was used to assess the intra-patient CTC heterogeneity. 138 CTCs from various classified CTC subtypes (ranging from 11 to 30 CTCs per subtype) were single cell sequenced for copy number alterations.

**Results:** CTCs were detected in 77.4% (178/230) of mBCa patients and clustered into 7 phenotypic CTC subtypes. Subset of CTCs from TNBC pts had larger nuclear areas and higher CK expression and shared with other mBCa pathological subtypes. A range of CTC phenotypic heterogeneity was observed across patients, with Shannon Index ranging from 0 (low heterogeneity) to 4.77 (high heterogeneity) and median of 0.95. Further, we observed a wide range of genomic instability scores and specific genomic alterations such as ERBB2 gain, FGFR1 gain, BRCA2 loss, and CDH1 loss were all observed in mBCa pathological subtypes. A range of CTC phenotypic heterogeneity was observed across patients, with Shannon Index ranging from 0 (low heterogeneity) to 4.77 (high heterogeneity) and median of 0.95. Further, we observed a wide range of genomic instability scores and specific genomic alterations such as ERBB2 gain, FGFR1 gain, BRCA2 loss, and CDH1 loss across phenotypic CTC subtypes.

**Conclusions:** Diverse inter- and intra-patient phenotypic CTC heterogeneity is observed across multiple cohorts with specific genomic profiles detected for different CTC subtypes. We seek to determine if patients with high heterogeneity might be better candidates for hormonal and targeted therapies. Studies linking heterogeneity to therapeutic efficacy and patient outcome are ongoing.

**Conflict of interest:** Other Substantive Relationships: We are employee of Epic Sciences.

**439 (PB-054)**

**Poster**

**Anagrelide, a novel anticancer compound for gastrointestinal stromal tumor**

O.P. Pulkka1, Y. Gebreyohannes2, A. Wozniak 2, J.P. Mpindi 3, O. Tynninen 4, K. Wennerberg5, M. Varjosalo 6, P. Laakkonen 7, R. Lehtonen 5, S. Hautanen4, O. Kallioniemi4, P. Scholtes7, H. Hoensuu6, H. Juonsemi6, 1University of Helsinki, Laboratory of Molecular Oncology, Translational Cancer Biology Research Program, Helsinki, Finland; 2KU Leuven & University Hospitals Leuven, Department of Oncology & Department of General Medical Oncology, Leuven, Belgium; 3University of Helsinki, Institute for Molecular Medicine Finland, Helsinki, Finland; 4University of Helsinki and HUSLAB, Department of Pathology, Helsinki, Finland; 5University of Helsinki, Genome-Scale Biology Research Program, Department of Biochemistry and Developmental Biology, Helsinki, Finland; 6University of Helsinki, Institute of Biotechnology, Helsinki, Finland; 7University of Helsinki, Translational Cancer Biology Research program, Helsinki, Finland; 8University of Helsinki & Helsinki University Hospital, Laboratory of Molecular Oncology, Translational Cancer Biology Research program and Comprehensive Cancer Center, Helsinki, Finland

**Background:** KIT and PDGFRα targeting tyrosine kinase inhibitors such as imatinib are highly effective in a therapy of gastrointestinal stromal tumor (GIST). However, advanced GISTs progress frequently on these therapies and there is dire need to find new therapeutic options for the patients. We investigated role of phosphodiesterase 3 family (PDE3) as a potential therapy target in GIST.

**Material and Methods:** PDE3A and PDE3B mRNAs were exceptionally highly expressed in GIST based on an in silico transcriptome database analysis of 9,763 human tissue and cancer samples. A high PDE3 expression was verified on protein level by using immunohistochemistry on tissue microarrays (TMAs) consisting of 630 human tumor samples. GIST882 and GIST46 cell lines were screened for sensitivity to 217 anticancer compounds, and the efficacy of PDE3-specific compounds was investigated further in GIST cell lines. The efficacy of anagrelide, the most potent PDE3-specific compound in GIST cell lines, was studied further in four GIST xenograft mouse models.

**Results:** A high PDE3 expression is frequent in GIST in comparison with other human tumor types. Anagrelide reduces tumor cell viability and promotes cell death by targeting PDE3A in GIST882 cell line. Anagrelide inhibits also tumor growth and reduces tumor volume in GIST xenograft mouse models.

**Conclusion:** PDE3A and PDE3B are expressed frequently in GIST and there is dire need to find new therapeutic options for the patients. We investigated role of phosphodiesterase 3 family (PDE3) as a potential therapy target in GIST.
GIST xenograft mouse models. Further testing of anagrelide in a clinical trial is warranted.


393 (PB-056) Poster
Concurrent KIT and PI3 K signaling inhibition with imatinib and copanlisib as front-line treatment in gastrointestinal stromal tumors (GIST)

L.A. Garcia Valverde 1, J. Rosell 1, G. Serna 1, C. Valverde 1, J. Carles 1, P. Nuñocoro 1, J. Arribas 1, D. Zapf 2, O. Polit 2, C. Serrano 1, 1Vall d’Hebron Institute of Oncology, 2Preclinical Research Program, Barcelona, Spain; 3Vall d’Hebron Institute of Oncology, Molecular Oncology Group, Barcelona, Spain; 4Vall d’Hebron Institute of Oncology, Medical Oncology, Barcelona, Spain; 5Institució Catalana de Recerca i Estudis Avançats ICREA, Recerca, Barcelona, Spain; 6Bayer AG, Therapeutic Research Groups Oncology, Berlin, Germany

Background: Most GISTs depend upon continuous KIT oncogenic signaling. KIT downstream PI3K/AKT pathway plays a critical role in GIST, thus emerging as an attractive target. However, PI3K inhibition has shown so far modest benefit in GIST patients. Here we evaluate preclinically copanlisib, a novel pan class-I PI3K inhibitor with predominant activity on PI3Kα, in a preclinical GIST model and p110α

Material and Methods: One IM-sensitive (GIST-T1) and 2 IM-resistant (GIST430/654, GIST-T1/670) cell lines were studied. Cells were treated with copanlisib alone or in combination with IM and studied for cell viability, proliferation (BrdU), pathway inhibition (immunoblotting) and apoptosis (caspase assay and immunoblotting). GIST-T1 and GIST-T1/670 xenografts were treated likewise and tumors analyzed for PI3K/AKT pathway inhibition by immunohistochemistry (IHC) at days 1 and 21.

Results: In cell viability assays copanlisib as single agent displayed IC50 values between 5.45 nM (GIST-T1) and 27.8 nM (GIST-T1/670). Immunoblot confirmed profound PI3K/AKT pathway inhibition. Immunoblot, proliferation and apoptosis assays revealed mild impact in proliferation and lack of pro-apoptotic activity, probably due to the unexpected cross-activation of MAPK signaling. Combined KIT and PI3K inhibition with IM and copanlisib, respectively, was synergistic, resulting in higher tumor proliferation impairment in both IM-sensitive and IM-resistant GIST cell lines, but only synergistic increase in apoptosis in GIST-T1.

GIST-T1 and GIST-T1/670 subcutaneous murine xenografts were treated for 21 days with IM and copanlisib both as single agents and in combination. Single-agent copanlisib significantly delayed tumor growth in both GIST-T1 and GIST-T1/670, and the combination showed a non-significant trend towards additive effect in GIST-T1, but not in GIST-T1/670. AI arms in the in vivo treatment were overall well tolerated.

Phospho-S6 assessment by IHC was used to evaluate the impact of GIST-T1 treatment. ARQ 751 was well tolerated at the 25 mg/kg QD dose level. Phospho-S6 assessment by IHC is a reliable biomarker for tumor growth inhibition in GIST.

Conflict of Interest: Corporate-sponsored Research: This project has been partially funded with a Bayer Research Grant.

394 (PB-057) Poster
A SMO inhibitor DCB-HDG2-411 with potent Hedgehog signaling pathway antagonist activity overcomes the Smo drug-resistant mutations

Y.S. Lee 1, M.Y. Kuo 1, C.W. Liu 1, Y.Y. Lu 2, 1Development Center for Biotechnology, Institute of Pharmaceuticals, Division of Bioactivity Identification, Taipei City, Taiwan; 2Development Center for Biotechnology, Institute of Pharmaceutics, Division of Medicinal Chemistry, Taipei City, Taiwan

The Hedgehog (Hh) signaling pathway is a critical regulator of embryonic patterning, and aberrant Hh pathway activation has been implicated in a diverse spectrum of cancers. Therefore, components of the Shh pathway (such as Shh, SMO, and GLI1/2) are viable therapeutic targets for anti-tumor strategy. SMO antagonists such as GDC-0449 and NVP-LDE225 have received FDA approval for treating basal cell carcinoma. However, primary and acquired SMO mutation-mediated resistance has emerged as a challenge to targeted therapeutics and may limit their anti-cancer efficacy. We report here the development of a potent SMO inhibitor designated DCB-HDG2-411 with potent Hh signaling pathway inhibition activity. DCB-HDG2-411 demonstrated Hh signaling pathway antagonist activity in a 293 cell-based Gli1-luciferase inhibition assay upon agonist treatment (IC50 = 4.7 nM), and retains inhibition activity against the SMO wild-type and D473H mutant (this mutant is responsible for resistance to vismodegib in medulloblastoma patients) co-transfection, with IC50 of 1.8 and 8.1 nM, respectively. The inhibition activity of DCB-HDG2-411 toward other SMO drug-resistant mutations such as SMO-G497W and SMO-W535L is under investigation. DCB-HDG2-411 competed with BODIPY-cyclopamine binding using SMO-expressing Hela cells with IC50 of 5.2 nM, indicating the compound occupy the same binding site as cyclopamine. DCB-HDG2-411 can also inhibit Hedgehog signaling in human HEPM cells with IC50 of 1.4 nM in reducing the amount of Gli1 mRNA. Treatment of a PTCH1-/- medulloblastoma allograft model with DCB-HDG2-411 (once-daily oral dosing at 10, 20, 40 mg/kg for 10 days) showed an effective dose-related antitumor activity mediated by inhibition of the Hh pathway. The DCB-HDG2-411 can therefore be evaluated further and serve as a potential drug development candidate for treatment of Hh pathway-driven cancers.

No conflict of interest

395 (PB-058) Poster
A Phase 1 dose escalation study of ARQ 751 in adult patients with advanced solid tumors with AKT1, 2, 3 genetic alterations, activating PI3 K mutations, PTEN-null, or other known actionable PTEN missing mutations

S. Pant 1, V. Subbiah 1, J. Rodon 1, F. Janku 2, D. Hong 2, K. Kapoor 3, S. Patel 1, A.M. Tsimberidou 1, A. Naimj 2, Fu 4, R. Savage 2, F. Chari 2, Y. Yu 2, K. Tibb 2, R. Attalal 2, B. Schwartz 2, F. Meric-Bernstam 1, Y. Yap 1, MD Anderson Cancer Center, Investigational Cancer Therapeutics, Houston, USA; 2ArQule, Inc., Clinical Development, Burlington, USA

Background: Dysregulation of the PI3K-AKT signaling pathway plays a critical role in cancer initiation and progression. AKT can be activated through activating PI3 K mutations, PTEN-null, or other known actionable PTEN missing mutations in advanced solid tumors with AKT1, 2, 3 genetic alterations, or activating PI3K mutations, PTEN-null, or other known actionable PTEN missing mutations in advanced solid tumors with AKT1, 2, 3 genetic alterations.

Material and Methods: This is a Phase 1 dose escalation study to assess the safety, tolerability, PK and preliminary anti-tumor activity of ARQ 751 in patients with advanced solid tumors with selected known genetic alterations. Treatment emerging adverse events (TEAE) were assessed per NCI CTCAE v. 4.03. Tumor response was evaluated per RECIST 1.1.

Results: 29 pts have been enrolled (84% White; 9 breast, 4 endometrial, 2 colon, 2 prostate and 9 others; median age 60 years; 39% White; 9 breast, 4 endometrial, 2 colon, 2 prostate and 9 others; median age 60 years). Preliminary anti-tumor activity was observed. Updated safety, PK, and tumor responses including 4 ER+ PR+/Her2− tumors, 1 PR, 2 SD, and 2 CR were reported. Among 26 evaluable pts, there were generally ranged from 16 to 22 h.

Conclusions: ARQ 751 demonstrated a manageable safety profile to date. Preliminary anti-tumor activity was observed. Updated safety, PK, biomarker and efficacy data will be presented.

Conflict of interest: Corporate-sponsored Research: Brian Schwartz, Ronald E. Savage, Feng Chai, Yi Yu, Kate Tihl are employee of the clinical trial sponsor, ArQule, Inc.
Cell-free DNA landscape of genomic alterations in over 3,000 patients with advanced breast cancer

L. Kiedrowski1, A. Bardia2, J. O’shaughnessy3, B. Nagy1, R. Lanman1, M. Cristofanilli4, J. Guardant Health, Medical Affairs, Redwood City, USA; 2Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, USA; 3Baylor University Medical Center, Texas Oncology, US Oncology, Dallas, USA; 4Northwestern University Feinberg School of Medicine, Robert H. Lurie Comprehensive Cancer Center, Chicago, USA

Background: Plasma cell-free DNA (cfDNA) can provide somatic genomic information non-invasively and in real-time for patients (pts) with breast cancer. Current tissue-based genomic sequencing companionably typically includes early stage, relatively treatment-naïve cohorts. We performed descriptive analysis of genomic data from a large and generally pretreated pt cohort with stage III/IV breast cancer to better understand the landscape of cfDNA alterations in advanced breast cancer.

Materials and Methods: Deidentified aggregate genomic data from clinical samples submitted for cfDNA next-generation sequencing (NGS) analysis with Guardan360® (Guardant Health, Inc; Redwood City, CA, USA) between 11/7/2016 and 2/28/2018 with an indicated diagnosis of stage III/IV breast cancer was queried. Testing analyzed cfDNA for the presence of at least one variant across 18 genes, and fusions in 6 genes.

Results: We identified a total of 3044 unique pts with advanced breast cancer who had 3695 consecutive test results, including serial specimens for some pts. The average pt age was 58 (range 18–95); 99% were female. 3242 tests (88%) had ≥1 somatic alteration (alt) detected; of these, a median of 4 alts were identified (range 1–85). The median mutant allele fraction (MAF) per alt was 0.50% (range 0.01–93.66%). Of the 2654 pts with ≥1 nonsynonymous (NS) alt detected, NS sequence alts were most frequently detected in TP53 (86%), PIK3CA (37%), ESR1 (24%), and NF1 (14%). CNAs were most frequently detected in MYC (17%), FGFR1 (16%), and CCND1 (14%). Fusions were identified in 11 pts involving FGFR3 (5), ALK (2), NTRK1 (2), RET (1), and ROS1 (1). Of the 649 pts with ESR1 NS alts detected, 219 (34%) had multiple NS ESR1 alts (range 2–9). ERBB2 (HER2) CNAs and NS sequence alts were identified in 8% and 10% of pts, respectively. NS alterations were also identified in other genes with matched therapies, including BRCA2 (9%), BRCA1 (6%), ATM (4%), and AKT1 (3%).

Conclusions: cfDNA can provide a noninvasive alternative to tissue biopsy to interrogate tumor genomics in pts with advanced breast cancer.
Results: Sixty six patients were treated in 8 dose-escalation cohorts (0.5, 1, 2.5, 3.5, 5, 7.5, 10, and 12.5 mg/kg) once every 3 weeks and an expansion cohort at 1 mg/kg. The trial assessed navicixizumab in refractory multiple solid tumor pts. The response rate in heavily pretreated ovarian cancer pts was 25% (3/12 PR). Pts with FFPE were ovarian (8), breast, uterine, endometrial (4 each) and other cancers. Expression levels of Notch, VEGF, and angio genesis pathway genes in baseline FFPE samples were analyzed to investigate mechanism of action among the heterogeneous tumor types and explore trends with clinical response. Exploratory mutation analysis identified b-catenin mutations in several pts. Pharmacodynamic analysis in surrogate tissues demonstrated Notch and VEGF genes were significantly modulated in whole blood, including upregulation of ANK1, FOXO3, BCL2L1 and downregulation of HEY1, consistent with engagement of both pathways by navicixizumab. Gene set enrichment analysis corroborated these results and showed that there was significant down-regulation of DLL4 and VEGF pathway genesets.

Conclusion: Biomarker analysis of pts from the Phase1a trial of navicixizumab in previously treated solid tumors demonstrated target engagement of both DLL4 and VEGF. Significant modulation of Notch and VEGF pathway genes by navicixizumab was observed in surrogate tissues. Updated biomarker results will be presented. Phase1b studies of navicixizumab plus chemotherapy are ongoing.

No conflict of interest

400 (PB-063) Poster
Super-enhancer landscapes of ovarian cancer reveal novel epigenomic subtypes and targets
M. Eaton1, B. Johnston1, K. Piotrowska1, C. Collins1, J. Lopez1, S. Knutson1, E. di Tomaso1, J. Carulli1, E. Olson1, M. Eaton1, B. Johnston1, K. Piotrowska1, C. Collins1, J. Lopez1, S. Knutson1, E. di Tomaso1, J. Carulli1, E. Olson1,1Syros Pharmaceuticals, Inc., Cambridge, MA, USA

Background: There is a critical unmet need for targeted therapies in ovarian cancer, especially high-grade serous ovarian cancer (HGSOC). We used enhancer mapping combined with transcriptomics and mutations to identify novel ovarian cancer subtypes and associated targets.

Material and Methods: With ChIP-seq for H3K27Ac, we profiled the enhancer landscape of 101 primary tumor samples from 7 ovarian cancer subtypes with a focus on HGSOC, 29 cell line models, 3 PDXs, and 8 non-cancerous samples of ovarian and fallopian tube tissue. We also profiled many of these samples through RNA-seq and a focused NGS-based mutational panel. We used matrix factorization methods to reveal novel subgroups of ovarian cancer patients and predicted their associated transcriptional circuitry.

Results: Through a computational deconvolution of enhancer maps, we identified novel enhancer-defined patient subtypes of ovarian cancer. While some known subtypes, such as granulosa cell, associated uniquely with their own enhancer profile, the majority of the primary tumor samples fell into 4 clusters that did not correlate with histological subtype or with known high-frequency ovarian cancer mutations. Each cluster was associated with its own unique super-enhancer (SE) signature, implying that each is driven by a unique transcriptional circuitry. There was a striking cluster-specific pattern of many known ovarian cancer related genes such as FOXM1, CD47, and MYC, and genes linked to pathways known to be dysregulated in ovarian cancer. Super-enhancers SEs were linked to the RB pathway gene CYCN1. Furthermore, many additional cluster-specific SEs were discovered representing novel potential therapeutic targets. Interestingly, while we could assign ovarian cancer cell line models to these novel subtypes, many cell lines’ enhancer landscapes appeared to be distinct from those of primary tumor cells.

Conclusions: Together, our results comprise the largest ovarian cancer enhancer mapping effort to date, and demonstrate how an integrated analysis of enhancers, transcriptomes, and genotypes together can yield transcriptional circuitry that reinforces the role of known pathways associated with ovarian cancer progression and treatment, can be used to select cell models that best recapitulate the enhancer landscape of primary tumors, and can be mined to identify novel targets and biomarkers. Cluster-specific SEs at MYC and CCNE1 suggest that some tumors have increased transcriptional dependency on these loci as well as the components of the corresponding transcriptional machinery. The role of one such component, CDK7, is currently being evaluated in ovarian cancer patients with SY-1365, a first-in-class selective CDK7 inhibitor in Phase 1 (NCT 03134638).

Conflict of interest: Ownership: All authors were shareholders and employees of Syros Pharmaceuticals at the time the work was conducted.

401 (PB-064) Poster
First-line treatment of metastatic non-small cell lung cancer (NSCLC): A randomized, double-blind, phase 2 trial of denosumab in combination with chemotherapy
R. de Boer1, P. Pedrazzoli2, B. Bisemsa2, R. Natale3, C.K. Lee2, L. Zhu4, A. Glennane5, 1The Royal Melbourne Hospital, Oncology, Parkville, Australia; 2Pollicino San Matteo Pavia Fondazione, Oncology, Pavia, Italy; 3Jeroen Boschziekenhuis, Oncology, ’s Hertogenbosch, Netherlands; 4Cedars-Sinai Medical Center, Hematology/Oncology, Los Angeles, USA; 5St. George Hospital, Oncology, Kogarah, Australia; 6Amgen Inc., Biostatistics, Thousand Oaks, USA; 7Amgen Inc., Medical Affairs, Thousand Oaks, USA

Background: Lung cancer remains the leading cause of cancer death worldwide. The receptor activator of nuclear kappa-B ligand (RANKL) inhibition may also improve overall survival (OS) in NSCLC. This phase 2 double-blind randomized trial compared the effect of denosumab on OS in combination with first-line platinum-based chemotherapy in metastatic NSCLC with or without bone metastases (NCT01951856).

Methods: Adults with metastatic NSCLC were randomized 1:1 to receive denosumab 120 mg or placebo subcutaneously every 3–4 weeks plus a loading dose on study day 8 in combination with a first-line platinum doublet. Randomization was stratified by presence of bone metastasis, histology, and geographic region. Key criteria for patient eligibility were confirmed stage IV NSCLC, favorable ECOG status (0–1), radiographically evaluable disease, and adequate organ function. The primary study objective was to estimate the effect of denosumab plus standard of care (SOC) vs SOC alone on OS. Key secondary endpoints included correlation between tumor tissue RANK expression with objective response rate (ORR) or OS; ORR (complete or partial responses based on modified RECIST 1.1), progression-free survival (PFS), clinical benefit rate (patients with objective response plus those with stable disease or better for ≥16 weeks), and safety.

Results: A total of 226 patients were randomized to denosumab (n = 148) or placebo (n = 78). Baseline demographics and disease characteristics were balanced between the two arms. Median OS was similar in the denosumab (10.7 months) and placebo arms (10.9 months; HR [95% CI] = 1.06 [0.75–1.50]). No significant correlation was detected between tumor RANK/RANKL expression and relative benefit from denosumab plus SOC vs SOC alone on OS or ORR. Median PFS was similar in the denosumab (5.2 months) and placebo arms (5.7 months; HR [95% CI] = 1.05 [0.78–1.43]). The ORR was 36.8% for denosumab and 43.4% for placebo (odds ratio [95% CI] = 0.76 [0.43–1.35]). The clinical benefit rate was 47.9% for denosumab and 53.9% for placebo (odds ratio [95% CI] = 0.81 [0.46–1.43]). Overall incidences of AEs were similar between the two treatment arms. Hypocalcemia (18.6% vs 5.3%) and positively-adjudicated osteonecrosis of the jaw (2.9% vs 0%) were more frequently reported in denosumab vs placebo recipients.

Conclusions: The addition of denosumab to SOC showed no clinical benefit in patients with NSCLC. There was no correlation of improved treatment effect on OS with high RANK/RANKL expression levels. Overall safety results from this study were similar between the two treatment arms and comparable to those seen in other studies with denosumab 120-mg dosing. In this setting, denosumab should be used for management of bone metastases and their complications.

Conflict of interest: Other Substantive Relationships: Bonnie Bisemsa is employed by the Amgen Inc. and affiliated with the Amgen Inc. Employee Benefits Bureau with Amgen, Novartis, Merck, and Roche. Anthony Glennane and Li Zhu report employment and stock ownership from Amgen Inc. Chee Khoon Lee reports research grants from AstraZeneca and consulting fees from Roche, AstraZeneca, Takeda, and Novartis. Ronald Natale reports research grants from AbbVie, AstraZeneca, and Bristol-Myers Squibb. Paolo Pedrazzoli has received consulting fees from Baxter, and has participated in speakers bureau with Lilly and Baxter.

402 (PB-065) Poster
Post-transcriptional regulation of TSC1 in oral cancer
K. Malliga1, A. Tiwari1, S. Swamy2, K.S. Gopinath3, A. Kumar1, 1Indian Institute of Science, Department of Molecular Reproduction, Development and Genetics, Bangalore, India; 2HCG-Bangalore Institute of Oncology, Surgical Oncology, Bangalore, India

Background: Cancer is the most common form of Head and Neck cancer with a highest number (nearly 20%) of cases in India and ranks among the top three cancers. Role of the PI3K/AKT/mTOR pathway in the
therapy including chemotherapy, immunotherapy and MET inhibitors (METi), characteristics at presentation, tx patterns, overall response rate to systemic ≥ with ≥ and/or gene/centromere ratio skipping mutations, MET characteristics, tx patterns, and outcomes in metastatic NSCLC pts. This study aims to characterize disease and pt vice versa accompanied with a high mTORC1 activity and 130a with the 3′UTR of TSC1 and miR-130a was done by real-time PCR in matched oral normal and tumor samples. Results: Overexpression of miR-130a in oral cancer cells (SCC131 and SCC084) significantly reduced the levels of TSC1 and hence increased cell proliferation. Dual luciferase reporter assay confirmed the interaction of miR-130a with the 3′UTR of TSC1. We observed an inverse correlation between the expression levels of miR-130a and TSC1 mRNA. Conclusion: Upregulation of miR-130a led to a decreased level of TSC1 accompanied with a high mTORC1 activity and vice versa. Our study suggests that inhibition of miR-130a may suppress the tumorigenic potential through the regulation of TSC1/mTOR axis in oral cancer, and provide a novel therapeutic target.

No conflict of interest

403 (PB-066) Poster Natural history, treatment (tx) patterns, and outcomes in MET dysregulated non-small cell lung cancer (NSCLC) patients (pts)

J. Wolf1, C. Baik2, R.S. Heist2, J.W. Neal3, A.S. Mansfield4, R. Buettner5, K.L. Davis5, M. Giovannini8, A. Mutebi9, M.M. Awad10, 1University Hospital Cologne, Department I of Internal Medicine, Cologne, Germany; 2University of Washington, Thoracic, Head and Neck Medical Oncology, Seattle, USA; 3Massachusetts General Hospital, Cancer Center, Boston, USA; 4University Hospital Cologne, Institute for Pathology, Cologne, Germany; 5Simon Medical Center Columbus, Columbus, USA; 6University Hospital Cologne, Department of Medicine, Division of Oncology, Stanford, USA; 7Mayo Clinic, Medical Oncology, Rochester, USA; 8University Hospital Cologne, Institute for Pathology, Cologne, Germany; 9RTI Health Solutions, Health Economics, Research Triangle Park, USA; 10Novartis Pharmaceuticals Corporation, Global Clinical Development, East Hanover, USA; 11Novartis Pharmaceuticals Corporation, US Health Economics and Outcomes Research, East Hanover, USA; 12Dana-Farber Cancer Institute, Medical Oncology, Boston, USA

Background: MET dysregulation through exon 14 skipping mutations, high-level gene amplification, or concurrent mutation with amplification occurs in ≥3% of NSCLC and may be a negative prognostic factor. Currently, limited data are available to characterize the natural history of MET-dysregulated metastatic NSCLC pts. This study aims to characterize disease and pt characteristics, tx patterns, and outcomes in MET-dysregulated metastatic NSCLC.

Material and Methods: This is an ongoing multinational, retrospective, real-world medical record analysis of pts with advanced EGFR/MET MET-dysregulated NSCLC defined, as MET mutated through exon 14 skipping mutations, MET amplification with gene copy number (GCN) ≥6 and/or gene/centromere ratio ≥2.2, or both mutation and amplification. Pts were included if they were ≥18 years old at diagnosis of metastatic NSCLC with ≥12 months of follow-up. Study measures include pt and disease characteristics, tx patterns, overall response rate to systemic therapy including chemotherapy, immunotherapy and MET inhibitors (METi), and Kaplan-Meier (KM) analyses of progression-free survival and overall survival (OS) across lines of therapy.

Results: This interim analysis included data from 131 pts (mutated [n = 70], amplified [n = 44], and concurrent mutated and amplified [n = 17]). Characteristics of mutated vs amplified vs concurrent mutation/amplification cancers are as follows: median age (yrs), 75 vs 64 vs 72; male, 47% vs 57% vs 59%; never smoker, 41% vs 7% vs 29%; squamous, 6% vs 2% vs 6%; median follow-up time from the first diagnosis of metastatic NSCLC (months), 12.1 vs 6.7 vs 13.4; stage IV at NSCLC diagnosis, 70% vs 68% vs 77%; brain metastases at metastatic NSCLC diagnosis, 20% vs 18% vs 47%; median number of systemic therapies, 2 vs 1 vs 3. METi were received by 37% vs 11% vs 59% pts, respectively. OS data are shown below (Table). Data on patterns of concurrent molecular alterations and additional tx specific efficacy data will be presented during the meeting.

Conclusions: MET exon 14 skipping mutations and high-level MET amplification define distinct NSCLC subgroups with poor prognosis. Tx with METi confers a survival benefit in both MET-dysregulated groups. Further study of METi in these populations is warranted.

Conflict of interest: Ownership: Reinhardt Buettner (co-founder and co-owner of Targos Molecular Pathology Inc, Kassel/Germany), Alex Mutebi (Novartis stockholder). Advisory Board: Juergen Wolf (Abbvie, AstraZeneca, BMS, Boehringer-Ingelheim, Chugai, Ignyta, Lilly, MSD, Novartis, Pfizer, Roche). Christina Baik (Astra Zeneca, Novartis), Rebecca S Heist (Tarveda), Joel W Neal (ARIAD/Takeda, AstraZeneca, Genentech/Roche, Eli Lilly), Aaron S Mansfield (Genentech, BMS), Mark M Awad (Bristol-Myers Squibb, Merck, Genentech, AstraZeneca, Pfizer, Nektar). Corporate-sponsored Research: Juergen Wolf (BMS, MSD, Novartis, Pfizer), Christina Baik (BluePrint, Novartis, Loxo, Pfizer, AstraZeneca, Spectrum, Celgene, Genentech/Roche), Rebecca S Heist (Genentech/Roche, Celgene, Mirati, Peregrine, Abbvie, Debiopharm, Millenium, Novartis, Dacchi, Coruxis, Elexisios), Joel W Neal (Genentech/Roche, Merck, Novartis, Boehringer Ingelheim, Elexisios, ARIAD/Takeda, Nektar Therapeutics), Aaron S Mansfield (Novartis), Keith L Davis (Novartis), Mark M Awad (Bristol-Myers Squibb). Other Substantive Relationships: Rebecca S Heist (Boehringer Ingelheim), Monica Giovanni (Novartis full time employee involved in clinical development of MET inhibitor), Alex Mutebi (Novartis employee).

404 (PB-067) Poster Ibrutinib in combination with sorafenib synergistically inhibits hepatocarcinogenesis by targeting EGFR signaling pathway

A. Noonan1, C.H. Lin1, K. Elkholly1, N. Wani1, D. Li1, P. Hu1, J.M. Barajas1, L.B. Yu1, X. Zhang1, P. Maciej2, S.T. Jacob2, K. Ghoshal1. 1The Ohio State University, The James Comprehensive Cancer Center, Department of Pathology, Columbus, USA; 2The Ohio State University, The James Comprehensive Cancer Center, Department of Cancer Biology and Genetics, Columbus, USA; 3The Ohio State University, The James Comprehensive Cancer Center, Department of Biomedical Informatics, Columbus, USA

Background: Hepatocellular carcinoma (HCC) is the second major cause of cancer related death worldwide because of limited therapeutic options. Our goal was to identify a novel combination therapy that can potently act on sorafenib (SOR), currently the only FDA-approved first-line targeted therapy for advanced HCC.

Materials and Methods: Ibrutinib (IBT), approved for B cell malignancies, is an irreversible inhibitor of both TEC (ITK) and ERBB1-4 (EGFR, Her2) families of tyrosine kinases. SOR inhibits PDGFR and Raf family kinases. The efficacy of IBT and SOR combination therapy was evaluated in vitro and in vivo. HCC cell lines (HepG2, Hep3B, PLC/PRF/5, SNU-182, SNU-449, Hep-7, Hepa-1-6 and HCCLM3 were used. NSG (NOD scid IL2R γ−) mouse model with HCCLM3 xenografts and C57BL/6NcrBRB mouse orthotopic model with HCCLM3 and Hepa-1-6 cells were used. Drug synergy was explicitly assessed for.

Table (abstract 403 PB-066): KM Estimates of OS by Tx and Dysregulation Status

<table>
<thead>
<tr>
<th>All (n = 131)</th>
<th>Mutated (n = 70)</th>
<th>Amplified (n = 44)</th>
<th>Concurrently Mutated and Amplified (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>From 1st diagnosis of metastatic NSCLC</td>
<td>Received METI</td>
<td>Not Received METI</td>
<td>Received METI</td>
</tr>
<tr>
<td>n (%) of pts with event</td>
<td>25 (61)</td>
<td>73 (81)</td>
<td>13 (50)</td>
</tr>
<tr>
<td>Median OS, months (95% CI)</td>
<td>25.1 (17.8, 30.6)</td>
<td>7.9 (5.6, 9.9)</td>
<td>29.3 (12.5, 53.0)</td>
</tr>
</tbody>
</table>

Poster Session (Friday, 16 November 2018) Abstracts, 30th EORTC-NCI-AACR Symposium e131
Results: IBT exhibits anti-HCC functions in vitro and ex vivo. More importantly, IBT–SOR co-treatment synergistically inhibited HCC cell proliferation and clonogenic survival by inducing apoptosis and suppressing tumor-sphere formation and expression of cancer stem cell markers. This combination therapy significantly suppressed the growth of highly aggressive HCCLM3 subcutaneous xenografts in immunodeficient mice, and increased survival of these mice bearing orthotopic HCCLM3 xenografts and immunocompetent mice bearing orthotopic mouse Hepat-1 tumors. Inactivation of EGFR and its downstream Akt and ERK signaling mediates anti-HCC effect of IBT. RNA-seq analysis showed the expression of several genes involved in cell proliferation, migration, anti-apoptosis, and stemness was downregulated whereas genes promoting differentiation were upregulated by these tyrosine kinase inhibitors.

Conclusion: Our results in preclinical models demonstrate that the combination of IBT and SOR suppressed tumor growth in HCC cell lines and mouse models, and particularly in SOR-resistant models with activated EGFR/Akt/ERK signaling. A phase III clinical trial of ibritinib-sorafenib combination in patients with HCC is planned to assess this hypothesis.

No conflict of interest

405 (PB-068) Poster
Targeting the TFE3 pathway in translocation renal cell carcinoma with the antibody-drug conjugate Glambatumumab vedotin
M. Lang1, D. Wei1, B.K. Gibbs1, L. Ohanjanian1, C.J. Ricketts1, S. Bourjaily1, L.S. Schmidt1, W.M. Linehan1, 1Urologic Oncology Branch, National Cancer Institute, Bethesda, USA; 2Basic Science Program, Leidos Biomedical Research Inc., PNL, Frederick, USA

Background: Renal cell carcinoma (RCC) consists of distinct subtypes with characteristic histologies, genetic mutations and clinical behaviors. The RCC subtype harboring an Xp11.2 chromosomal rearrangement (tRCC) is characterized by translocations of the TFE3 gene, a transcription factor that regulates cell growth and differentiation. This RCC subtype is mainly seen in young patients and can show aggressive behavior. The chromosomal rearrangements characterizing tRCC result in a gene-fusion product that leads to increased TFE3 expression, nuclear localization and transcriptional activity. Key signaling pathways in TFE3-fusion RCC are unregulated and drug therapies are yet to be identified. Glycoprotein NMB (GPNMB) is a transcriptional target of TFE3 and a fully human antibody-drug conjugate (ADC) has been developed against GPNMB. This ADC, Glambatumumab vedotin (CDX-011), has shown pharmacologic effect against breast cancer and melanoma with high expression levels of GPNMB.

Material and Methods: GPNMB expression was validated at the RNA and protein level in IRCC tumor tissues and normal kidney tissues, IRCC-derived cell lines and cells with inducible expression of TFE3-fusion genes. Flow cytometry was used to show GPNMB localization to the extracellular membrane and a cytotoxicity assay was used to prove the targeted action of the ADC. Susceptibility of tRCC cells to CDX-011 was performed in vitro in 2D and 3D spheroid culture models and in vivo in a xenograft model.

Results: Previously, our lab has shown that TFE3 can directly regulate GPNMB expression and data provided by the TCGA project confirmed that kidney cancer samples with TFE3 translocations have high expression of GPNMB. Here we confirm that kidney tumors and cell lines carrying TFE3-fusions express high levels of GPNMB, while other kidney cancer cell lines are negative for the protein. We show that GPNMB localizes to the outer cellular membrane and is recognized by the human antibody used to produce the ADC. tRCC cells are sensitive to Monomethyl auristatin E, the tubulin inhibitor conjugated with the anti-GPNMB antibody. Unlike the non-conjugated antibody, CDX-011 is cytotoxic to GPNMB-expressing cells. The tRCC cell lines UOK120 and UOK124 show significant decrease of viability after treatment with 10 μg/ml CDX-011 in 2D and 3D spheroid culture. Tumor growth is significantly inhibited in vivo upon CDX-011 treatment of mice carrying UOK124 xenografts.

Conclusions: No drug has previously demonstrated convincing efficacy in in vivo models for IRCC. Here we show that TFE3-fusion kidney cancers express high levels of GPNMB and can therefore be specifically and directly targeted with the antibody-drug conjugate Glambatumumab vedotin. Our data therefore provide a promising therapeutic option for patients with IRCC with a drug that is currently undergoing stage 2 clinical trials.

No conflict of interest

406 (PB-069) Poster
Identifying predictive markers and novel combinations for TORC1/2 inhibition in ovarian and endometrial cancer
N. Grosenbacher1, S. Crafton1, J. David1, C. Stelts1, B. Demore2, J. Chen2, J. Hays3, 1The Ohio State University Comprehensive Cancer Center, Medical Oncology, Columbus, USA; 2The Ohio State University Comprehensive Cancer Center, Gynecologic Oncology, Columbus, USA; 3The Ohio State University Comprehensive Cancer Center, Medical and Gynecologic Oncology, Columbus, Ohio, USA

Background: In an effort to overcome the poor efficacy of targeted agents as monotherapy in endometrial and ovarian cancers, interest has turned to combination therapy. Significant fractions of endometrial and ovarian cancers have de-regulation of the PI3K/AKT/MTOR pathway due to inactivating mutations in PTEN or activating mutations in PIK3CA, AKT or MTOR. We examined a panel of endometrial and ovarian cancer cell lines to determine response to TAK228, a dual TORC1/2 inhibitor and utilized mutational analysis and reverse phase protein arrays (RPPA) to identify possible mechanisms of resistance and synergy.

Material and Methods: We analyzed 5 endometrial (AN3CA, ECC, HEC1A, HEC1B, RL952) and 6 ovarian cancer cell lines (OVCIO, SKOV3, OVCA83, OVCAR8, HEYA8, CAOV3) for sensitivity to AKT (MK2206) and MTOR inhibition (TAK228) utilizing XTT, scratch, and attachment independent growth assays. Co-operativity assays were performed according to the method of Chou-Talalay. Cell lysates before and after treatment were analyzed for protein expression of >300 proteins utilizing RPPAs and published mutational data was collected for each cell line.

Results: All cell lines showed sensitivity to both agents, with TAK228 being significantly more toxic in XTT and scratch assays than the AKT inhibitor MK2206 (mean IC50 20.2 nM vs 12 μM, respectively). TAK228 was also significantly more toxic than the TORC1 specific inhibitor rapamycin (mean IC50 32 nM vs 21 μM, respectively). Analysis of RPPA proteomics data identified VEGFR and SMAD1 pretreatment protein levels as being strongly correlated with sensitivity to TAK228. Also, a panel of growth stimulatory factors were associated with sensitivity in both pre-treatment protein samples and when looking at changes before and after treatment. Mutational analysis was not predictive of response. Modulation of the VEGF/VEGFR axis with antibody and small molecule inhibitors demonstrated synergy across multiple cell lines. Cell cycle modulation with CHK1/2 inhibitors also revealed marked synergy across multiple cell lines (mean CI <0.5).

Conclusions: Single agent targeted therapy in gynecologic malignancies has proven largely ineffective with the exception of PARP inhibition in select populations. Here we identify novel combinations with targeted agents in ovarian and endometrial cancer that can be quickly translated to clinical testing. A clinical trial with bevacizumab and TAK228 in recurrent ovarian and endometrial cancer is currently underway.

No conflict of interest
Results: EG-011 presented a median IC50 of 2.25 mM across 62 lymphoma cell lines, including 11/15 3 cell lines with greatest synergy between afatinib and dasatinib (A&D) were sensitive to both drugs. Dasatinib had the most effective growth inhibition in both cell lines (>70%) and this effect was further investigated. The selective anti-lymphoma activity, in both in vitro and in vivo models, was observed in in vitro synergistic effects with FDA approved targeted agents make EG-011 a novel intriguing drug candidate deserving further preclinical studies.

No conflict of interest

Background: SRC was recently identified as driver in IDH mutant Intrahepatic Cholangiocarcinoma (ICC). While SRC transitions between globally "open" (active) and globally "closed" (inactive) conformations, the structural mechanisms underlying conformational transitions are not well understood, and approved inhibitors stabilize SRC in the globally open conformation. We sought to develop inhibitors that stabilize SRC in the globally closed conformation, elucidate allosteric networks, and validate the SRC-dependency of IDH mutant ICC.

Materials and Methods: Small molecule inhibitors of SRC were identified through biochemical, biophysical, and virtual screening. Protein constructs were crystallized with inhibitors. Compound-bound structures were determined using X-ray crystallography, and binding kinetics were evaluated using Surface Plasmon Resonance. RBE and SNU-1079 cell lines, derived from patients with IDH mutant ICC, were used for in vitro studies. Phosphorylation state of SRC (Y419) and S6 (S235/236) were used to monitor pathway inhibition, and Cell Titer-Glo was used to assess cellular viability. CRISPR/ Cas9 was used to introduce the SRC gatekeeper mutation T341I into cells.

Results: We developed small molecule inhibitors that stabilize SRC in globally open or closed conformations. In the closed conformation, the SH2 and SH3 domains dock against the catalytic domain and decrease catalytic activity by a long-range allosteric mechanism. In the open conformation, the SH2 and SH3 domains are repositioned, referred to as the R-spine, align. These changes bring the catalytic machinery in close proximity, forming the fully activated state. A subset of molecules uncouple the global state from the local arrangement of the catalytic machinery, allowing us to confirm Trp283 as a central hub of allosteric information transfer. Subtle shifts in the R-spine residue Leu 328 and residue Tyr 385, outside of the R-spine, were found to have key interactions balancing the opening and closing mechanism. Treatment of IDH mutant ICC cells with molecules stabilizing the closed conformation inhibited pathway signaling and proliferation with IC50 < 500 nM. Introduction of the SRC (T341I) gatekeeper mutation rescued cells from the effects of tool compounds, confirming SRC as a critical target in IDH mutant ICC.

Conclusions: We developed small molecule inhibitors that stabilize SRC in the closed conformation, elucidated underlying allosteric networks, and confirmed the SRC dependence of IDH mutant ICC cells. Further evaluation of this mechanism for the treatment of patients with IDH mutant ICC is warranted.

Conflict of interest: Corporate-sponsored Research: All authors are full-time employees and shareholders of Relay Therapeutics.

410 (PB-073) Poster MET mutant allele frequency (MAF) is correlated with glesatinib anti-tumor activity in patients with advanced non-small cell lung cancer (NSCLC) harboring MET alterations

H. Der-Torossian1, G. Shapiro2, P. Janne3, M. Awad4, K. Reckamp6, W. Miller6, D. Hong2, M. Hussein1, I. Percient6, L. Bazenjova4, A. Dowlait10, K. Dong-Wan11, B.C. Cho12, K. Park9, V. Tasselli13, R. Chao1, D. Faltaos14, J. Christensen15, Miriti Therapeutics, Medical, San Diego, USA; 2 Dana Farber Cancer Institute, Early Drug Development Center, Boston, USA; 3 Dana Farber Cancer Institute, Medical Oncology, Boston, USA; 4City of Hope, Clinical Research Operations, Duarte, USA; 5Jewish General Hospital, Department of Medicine, Department of Medicine, Canada; 6MD Anderson, Department of Investigational Cancer Therapeutics – Division of

Poster Session (Friday, 16 November 2018)
Poster Session (Friday, 16 November 2018)
Background: The receptor tyrosine kinase AXL, is overexpressed in many human cancers and its expression is associated with a poor prognostic outcome for patients. AXL is a member of the TAM family of kinases (AXL, MER, TYRO3), which are involved in multiple aspects of tumorigenesis. Increased expression of AXL is associated with increased oncogenic transformation, cell survival, proliferation, migration, angiogenesis, cellular adhesion and avoidance of the immune response. We discovered and are developing a small molecule AXL kinase inhibitor, TP-0903, and have shown the effectiveness of TP-0903 in cell-based and animal models of human cancers. TP-0903 has low nanomolar (IC50 < 12 nM) activity against the AXL kinase in biochemical assays. In other biochemical evaluation, TP-0903 is shown to inhibit the entire TAM family of kinases at nM potencies. Recent studies have indicated that AXL and other TAM family kinases are critical regulators of the innate immune response, and their activation may mediate immune-suppressive activity seen in many cancers.

Material and Methods: To explore the immune activating potential of TP-0903 we tested TP-0903 in several syngeneic mouse models of solid tumors. Markers of infiltrating immune cells in these models were assessed on both formalin-fixed and fresh tissues using standard immunohistochemical and real-time PCR techniques. Effects of TP-0903 on regulatory T-cells was also assessed in vitro using standard viability assays and multiplexed cytokine arrays.

Results: Inhibition of TAM kinase activity by TP-0903 was shown to enhance host immunity in these tumor models. The immune response effects of TP-0903 were associated with dose-related increases in the percent of tumor-infiltrating effector CD4+ and CD8+ T-cells and enhanced therapy responses to immune checkpoint inhibitors. In addition, TP-0903 treatment results in an increase in activated dendritic cells and a reduction in immune-suppressive regulatory T-cells. We are currently analyzing the effect of TP-0903 on markers of immune suppression in a multi-center, open-label, Phase 1/1b first-in-human study of TP-0903.

Conclusions: Taken together, these preclinical data support the potential therapeutic activity of TP-0903 as an immune modulating agent capable of enhancing host immune response in cancer patients as a single agent and when combined with therapies targeting immune checkpoints. Expansion cohorts from the current clinical study will further evaluate the potential of TP-0903 to enhance the activity of immune checkpoint inhibitors. Clinical trial information: NCT02729298.

Conflict of interest: Other Substantive Relationships: Employment.

414 (PB-077)  Poster

Phase I trial of enzalutamide in combination with gemcitabine and nab-paclitaxel in the management of advanced pancreatic cancer

A. Kommalapat 1, R. Kim 2, T. Sri Harsha 3, B. Berteis 1, G. Wapinsky 2, N. Burke 1, A. Neuger 2, G. Springett 2, K. Almhaana 1, A. Mahipal 1

1University of South Carolina School of Medicine, Internal Medicine, Columbia, SC, USA; 2Moffitt Cancer Center, Hematology/Oncology, Tampa, USA; 3University of South Carolina Cancer Center, Internal Medicine, Columbia, USA; 4Mayo Clinic, Hematology/Oncology, Rochester, MN, USA

Background: Androgens were shown to play a key role in the growth and progression of pancreatic cancer. In this Phase I trial involving metastatic pancreatic cancer patients, we evaluated the safety and tolerability of the combination of enzalutamide, a novel androgen receptor (AR) antagonist with gemcitabine and nab-paclitaxel as a first-line treatment.

Methods: We used the standard 3+3 dose escalation design with cohort expansion to evaluate 2 dose levels of enzalutamide: 80 mg and 160 mg/day orally. In the expansion phase, AL故事 was a pre-requisite criterion. Gemcitabine (1000 mg/m²) and nab-paclitaxel (125 mg/m²) was administered IV on days 1, 8 and 15 of a 28-day cycle. The dose-limiting toxicity (DLT) period was 28-days or until the beginning of the second cycle for the phase la part. In the phase 1 b portion, only patients with tumor-positive for AR were included. We evaluated the full pharmacokinetic (PK) profile for nab-paclitaxel after the initial dose through 48 hours post-dose while enzalutamide was tested on day 1 at 1 and 2 hours post-dose along with levels every 7 days to follow achievement of a steady state by day 29 (Cycle 2 Day 1).

Results: We enrolled 25 patients (16 males and 9 females) with metastatic pancreatic cancer, 12 patients at the first dose level and 13 patients at the second dose level. The median age was 68 years (32–84 years). No DLTs were observed. Grade 3/4 treatment related adverse events included neutopenia (44%), anemia (40%), leukopenia (24%), nausea and vomiting (20%), diarrhea (16%), infections (12%), thrombocytopenia (8%), thromboembolic event (8%), hypertension (8%), hypokalemia (8%), hyponatremia (8%), ALT-elevation (8%), pneumonitis (4%), skin infection (4%) and hypoxia (5%). Median overall survival and progression-free survival was 9.73 (95%CI: 7.93–13.5) and 7.53 (95%CI: 6.05–12.8) months, respectively. PK analysis suggests that the combination therapy does not impact the kinetics of either drug evaluated. Enzalutamide reached steady-state levels between day 22 and 29 and the mean half-life of nab-paclitaxel was 19.6 ± 4.7 hours. All other PK parameters estimates are similar to historical data.

Conclusions: Enzalutamide 160 mg daily in combination with gemcitabine and nab-paclitaxel can be safely administered with no unexpected toxicities. We also noticed preliminary signals of efficacy with this combination. We continue to enroll patients with AR+ tumors in phase 1b part.

No conflict of interest

415 (PB-078)  Poster

Assessing of Combretastatin A-4 Phosphate Activity for H1299 Lung Cancer

L. Liu 1, D. Yang 1, J. Chen 1, J. Campbell 1, S. Zhong 2, L. Zhang 2

1The University of Texas Southwestern Medical Center, Radiology, Dallas, USA; 2The University of Texas at Dallas, Biological Sciences, Richardson, USA

Background: Combretastatin A-4 phosphate (CA4P) (Millpore Sigma) is a well-characterized vascular disrupting agent that inhibits microtubule polymerization, which selectively reduces the tumor blood flow, causing ischemia and cell death. CA4P is a water-soluble prodrug of the cis-stilbene CA4 originally isolated from the tree Combretum caffrum. In this study, we apply multimodality imaging to investigate the effect of CA4P on tumor oxygenation in human lung H1299-luc xenograft tumor model.

Material and Methods: 1 million H1299-luc cells were subcutaneously injected in the right flank of female and male 8 week-old nude mice (n = 6). After 3–6 weeks, when the tumor size reaches 8–10 mm in diameter, at baseline, 1, 3, and 24 h after control saline or CA4P administration, blood oxygenation was assessed in vitro using standard viability assays and multiplexed cytokine arrays.

Results: After control saline administration in a stepwise manner: disruption of the vasculature (3 h), tumor necrosis (8 h), reduced oxygenation (3 h), and cell death (24 h). This result illuminated the molecular events leading to tumor hypoxia and variations within one tumor and among different tumors.

Conclusions: Multimodality imaging characterized the effects of CA4P administration in a stepwise manner: disruption of the vasculature (3 h), reduced oxygenation (3 h), cell death (24 h). This result implies the molecular events leading to tumor hypoxia and variations within one tumor and among different tumors.

No conflict of interest
TRAILR2 is a pro-apoptotic receptor widely expressed in cancers and its ligand-induced oligomerization results in the induction of apoptosis which is potentiated upon the formation of larger clusters. Several agonistic molecules have reached the clinic but were terminated due either to lack of efficacy or liver toxicity. CDH17 is a cell surface molecule that is absent in liver and co-expressed with TRAILR2 in several cancer types. BI 905711 is a tetravalent bispecific molecule targeting both TRAILR2 and CDH17, and is designed to selectively induce apoptosis in CDH17 expressing tumor cells via CDH17-dependent clustering of TRAILR2, while avoiding the hepatotoxicity associated with clustering of TRAILR2 in liver. Here, we report the preclinical activity of BI 905711 using colorectal cancer (CRC)-derived cell lines both in vitro and in vivo as tumor xenografts implanted in mice.

We showed that, in a CDH17-dependent manner, BI 905711 triggered apoptosis in several CDH17 positive CRC tumor cells in vitro, but not in CDH17 negative/TRA1L sensitive liver-derived cells. BI 905711 in vivo efficacy was demonstrated using disease-related CRC xenograft models, where sustained tumor regressions were observed after administration of a single dose. Selective pathway engagement was assessed by measuring the downstream apoptosis biomarkers caspase 8 and 37. Significant activation of caspases was demonstrated not only in the tumor tissue, but also active caspases released from dying tumor cells could be detected in plasma samples.

In summary, we demonstrated that BI 905711 triggers apoptosis specifically in CDH17 positive tumor cells, demonstrating the potential for a favorable safety and efficacy profile. BI 905711 represents a promising new approach for the treatment of CRC and additional CDH17 positive oncological indications.
Conflict of interest: Ownership: Drs. Koropatnick and Vincent are co-owners of Sarissa, Inc., which owns the license for IBR2 and IBR120.

420 (PB-083) Poster
Tipifarnib is highly active in HRAS-mutant HNSCC tumor models
M. Gianlò1, Z. Wang1, L. Kessler2, M. Janes3, Y. Chen3, A. Guaita2, Y. Lu3, S. Gutkind4. 1University of California, San Diego, USA; 2Kura Oncology, Clinical, San Diego, USA; 4Wellspring Biosciences, Biology, San Diego, USA
Tipifarnib is a potent and highly selective inhibitor of farnesyltransferase (FT). FT catalyzes the post-translational attachment of farnesyl to signaling proteins that are requisite for localization to the cell inner membrane. While all RAS isoforms are FT substrates, only HRAS is exclusively dependent upon farnesylation for membrane localization and signaling activation, making HRAS mutant tumors uniquely susceptible to tipifarnib mediated inhibition of FT. Based upon this rationale, the efficacy and safety of tipifarnib in patients with HRAS mutant head and neck squamous cell carcinoma (HNSCC) is currently being evaluated in a multi-institutional, open-label Phase II trial (NCT02383927).

Herein we report the characterization of tipifarnib in preclinical models of HRAS mutant HNSCC. Tipifarnib was screened in a panel of 20 HNSCC cell lines to identify FT-dependent inhibition of proliferation in 4/4 HRAS mutant lines tested in conventional monolayer assays, and the robustness and selectivity of the response was further enhanced in the 3D anchorage-independent format, as reported for other RAS inhibitors. Tipifarnib displaced HRAS, but not KRAS, from membranes and inhibited MAPK signaling in HNSCC cells in vitro.

Tipifarnib displayed robust antitumor activity in nine of ten cell line- and patient-derived xenograft (PDX) models of HRAS mutant HNSCC, including several that were resistant to chemotherapy and/or cetuximab, suggesting that tipifarnib may offer improved outcomes in this subset of HNSCC. Tipifarnib treatment in vivo resulted in downregulated MAPK kinase activity that was associated with reduced proliferation, activation of apoptosis and other phenotypic changes consistent with blockade of mutant HRAS signaling.

These preclinical findings compliment preliminary data from the ongoing Phase 2 clinical study of tipifarnib in which confirmed partial responses have been observed in HRAS mutant HNSCC patients who were relapsed and/or refractory to prior therapy, including platinum, immunotherapy and cetuximab. STEAP4. We have discovered that treatment of breast cancer cell lines, displaying potent inhibition of proliferation in 4/4 HRAS mutant lines, with HRAS mutant tumors uniquely susceptible to tipifarnib mediated inhibition of h/ft.

Conflict of interest: Ownership: Kura Oncology, Inc., Wellspring Biosciences, Inc.

421 (PB-084) Poster
Targeting sorting nexins to treat ErbB-dependent breast cancer
B. Atwell1. 1University of Arizona, Molecular and Cellular Biology, Tucson, USA
The Epidermal Growth Factor Receptor (EGFR) is a powerful driver of transformation and metastasis, being overexpressed and amplified in 60% of triple negative breast cancer. Yet, therapies designed to target EGFR have been successful in only a small subset of these cases. We and others have worked to understand why EGFR is so difficult to target in breast cancer, and have found that it is frequently mis-trafficking during cancer progression and can function internally in a kinase-independent manner. Overexpression of adapter proteins (such as MUC1) drive EGFR off of the cell surface where it undergoes retrograde trafficking to locations including long-lived endo-

omes, the mitochondria and the nucleus. Once in these novel locations, EGFR drives migration, alters mitochondrial function and acts as a transcription co-factor for genes such as Cyclin D1. Blocking this retrograde trafficking results in the complete ablation of EGFR’s ability to drive EGFR-
dependent migration. We are investigating the role of the Sorting Nexus (SNX) complexes to regulate EGFR trafficking, and have developed a peptide-based therapeutic to reintroduce normal trafficking of EGFR to the lysosome (SNX1.3). We have discovered that treatment of breast cancer cell lines with SNX1.3 results in a reduction of EGFR-dependent migration. Furthermore, SNX1.3 treatment results in the degradation of EGFR and HER2, but not HER3, indicating a specificity in the interaction. We are currently working to better define the proteins driving the interactions between SNX and EGFR, stabilize the peptide and test its efficacy in mouse models of breast cancer.

No conflict of interest

Poster Session (Friday, 16 November 2018)
Abstracts, 30th EORTC-NCI-AACR Symposium
Results: Characterization of TAS6417 against uncommon mutations including ex20ins, G719X, and L861Q were performed. MS analysis and biochemical assay demonstrated that TAS6417 may exert inhibitory activity against EGFR D770_N771insNPg through covalent modification of the C797 residue. In cell viability assays, the G50 values of TAS6417 for LXF 247TL cells harboring EGFR V790M_D770insN771cys and human primary keratinocytes (NHEK-NEO), of which WT EGFR is implicated in the growth, were 86.5, 45.4, and 729 nM, respectively. Further analysis with In-Cell Western demonstrated that TAS6417 had potent inhibitory activity against EGFRs with other uncommon mutations such as G719A and L861Q even if complexed with T790M acquired resistant mutation. Consistently, TAS6417 inhibited respectively. Further analysis with In-Cell Western demonstrated that Baf-B3 cells expressing EGFR with G719A ± T790M or L861Q ± T790M with IC50 values ranging from 6.55 to 37.5 nM. In contrast, the IC50 value for WT EGFR was 676 nM. Selectivity for these mutations was superior to other representative EGFR-TKIs such as afatinib and osimertinib. Furthermore, in an in vivo model using NGRG3 EGFR G719A cells, TAS6417 exerted marked antitumor efficacy at 50 mg/kg or more without severe body weight loss, comparable to efficacy shown in xenograft models of EGFR ex20ins mutations.

Conclusions: TAS6417 exerts mutant-selective inhibition against uncommon EGFR mutations including ex20ins, G719X, and L861Q while sparing WT. This mutation-selective characteristic led to a significant antitumor efficacy in mouse model, suggesting a promising therapeutic option for NSCLC patients with uncommon EGFR mutations.

No conflict of interest

425 (PB-088) Poster Phloretin inhibits hepatic cancer via downregulation of JAK2/STAT3 signaling and induction of apoptosis
S. Saraswati1, A.A. Alhaider1. 1King Saud University, Pharmacology, Riyadh, Saudi Arabia

Background: Phloretin possess numerous biological properties, including anti-angiogenic, anti-inflammatory, antiproliferative, and protein kinase C inhibition activity, but its effect of hepatic cancer is not explored yet.

Material and Methods: We investigated the anti-cancer effect of phloretin on an experimental carcinogenesis model of liver cancer by studying the anti-oxidant, anti-inflammatory, anti-proliferation, pro-apoptotic activities of phloretin in vivo. We evaluated the effects of phloretin on N-nitrosodiethylnitrosamine (DENA) induced hepatic carcinoma in rats. We initiated hepatic carcinogenesis by intraperitoneal injection of diethylnitrosamine (DENA) followed by promotion with phenobarbital.

Results: Administration of phloretin at doses of 15, 30, and 50 mg/kg/day was started 4 weeks prior to the DENA injection and was continued for 22 weeks. Phloretin decreased the incidence, total number, multiplicity, size and volume of preneoplastic hepatic nodules in a dose-dependent manner. Furthermore, phloretin counteracted DEN-induced oxidative stress in rats as determined by restoration of superoxide dismutase, catalase, and glutathione-S-transferase levels and diminishing of myeloperoxidase activity, malondialdehyde and protein carbonyl formation in liver. Phloretin resulted in a normal carcinogenesis of level of IL-2, IFN-γ, AFP and AFU. We found that JAK2/STAT3 signaling was significantly up-regulated in DENA-treated group compared to that in control group. Western blot analysis showed that phloretin inhibited phosphorylation of STAT3 and its principal upstream kinase, JAK2. Furthermore, it also decreased expression of cyclin D1 and Bcl-2 with activation of caspase-3 and increased expression of Bax. Immunohistochemical demonstrated the decreased expression of the PCNA, VEGF cyclooxygenase 2, iNOS, nuclear factor-kappa B p65.

Conclusions: Phloretin exerts a significant chemopreventive effect against liver cancer via inhibition of cell proliferation and induction of apoptosis mediated by JAK2/STAT3 signaling pathway. This study also demonstrated that phloretin protects rat liver from cancer via modulating oxidative damage and suppressing inflammatory response.

No conflict of interest

427 (PB-090) Poster Effects of treatment regimens on antitumor activity of lenvatinib and anti-PD-1 antibody combinations in the CT26 mouse colon cancer syngeneic model
Y. Kato1, T. Kimura1, K. Tabata1, Y. Horii1, K. Yamada1, T. Uehara1, J. Ito1, Y. Ozawa1, K. Ishikawa1, J. Matsu1, Y. Funahashi1, K. Nomoto1. 1Eisai Co., Ltd., Tsukuba Research Laboratories, Tsukuba, Japan; 2Eisai Inc., Oncology Business Group, Woodcliff Lake, NJ, USA

Background: Lenvatinib (LEN) is a multitargeted tyrosine kinase inhibitor that selectively inhibits vascular endothelial growth factor (VEGF) receptor (VEGFR) –1,–3, fibroblast growth factor receptor (FGFR) –1,–4, platelet derived growth factor receptor (PDGFR) α, RET and KIT. Phase 1b/2 clinical trials investigating combinations of LEN and anti-PD-1 antibodies are ongoing in selected cancer types including renal cell carcinoma and hepatocellular carcinoma and other cancers. We previously reported inhibitory effects of LEN on tumor-associated macrophages and antitumor activities of LEN combined with anti-PD-1 antibody (Ab) in mouse syngeneic tumor models. In this study, treatment regimens and mechanisms of immune modulatory effects of the LEN plus anti-PD-1 Ab combination were investigated in CT26 mouse colon cancer syngeneic model.

Methods: Antitumor activities of monotherapy, sequential and combination treatments of LEN (10 mg/kg, qd) and anti-PD-1 Ab (200 μg/mouse, twice weekly) were examined in the CT26 colon cancer syngeneic model. Tumor-infiltrated lymphocytes were analyzed by flow cytometry to assess immune cell populations. Antitumor activities in mice were assessed by gene expression analyses using RNA sequencing and quantitative PCR.

Results: Combinations of LEN and anti-PD-1 Ab showed greater antitumor activity in the CT26 model compared with each monotherapy. In addition, prior treatment with LEN followed by anti-PD-1 Ab showed greater antitumor activity compared with anti-PD-1 Ab single agent treatment, particularly in mice whose tumor growth was strongly inhibited by LEN alone. Flow cytometric analysis showed that LEN or LEN plus anti-PD-1 Ab increased memory T cell populations compared with non-treatment. In addition, LEN or LEN plus anti-PD-1 Ab also increased Granzyme B-expressing CD8+ T cells in tumor. Gene expression analysis of CT26 tumors treated with LEN plus anti-PD-1 Ab showed upregulation of cytotoxicity molecules, Prf1 (perforin) and GzmB (granzyme B), and genes related to memory T cells.

Abstracts, 30th EORTC-NCI-AACR Symposium Poster Session (Friday, 16 November 2018)
Conclusions: These results indicated greater antitumor activity of combinational therapy than monotherapy and sequential treatments. The results from sequential treatment suggested that LEN may modify tumor microenvironment to lead to more sensitivity to anti-PD-1 Ab. The antitumor activity of the LEN plus anti-PD-1 Ab combination was accompanied by an immune modulatory response characterized by upregulation of cytotoxic molecules from T cells and memory T cells and genes related to memory T cells.

Conflict of interest: Corporate-sponsored Research: This research is funded by Eisai. Co., Ltd. Other Substantive Relationships: We are employees of Eisai Co., Ltd. or Eisai Inc.

428 (PB-091) Poster Identification of robust E-cadherin-related synthetic lethal interactions in breast cancer

I. Baranov1, C. Lord2,1. Institute of Cancer Research, Breast Cancer Now, London, United Kingdom; 2The Institute of Cancer Research, Breast Cancer Now, London, United Kingdom

Background: The E-cadherin (CDH1) tumour suppressor gene encodes a calcium-dependent cell-cell adhesion glycoprotein, which has roles in maintaining cell polarity, differentiation, cell migration, and survival. E-cadherin dysfunction is a feature common to many epithelial tumours, with the highest incidence occurring in diffuse gastric cancer (50%) and lobular breast cancer (63%) and can occur via CDH1 mutation, deletion or epigenetic silencing. Although E-cadherin dysfunction is a feature common, precision medicine approaches that exploit this pathogenic alteration are not yet available.

Materials and Methods: We used a series of integrated approaches to identify synthetic lethal interactions that operate in a diverse set of models of E-cadherin deficiency in breast cancer. These included: (i) high-throughput in vitro genetic perturbation screens in isogenic and non-isogenic cell lines; (ii) analysis of ex vivo breast cancer explants; (iii) analysis of genetically engineered mouse models of invasive lobular carcinoma; (iv) analysis of mice bearing patient derived xenografted tumours.

Results: Perturbation screens in breast tumour cells with CRISPR/Cas9-engineered CDH1 mutations identified a synthetic lethality between E-cadherin deficiency and inhibition of the receptor tyrosine kinase ROS1. Data from large-scale genetic screens in molecules diverse breast tumour cell lines established that the E-cadherin/ROST synthetic lethality was not only robust in the face of considerable molecular heterogeneity but was also elicited with clinical ROS1 inhibitors, including foretinib and crizotinib; these effects also operated in ex vivo breast cancer explants; (ii) analysis of genetically engineered mouse models of invasive lobular carcinoma; (iv) analysis of mice bearing patient derived xenografted tumours.

Conclusions: These data provide the preclinical rationale for assessing ROS1 inhibitors, such as the licensed drug crizotinib, in appropriately stratified patients. The phase 2 ROLO clinical trial will now assess the effect of crizotinib treatment when combined with fulvestrant in patients with advanced, E-cadherin deficient, breast cancer.

No conflict of interest

429 (PB-092) Poster INC280 inhibits Wnt/beta-catenin and EMT signaling pathways and its induce apoptosis in diffuse gastric cancer positive for c-MET amplification

S.H. Sohn1, Y. Kim1, B. Kim2, H.J. Su1, H.S. Kim2, H. Kim2, J.B. Seo2, Y. Koh1, D.Y. Zang1, 1Hallym Translational Research Institute, Hallym University Medical Center Hallym University College of Medicine, Anyang-si, South Korea; 2Department of Internal Medicine, Hallym University Medical Center Hallym University College of Medicine, Anyang-si, South Korea; 3Department of Biomedical Sciences, Sungkyunkwan University, Suwon, South Korea; 4Korea Basic Research Institute, Seoul Center, Seoul, South Korea; 5Department of Bio-medical Gerontology, Ilson Institute of Life Sciences, Hallym University, Anyang, South Korea

Background: Intestinal-type gastric cancer is related to Helicobacter pylori infection whereas diffuse-type gastric cancer is more frequently related to genetic predisposition. Previous studies have shown that Runt-related transcription factor 3 (RUNX3) expression was significantly down-regulated in gastric cancer tissues compared with matched normal tissues. We showed that decreased levels of RUNX3 are significantly associated with hepatocyte growth factor receptor (MET) (r = 0.4216, P = 0.013). In addition, c-MET is potentially a highly plausible target for gastric cancer therapy. Therefore, in the present study, the anti-cancer effects of the c-MET inhibitor on gastric cancer cells with or without c-met amplification were evaluated.

Material and Methods: c-Met inhibitors such as crizotinib, foretinib, cabozantinib, tivantinib and tepotinib were purchased from Selleck Chemical and INC280 was gratefully supplied by Novartis. SNU620 and MKN45 were purchased for c-Met amplification, but MKN28 was taken as a control. Each cell-line was treated with c-Met inhibitors. We measured cell viability, migration ability, apoptosis, mRNA expression and protein levels in each cell-line.

Results: INC280 treatment inhibits growth of a c-MET-amplified cell line. Dose-response non-linear regression analysis revealed that the half-maximal inhibitory concentration (IC50) of INC280 was 1.7 nM or 2.4 nM in the c-MET-amplified MKN45 and SNU620 (diffuse type) cell-line, respectively. Next, migration and apoptosis analysis demonstrated that INC280 showed the best inhibition and apoptotic rates with the smallest IC50s in MKN45 cells but not in c-MET-reduced MKN28 (intestinal type) cells. We also showed that INC280 inhibits the WNT signaling pathway and SNAIL expression in MKN45 cells.

Conclusions: INC280 shows significant inhibitory activity against c-MET-expressed diffuse gastric cancer. Our in vitro study strongly supports that INC280 could be used as therapeutic agents for diffuse gastric cancer with c-MET amplification.

No conflict of interest

430 (PB-093) Poster TAS0612, a novel and highly potent RSK, AKT, and S6K inhibitor, exhibited strong antitumor effect in preclinical tumor models with deregulated RAS and PI3K pathway activities

K. Ichikawa1, S. Ito1, T. Machida1, E. Kato1, M. Yamada1, R. Fujita1, Y. Nakatsum1, H. Sato1, G. Tanaka1, K. Wakayama1, H. Jona1, T. Sugimoto1, K. Matsu1, K. Miyadera1, T. Tsugui1, Y. Iwasawa1, 1Taiho Pharmaceutical Co., Ltd., Discovery and Preclinical Research Division, Tsukuba, Japan; 2Taiho Pharmaceutical Co., Ltd., Discovery and Preclinical Research Division, Takushkima, Japan

Background: The PI3K/AKT/mTOR pathway plays a crucial role in cancer cell growth and survival. Although molecular targeted drugs for PI3K, AKT and mTOR are under clinical development, it has been demonstrated that these drug show insufficient clinical response due to cancellation of negative feedback or the coexistence of mutations in driver genes such as KRAS and BRAF. TAS0612 was identified as a novel, highly potent, and orally bioavailable inhibitor of RSK, AKT, and S6K kinases. RSK has a key role in cell growth, migration, and transcription under the RAS pathway. Recent studies revealed that RSK is involved in the resistance to treatment of various cancers, e.g. breast, lung, and prostate cancer. Here we present a unique anticancer effect of TAS0612 in various cancer cells unresponsive to molecular targeted drugs and chemotherapy agents.

Material and Methods: The kinase selectivity profiling of TAS0612 was conducted in a kinase panel including more than 200 kinases. Cell growth inhibition was assayed by measuring cellular ATP. For pharmacodynamic marker inhibition and antitumor efficacy in vitro, human tumor cell lines were subcutaneously transplanted into the flank of nude mice. Dosing of compound was started when tumor size reached ~150 mm3. The total and phospho-protein level were determined by Western blot and ELISA analysis.

Results: TAS0612 inhibited enzymatic activity of AKT isoforms at a single digit nM level and inhibited RSK and S6K isoforms at sub nM level. Pharmacological inhibition of RSK, AKT, and S6K by TAS0612 was confirmed by the reduction of their phospho-substrates. TAS0612 showed stronger IC50 values than those of AKT, PI3K and PI3K/mTOR inhibitors regardless of whether cancer cell carried mutations in KRAS or BRAF gene. In vitro, PI3K, AKT, MEK, BRAF inhibitors couldn’t inhibit downstream signals of cancer cells with complicated driver-gene mutations by a single or a combination treatment. However, TAS0612 clearly inhibited downstream signals and induced apoptosis. TAS0612 also demonstrated superior antitumor efficacy both in KRAS mutant (TOV21G and HEC-6) and BRAF mutant (A375) xenografts which were less sensitive to AKT inhibitors and PI3K/mTOR inhibitor. Furthermore, MEF319 xenograft tumor was refractory to platinum treatment (T/C was about 60% to CDDP and CBDDA at their maximum tolerated dose). In the same model, TAS0612 showed significant efficacy around 5–20% of T/C, suggesting the possibility for application in the treatment of cancer unresponsive to chemotherapy.

Conclusions: We identified a novel class and highly potent RSK/AKT/ S6K inhibitor, TAS0612. TAS0612 would be efficacious for cancers in which deregulated RAS and PI3K pathway activities
with the RAS pathway. Further evaluations are currently ongoing to support clinical development.

**Conflict of interest:** Corporate-sponsored Research: Our work was sponsored by Taiho Pharmaceutical Co., Ltd. (All authors are employees of Taiho Pharmaceutical Co., Ltd.).

**431 (PB-094)**

**Poster**

**ET-D5, first-in-class synthetic Protein Phosphatase 1 (PP1) inhibitor for the treatment of aggressive tumors**

A. Juhe0,1, M. Pastor1, D. Lecerclè1, J. Deverchère1, B. Bestgen1, A. Popov1. 1Écrins Therapeutics, R&D, La Tronche, France

**Background:** ET-D5 is a new chemical entity and the first selective PP1 inhibitor to be evaluated in a clinical trial. ET-D5 offers a comprehensive anti-cancer treatment, as the inhibition of PP1 produces anti-proliferative and anti-vascular activities. PP1 is highlighted as a major protein in control of cell cycle progression and the overexpression of PP1 is positively correlated with bad prognosis in different tumors types, such as sarcomas or lung adenocarcinomas.

**Material and Methods:** ET-D5 was discovered in a phenotypic screening based on the cell cycle perturbation. After cell phenotype characterization, different methods such as affinity chromatography, immunoblotting and siRNA were used for target identification and validation. We then evaluated ET-D5 in vitro and in vivo cancer models and refined its original mechanism-of-action. To develop this new chemical entity as an oral drug, we realized a formulation screening, and GLP regulatory toxicology studies were performed in two animal species, rats and dogs. ET-D5 efficacy is currently being evaluated in a comparative veterinary oncology Phase 1/2a clinical trial in companion dogs suffering from spontaneous sarcomas.

**Results:** Targeting the PP1, ET-D5 showed a strong in vitro (in a panel of different cancer cell lines) and in vivo anti-cancer activity in several mouse xenograft models (lung, thyroid, kidney, sarcomas …). MRI studies and dorsal window chamber experiments in mice demonstrated the anti-vascular activity of ET-D5. MR imaging can be used as the efficacy clinical biomarker. Nano-suspension formulation allowed us to increase the oral bioavailability from less than 5% to over 70%. GLP toxicology studies showed an excellent toxicity and safety profile.

**Conclusions:** Efficacy data, coupled with the successful completion of the regulatory toxicology studies provide a strong and relevant background for the development of ET-D5 as a first-in-class small molecule drug candidate in advanced cancer patients.

**No conflict of interest**

**435 (PB-098)**

**Poster**

**A phase I dose escalation multi-centre study of crizotinib (MET inhibitor) combined with binimetinib (MEK inhibitor) in patients with advanced solid tumours**

R. Wilson1, P. Hari Dass2, S. Van Schaeybroeck3, E. Elez Fernandez3, R. Jones4, A. Quinton5, J. Houlden5, L. Collins5, C. Roberts6, S. Love7, M. Lawler8, D. Gi Nicolantonio8, M. Grayson8, A. Bardelli8, P. Laurent-Puig6, T. Maughan11, J. Tabernero12, M. Peeters13, M. Middleton12, C. Rolfo14. 1Queen’s University Belfast, Centre for Cancer Research and Cell Biology, Belfast, United Kingdom; 2Waikato Hospital, Department of Oncology, Hamilton, New Zealand; 3Vall d’Hebron University Hospital, Medical Oncology Department, Barcelona, Spain; 4Veinlid Cancer Centre and University of Cardiff, Department of Oncology, Cardiff, United Kingdom; 5University of Oxford, Oncology Clinical Trials Office OCTO, Oxford, United Kingdom; 6University of Oxford, Department of Clinical Statistical Medicine, Oxford, United Kingdom; 7University of Turin, Division of Molecular Genetics, Turin, Italy; 8Northern Ireland Cancer Research, Consumer Forum, Belfast, United Kingdom; 9University of Candidolo, Department of Oncology IRCSS, Candidolo, Italy; 10Paris Descartes University, Department of Oncology, Paris, France; 11University of Oxford, CRUK/MRC Oxford Institute for Radiation Oncology, Oxford, United Kingdom; 12Antwerp University Hospital, Department of Oncology, Antwerp, Belgium; 13Churchill Hospital, Cancer and Haematology Department, Oxford, United Kingdom; 14University of Maryland School Of Medicine, Marlene and Stewart Greenebaum Comprehensive Cancer Centre, Baltimore, USA

**Background:** Inhibition of nuclear EGFR localization as a breast cancer therapeutic (C. Wiersma1, J. Schroeder2. 1University of Arizona, Cancer Biology, Tucson, USA; 2University of Arizona, Molecular and Cellular Biology, Tucson, USA

**Abstracts, 30th EORTC-NCI-AACR Symposium**

**Poster Session (Friday, 16 November 2018)**

**Inhibition of nuclear EGFR localization as a breast cancer therapeutic**

**C. Wiersma1, J. Schroeder2. 1University of Arizona, Cancer Biology, Tucson, USA; 2University of Arizona, Molecular and Cellular Biology, Tucson, USA

**Epidermal growth factor receptor (EGFR), a receptor tyrosine kinase, is frequently overexpressed in invasive carcinoma including glioblastoma, head and neck, and breast cancer. The primary role of EGFR is to transduce signals from the cell surface when a ligand (e.g., epidermal growth factor (EGF)) binds, resulting in increased proliferation, survival and migration. Membrane localized EGFR can also retrotranslocate to the nucleus where it can function as a transcriptional cofactor and alter gene expression directly. We have previously demonstrated that this activity is dependent on the oncogene MUC1, which colocalizes with EGFR at the cell surface and drives its retrotranslocation. Nuclear EGFR can act as a co-transcriptional activator to such oncogenes as Cyclin D1, INOS, and COX2. We hypothesize that it may be driving an epigenetic program to promote transformation and metastasis. To test this hypothesis, we have developed a peptide-based therapeutic against the nuclear localization signal (NLS) of EGFR, named ENLS4. We have found that treatment of breast cancer cell lines blocks the nuclear translocation of EGFR, resulting in a reduction in cell survival. We are now performing ChIP-seq to identify DNA binding sites targeted by EGFR, proteomic analysis to determine the interactions between chromatin remodeling proteins and nuclear EGFR determining the mechanism of action of ENLS4. Together, we expect that characterizing EGFR genomic targets and inhibition of EGFR nuclear localization holds the potential for improved therapeutics.

**No conflict of interest**
outcome-recommended phase II dose and schedule (RP2D). PK and PD (skin +/- tumour biopsies) were analysed.

Results: Twenty patients (ECOG performance status 0–1) were recruited to the dose escalation part of this phase I trial (Table 1). Three patients withdrew early for reasons other than DLTs. Of the 8 Cohort A patients, 6 were evaluable and 2 patients experienced DLT (grade III transaminis and creatinine kinase [CK] elevation). We reduced to interval dose scheduling of binimetinib where 2 of 5 Cohort B patients (grade III transaminis and Grade IV CK elevation) had DLTs. In Cohort C with reduced crizotinib dosing, 1 of 7 patients (grade III fatigue) was identified as DLT. The MTD was thus achieved at Cohort C. The most common treatment-related adverse events were diarrhoea (11%), rash (9%), CK elevation (9%), fatigue (7%), and nausea (6%).

Conclusions: Crizotinib 250 mg OD continuously combined with binimetinib 30 mg BD at interval dosing (days 1–21 every 28 days) was the RP2D. The drug doses are pharmacologically active and the combination has a manageable safety profile. A dose expansion cohort of patients with RAS MT CRC has recently been recruited (n = 29) and efficacy results will be presented.


436 (PB-099) Poster
Association of oestrogen receptor β with inflammatory mediators in female patients with colorectal cancer

G. Topl,1 S.R. Satapathy,1 R. Ehrnstrom2, M.L. Lydrup3, A. Siölander4,1
1Lund University, Skane University Hospital, Department of Translational Medicine, Division of Cell Pathology, Malmo, Sweden; 2Lund University, Skane University Hospital, Department of Translational Medicine, Division of Pathology, Malmo, Sweden; 3Lund University, Skane University Hospital, Department of Clinical Sciences, Division of Surgery, Malmo, Sweden

Background: The estrogen receptor β (ERβ) is the predominant ER in the colon mucosa. We have previously reported that high expression of ERβ is independently associated with a better prognosis in female patients with colorectal cancer (CRC). On the other hand, β-catenin plays a major role in CRC development. In addition, inflammatory mediators, such as cyclooxygenase-2 (COX-2), cysteinyl leukotriene receptor 1 and 2 (CysLT1R, CysLT2R) and prostaglandin D2 (PGD2) have been associated with CRC. In this study we aimed to investigate the correlation between ERβ and inflammatory mediators in patients with CRC and cell line experiments.

Material and Methods: A tissue microarray (TMA) of primary CRCs from 320 female patients was stained with the following antibodies: anti-ERβ (1:100), anti-COX-2 (1:200), anti-CysLT1R (1:100), anti-CysLT2R (1:10), anti-β-catenin (1:200) and anti-PGD2 (1:25). Immunohistochemistry technique was used to evaluate the staining intensity. Two colon cancer cell lines were stimulated with 50 μM of ERβ agonist (ERβ-D4) for 48 h. The protein levels of interest inflammatory mediators were evaluated using the Western Blot method.

Results: Patients with high ERβ expression had significantly lower IRS for COX-2, CysLT1R and nuclear β-catenin expression and significantly higher IRS for CysLT2R, membrane β-catenin and PGD2 expression. ERβ was negatively correlated with COX-2 (r = −0.32; p < 0.0001), CysLT1R (r = −0.12; p = 0.03) and nuclear β-catenin (r = −0.28; p < 0.0001). ERβ was positively correlated with CysLT2R (r = 0.60; p < 0.0001), membrane β-catenin (r = 0.50; p < 0.0001) and PGD2 (r = 0.43; p < 0.0001) expression. Stimulation of colon cancer cell lines with ERβ agonist decreased the expression of CysLT1R and increased the expression of CysLT2R, which was stronger in the female colon cancer cell line HT-29.

Conclusion: High ERβ expression was significantly correlated with anti-proliferative and pro-apoptotic inflammatory mediators in female patients with CRC. These results strengthen our hypothesis for a beneficial role of ERβ in CRC patients. Further cell line experiments will be conducted to evaluate the cross talk between estrogen receptors and cysteinyli leukotriene receptors.

No conflict of interest

437 (PB-100) Poster
Identification of highly penetrant Rb-related synthetic lethal interactions in triple negative breast cancer

R. Brough1, A. Gutti3,1, S. Haider2, R. Kumar1, J. Campbell1, E. Knudsen2, S. Pettit5, C. Ryan1, C. Lord1, 1Institute of Cancer Research, Breast Cancer, London, United Kingdom; 2University of Arizona, Department of Medicine, Tucson, USA; 3University College Dublin, Systems Biology Ireland, Dublin, Ireland

Background: Although defects in the RB1 tumour suppressor are one of the more common driver alterations found in triple negative breast cancer (TNBC), therapeutic approaches that exploit this have not been identified. The availability of several, large-scale, shRNA and siRNA screens, conducted in multiple tumour cell lines, some of which are derived from TNBCs, now make it possible to predict highly penetrant RB1-related synthetic lethal effects.

Materials and Methods: We integrated molecular profiling data with data from multiple genetic perturbation screens and identified candidate synthetic lethal (SL) interactions associated with RB1 defects in TNBC. This was achieved using a refined data analysis pipeline that not only identified SL effects but also assessed their robustness or penetrance. We reasoned that highly penetrant synthetic lethal effects would be more robust in the face of molecular heterogeneity and would represent more promising therapeutic targets. Using protein-protein interaction and transcription factor binding data, we assessed the molecular connectivity of highly penetrant SL effects.

Results: A significant proportion of the highly penetrant RB1 SL effects involved proteins closely associated with RB1 function, suggesting that this might be a defining characteristic. These included nuclear pore complex components associated with the MAD2 spindle checkpoint protein, the kinase and bromodomain containing transcription factor TAF1, multiple TAF1 transcriptional targets and multiple components of the SCF^TOP Cul5 F box containing complex, including β-catenin and SKP2. Small molecule inhibition of SCF^TOP elicited an increase in p27^kip levels, providing a mechanistic rationale for RB1 SL. Transcript expression of SKP2, a SCF^TOP component, was elevated in RB1 defective TNBCs, suggesting that in these tumours, SKP2 activity might be a defining characteristic. These included nuclear pore complex components associated with the MAD2 spindle checkpoint protein, the kinase and bromodomain containing transcription factor TAF1, multiple TAF1 transcriptional targets and multiple components of the SCF^TOP Cul5 F box containing complex, including β-catenin and SKP2. Small molecule inhibition of SCF^TOP elicited an increase in p27^kip levels, providing a mechanistic rationale for RB1 SL. Transcript expression of SKP2, a SCF^TOP component, was elevated in RB1 defective TNBCs, suggesting that in these tumours, SKP2 activity might be a defining characteristic. These included nuclear pore complex components associated with the MAD2 spindle checkpoint protein, the kinase and bromodomain containing transcription factor TAF1, multiple TAF1 transcriptional targets and multiple components of the SCF^TOP Cul5 F box containing complex, including β-catenin and SKP2. Small molecule inhibition of SCF^TOP elicited an increase in p27^kip levels, providing a mechanistic rationale for RB1 SL. Transcript expression of SKP2, a SCF^TOP component, was elevated in RB1 defective TNBCs, suggesting that in these tumours, SKP2 activity might be a defining characteristic. These included nuclear pore complex components associated with the MAD2 spindle checkpoint protein, the kinase and bromodomain containing transcription factor TAF1, multiple TAF1 transcriptional targets and multiple components of the SCF^TOP Cul5 F box containing complex, including β-catenin and SKP2. Small molecule inhibition of SCF^TOP elicited an increase in p27^kip levels, providing a mechanistic rationale for RB1 SL. Transcript expression of SKP2, a SCF^TOP component, was elevated in RB1 defective TNBCs, suggesting that in these tumours, SKP2 activity might be a defining characteristic. These included nuclear pore complex components associated with the MAD2 spindle checkpoint protein, the kinase and bromodomain containing transcription factor TAF1, multiple TAF1 transcriptional targets and multiple components of the SCF^TOP Cul5 F box containing complex, including β-catenin and SKP2. Small molecule inhibition of SCF^TOP elicited an increase in p27^kip levels, providing a mechanistic rationale for RB1 SL. Transcript expression of SKP2, a SCF^TOP component, was elevated in RB1 defective TNBCs, suggesting that in these tumours, SKP2 activity might be a defining characteristic.
highly penetrant candidate Rb-related synthetic lethal effects. We identified a series of pharmacologically tractable effects, one of which, SKP2, we validated using both genetic and pharmacological methods. We also noted that a significant proportion of the highly penetrant Rb SL effects in TNBC involved proteins closely associated with Rb function, suggesting that this might be a defining characteristic. Importantly, this straightforward analysis pipeline can be applied to any gene of interest.

No conflict of interest

438 (PB-101) Poster
A phase I b study of the combination of MLN0128 (dual TORC1/2 inhibitor) and MLN8237 (Aurora A inhibitor, alisertib) in patients with advanced solid tumors, expansion cohort data
S.L. Davis, S. Leong, W. Messersmith, T. Purcell, E. Lam, B. Corr, A. Leal, C. O'bryan, A. Glode, N. Adler, N. Serkova, J. Diamond
1University of Colorado Cancer Center, Medical Oncology, Aurora, USA; 2University of Colorado Anschutz Medical Campus, Pharmacy, Aurora, USA; 3University of Colorado Anschutz Medical Campus, Radiology, Aurora, USA

Background: MLN0128 is an oral inhibitor of mTOR kinase and mTORC1/2 signaling. Alisertib is an oral inhibitor of Aurora A kinase. Senescence and up-regulation of genes in the PI3K/AKT/mTor pathway were observed in patient-derived xenograft models (PDX) treated with alisertib to resistance, and tumor growth inhibition was demonstrated in these models treated with MLN0128 in combination. An ongoing phase II clinical trial is evaluating the combination of MLN0128 and alisertib in patients with advanced solid tumors. Results of the dose escalation portion of this trial identifying the maximum tolerated dose (MTD) of the combination as MLN0128 2 mg daily on a continuous schedule and alisertib 30 mg BID days 1–7 of a 21-day cycle have been previously reported. Presented here are updated results from the dose expansion cohort of this trial.

Methods: Patients with advanced solid tumors, refractory to standard therapy, were treated with the combination of MLN0128 and alisertib at the MTD. In Cycle 1, patients were assigned to treatment with either MLN0128 or alisertib as a single-agent on days 1–7. For the remainder of the study, patients received combination treatment according to the MTD. Biopsies were performed in all patients prior to treatment initiation, Cycle 1 Day 7 (after single-agent lead-in), and Cycle 2 Day 7 (after combination treatment). Diffusion weighted MRI (DWI-MRI) was performed in a subset of patients with hepatic metastases at these time points, with assessment of apparent diffusion coefficient (ADC) as a potential biomarker of early treatment response. For a subset of patients who underwent baseline FDG-PET/CT for evaluation of disease, optional repeat assessment was performed Cycle 2 Day 7.

Results: Twenty patients with refractory cancers were treated in the dose expansion portion of this trial, with treatment ongoing in two patients. Represented tumor types include breast (9), colorectal (9), pancreatic (3), ovarian (2), renal cell (1), and uterine carcinoma (1). Median time on study was 2.5 cycles (range 1–15). No complete or partial responses were documented. Stable disease was observed in four patients (20%), including prolonged stable disease (15 cycles) in one patient with pancreatic cancer. The ADC of five patients who underwent DWI-MRI has been evaluated, with three patients with increased ADC values observed at Cycle 1 Day 7 noted to have decreased lesion size on Cycle 2 Day 7 imaging. Further assessment of this and additional correlatives is ongoing, with an up-date to be reported at the meeting.

Conclusions: In an expansion cohort of patients treated with the combination of MLN0128 and alisertib at the previously defined MTD, prolonged stable disease was observed in a patient with pancreatic cancer. Further expansion in a cohort of patients with refractory pancreatic adenocarcinoma is planned.

Conflict of interest: Other Substantive Relationships: Diamond: Grant funding for clinical trial, travel expenses for investigator meeting.

439 (PB-102) Poster
Targeting the PI3K pathway in HER2-positive gastric cancer
S. Toomey, A.M. Farrelly, M.J. Mezynski, A. Carr, P. Armstrong, C. Westmose Yde, M. Mau-Soerensen
1Royal College of Surgeons in Ireland, Molecular Medicine, Dublin, Ireland; 2Royal College of Surgeons in Ireland, Pathology, Dublin, Ireland; 3St. James’s Hospital, Medical Oncology, Dublin, Ireland

Background: HER2 is overexpressed in about 25% of gastric cancers and is correlated with more advanced tumours. PI3K pathway activating mutations have been reported in gastric cancer, however neither the association of HER2 overexpression with these mutations or the impact of the PI3K pathway signalling on trastuzumab responsiveness has been described. In HER2-positive breast cancer, targeting the PI3K pathway can overcome resistance to HER2-targeted therapies; however the role of PI3K inhibitors in sensitizing HER2-positive gastric cancers to trastuzumab or in overcoming trastuzumab resistance has yet to be addressed.

Materials and Methods: Gastric tumour samples were collected from 69 patients and stratified into HER2-positive and HER2-negative groups. Mass spectrometry-based genotyping (Agena Bioscience) was performed on 105 hotspot, non-synonymous somatic mutations in PIK3CA, EGFR, ERBB2, ERBB3 and ERBB4 in the tumours. A panel of HER2-positive gastric cell lines (N87, OE19, ES026 and SNU16) was profiled for the same mutations and their anti-proliferative response to the PI3K inhibitor copanlisib alone and in combination with the HER2-targeted therapies trastuzumab and lapatinib was assessed.

Results: Patients with HER2-positive gastric cancer had significantly poorer overall survival compared to HER2-negative patients (15.9 months vs. 35.7 months). EGFR and ERBB family mutations occurred more frequently in HER2-negative than HER2-positive tumours. Mutations in PIK3CA or ERBB family genes did not have any effect on either progression free or overall survival. Mutations in PIK3CA (Q546H) and ERBB4 (M772L) were identified in ES026 cells, while a mutation in ERBB2 (F254L) was identified in N87 cells. OE19 cells were resistant to copanlisib, while all other cell lines were sensitive, with IC50s ranging from 23.4 nm (N87) to 93.8 nm (SNU16). PIK3CA mutation status had no impact on copanlisib sensitivity. All cell lines except SNU16 were sensitive to lapatinib with IC50s ranging from 0.04 mg/ml to 0.15 mg/ml and SNU16 were resistant to trastuzumab. The combination of lapatinib and copanlisib is synergistic in ESO-26 and OE-19 cells (ED50 = 0.8 ± 0.19 and 0.88 ± 0.13, respectively) and additive in NC-N87 cells (ED50 = 1.01 ± 0.55). The combination of copanlisib and trastuzumab significantly improved growth inhibition compared to either therapy alone in N87, ES026 and OE19 cells (p < 0.05).

Conclusions: Copanlisib is an effective monotherapy in some HER2-positive gastric cancer cell lines. Combinations of copanlisib and trastuzu-

440 (PB-103) Poster
Mutational landscape assessed in tumor tissue and cell-free DNA during neratinib treatment of patients with HER2 mutated solid tumours
1Rigshospitalet, The Phase 1 Unit, Department of Oncology, Copenhagen, Denmark; 2Rigshospitalet, Center for Genomic Medicine, Copenhagen, Denmark; 3Rigshospitalet, Department of Pathology, Copenhagen, Denmark

Background: The pan-HER kinase inhibitor neratinib has shown clinical activity in a range of solid tumours, harboring activating HER2 mutations. However, anti-tumor activity varies between tumor types and different mutant variants. Here, we explore the dynamic changes in the mutational landscape of cell-free DNA (cfDNA) in serial blood samples and in pre- and on-treatment biopsies in patients with HER2 mutated cancers treated with neratinib.

Materials and Methods: Patients with refractory solid tumours were biopsied for whole exome sequencing as part of the Copenhagen Prospective Personalized Oncology (CoPPO) program to identify actionable driver mutations. Patients with activating HER2 mutations were offered treatment with neratinib as monotherapy or in combination with trastuzumab/ fulvestrant either in a basket trial or in a patient named program. Repeated plasma sampling for cfDNA analysis was performed to assess the dynamics of the variant allele frequency (VAF) of activating HER2 mutations during therapy. When possible archival, on-treatment and/or post-progression biopsies were collected for comprehensive genomic analysis.

Results: From May 2013 to June 2018, fourteen of 705 patients included in CoPPO were identified as having HER2 mutations. Eight patients received neratinib either as monotherapy or in combination with trastuzumab (and fulvestrant in cases of estrogen receptor positive breast cancer). One patient received epirubicin+trastuzumab and one patient received copanlisib as monotherapy. Three patients received no additional therapy due to rapid decline in performance status. Partial response was observed in 2/8 patients receiving neratinib, 3/8 had stable disease and 2 patients had progressive disease. One patient is awaiting first evaluation. In general, a decline in

Poster Session (Friday, 16 November 2018)
HER2 VAF was observed upon initiation of neratinib treatment. In case of progression, an increase in VAF was observed prior to radiological detection of cancer metastases, supporting potential utility of cfDNA analysis for tracking the clinical response of neratinib treatment. Analysis of co-mutated genes and emergence of possible acquired resistance mechanisms to neratinib is ongoing.

Conclusions: The results support the use of a neratinib-containing regimen in patients with somatic HER2 mutations. Analysis of HER2 mutation VAF in cfDNA from repeated plasma sampling during treatment shows promise for real-time monitoring of clinical response to HER2 targeting therapy.

Conflict of interest: Corporate-sponsored Research: Dr. Morten Mau-Soeensen has received a research grant from Puma Biotechnology. All other authors declare no potential conflicts of interest.

441 (PB-104) Poster
SEL120 – 34A, a potent and specific inhibitor of CDK8, as a potential treatment of acute myeloid and lymphoblastic leukemias
M. Mazan1, E. Majewska1, M. Mikula1, K. Wiklick1, M. Combik2, A. Golas3, M. Masiejczyk1, E. Fliedor1, F. Malusa1, A. Polak1, A. Paziewska1, M. Dąbrowska3, A. Białas3, K. Michaelik1, R. Windak1, J. Ostrowski1, P. Juszczynski1, K. Brzózka2, T. Rzymski2, Selvita S. A., R&D Biology, Krakow, Poland; Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Department of Genetics, Warsaw, Poland; Selvita S. A., Computational Drug Discovery, Krakow, Poland; Institute of Hematology and Transfusion Medicine, Department of Experimental Hematology, Warsaw, Poland; Medical Center for Postgraduate Education, Department of Gastroenterology, Hepatology and Clinical Oncology, Warsaw, Poland; Selvita S. A., Discovery Chemistry, Krakow, Poland; Selvita S. A., Clinical & Preclinical Operations, Krakow, Poland; Selvita S. A., Research & Development, Krakow, Poland

Background: Cyclin-dependent kinase 8 inhibitors (CDK8i) have anti-cancer activity in human acute myeloid leukemia (AML) cell lines that has been demonstrated both in vitro and in vivo. Inhibition of CDK8, Mediator-associated kinase, induces upregulation of Super-enhancer-associated genes in sensitive AML cell lines. Our preliminary results indicate also effectiveness of CDK8i in T-cell acute lymphoblastic leukemia (T-ALL) cell lines, however the exact mechanism of action here remains unknown. Due to poor outcome of current treatment in adult T-ALL, development of new therapies is urgently needed.

Material and Methods: Efficacy of the compound alone or in combination was tested in viability assays in a broad panel of cancer cell lines. Activity and mechanism of action of CDK8 inhibitor – SEL120-34A alone and in combination with other compounds was investigated by flow cytometry, western blotting and differential gene expression analysis. In vivo efficacy was tested in mice injected with leukemia cell lines. Sensitive and resistant cell lines panel was used for bioinformatic analysis of specific signatures.

Results: SEL120-34A showed differential activity in selected AML and T-ALL cells. We observed robust and dose-dependent inhibition of STAT5 phosphorylation on serine 726 (STAT5 pS726) in sensitive cell lines. Moreover, basal level of STAT5 pS726 in AML cell lines correlated with sensitivity to treatment with our compound. Differential gene expression analysis between sensitive and resistant leukemia cell lines demonstrated leukemia stem cell (LSC) – like signature present in responding cells that has been linked to resistance to standard therapies and relapsed disease. These findings were further corroborated by the presence of surface LSC markers in sensitive cells. Further comparison of transcriptomic changes upon treatment with SEL120-34A revealed changes in genes regulating differentiation and apoptosis. Prolonged treatment with SEL120-34A induced lineage commitment markers followed by reduced viability. We observed synergistic effects of SEL120-34A in several AML cell lines in combination with standard of care compounds like cytarabine and azacitidine and late phase experimental therapies effective in AML such as ABT-199.

Conclusions: SEL120-34A effectively inhibits phosphorylation of STAT proteins and induce CDK8-specific transcriptomic changes not only in previously demonstrated AML cell lines, but also in T-ALL models. Differential activity on LSC-like cells and synergistic activity with standard of care therapies warrants further clinical development, particularly in relapsed and refractory leukemia.

No conflict of interest

442 (PB-105) Poster
Miransertib and ARQ751 exhibit superior cell-death-inducing properties compared to other AKT inhibitors, and can overcome resistance to other allosteric AKT inhibitors
E. Kostaras1, P. Cutillas2, Y. Yu3, R. Savage4, B. Schwartz5, L. Vivanco1
1The Institute of Cancer Research, Cancer Therapeutics, London, United Kingdom; 2Barts Cancer Institute, Centre for Haematology- Oncology, London, United Kingdom; 3Arque, Inc., Translational Research, Burlington, USA; 4Arque, Inc., Preclinical Development & Clinical Pharmacology, Burlington, USA

Background: The serine/threonine kinase AKT is a critical effector of the PI3K signalling pathway, and is an attractive therapeutic target because of its frequent activation in human tumours and its role in the regulation of several hallmarks of cancer. As such, there has been significant effort in developing AKT inhibitors as cancer therapeutics. However, the clinical success of these compounds as single agents has so far been disappointing. Existing clinical AKT inhibitors can be classified, based on their mode of inhibition, into either ATP-competitive or allosteric. Recent data from our laboratory suggest that the latter may be able to inhibit both catalytic as well as non-catalytic activities, which we have also shown to regulate cancer cell survival. Miransertib (ARQ092) and ARQ751 are two novel allosteric and selective inhibitors of AKT1/2/3 that have shown promising anti-proliferative activity in pre-clinical models.

Methods: We used miransertib, ARQ751, MK2206, GSK690693, and ipatasertib to treat breast cancer (MDA-MB-361, T47D, ZR75.1) and lung cancer cell lines (EBC1). Phosphoproteomic analysis was done using LC-MS.

Results: We have evaluated the cell-death inducing properties of these compounds in cell lines carrying activating PIK3CA mutations or MET gene amplification. Both compounds were highly active, and exhibited superior activity compared to MK2206, another allosteric AKT inhibitor, and two distinct ATP-competitive inhibitors. Interestingly, we have found that cell lines that have been made resistant to MK2206 through either chronic drug exposure or ectopic expression of an MK2206-binding-deficient AKT allele (W80A) remain sensitive to miransertib and ARQ751.

We found that the disappointing clinical performance of AKT inhibitors as single agents, the therapeutic focus has now shifted towards the use of AKT inhibitors in combination regimens. To identify potential new co-targets for AKT, we used phosphoproteomic analysis using cell lines treated with a variety of AKT inhibitors. Allosteric and ATP-competitive inhibitors generated similar phosphopeptide signatures that were distinct from those generated by allosteric inhibitors. Through kinase substrate enrichment analysis (KSEA), we found that AKT inhibition was associated with activation of kinases in cancer repair pathways, and that combined inhibition of AKT and ATM was synergistic.

Conclusions: Our data suggest that Miransertib and ARQ751 have significant single agent activity AKT-dependent cell lines, and are superior to other AKT-targeting agents currently in clinical development. We also find that these compounds might be able to overcome at least some mechanisms of resistance to other AKT inhibitors. Finally, our data suggest that combined inhibition of AKT and ATM might be a rational therapeutic strategy to maximize therapeutic benefit.

Conflict of interest: Corporate-sponsored Research: Miransertib and ARQ751 were provided by Arque, Inc. Funds for attendance to this conference were also provided by Arque, Inc.

443 (PB-106) Poster
Harnessing the anticancer activity of the stapled peptide ALRN-6924, a dual inhibitor of MDMX and MDM2, using rational combination strategies for breast cancer and other malignancies
A. Annis1, J.G. Ren1, L.A. Carvajal1, S. Santiago2, N. Narasimhan3, D. Sutton1, S.S. Pairawan2, V. Guerlavais1, F. Meric-Bernstam2, M. Alvado1, Aileron Therapeutics Inc., Research, Cambridge, USA; 2MD Anderson, Investigational Cancer Therapeutics, Houston, USA

Background: The purpose was to identify rational anticancer drug combinations with ALRN-6924.

Material and Methods: ALRN-6924 is a cell-penetrating stapled α-helical peptide that disrupts the interaction of the p53 tumor suppressor protein and its endogenous inhibitors, MDMX and MDM2. For TP53 wild-type (WT) tumors, this can restore p53-dependent cell cycle arrest and apoptosis leading to antitumor efficacy. ALRN-6924 was tested in combination with 29 drugs for synergistic in vitro anticancer activity. Select agents were further evaluated in vivo. Drugs listed in Table 1 were assayed in combination with ALRN-6924 using WST-1 and/or CyQUANT cell viability assays in
Comprehensively characterize RSP03 fusion driven cancers in order to gain insights to potential therapeutic biomarkers.

**Material and Methods:** Tumor DNA sequencing of 592 genes (NextSeq, Illumina, San Diego, CA) and RNA sequencing of 53 gene fusions ( ArcherDX FusionPlex Assay, ArcherDX, Boulder, CO) were used along with immunohistochemistry for PD-L1 expression in the tumor cells (SP142 antibody, Ventana Medical Systems, Tucson, AZ).

**Results:** Forty-one RSP03 fusion-positive cases were identified and included 36 cases arising in colon/rectum (CRC), 4 in small intestine and 1 esophageal. Only PTPTRR–RSP03 fusions were detected. The most common additional somatic alteration was a pathogenic TP53 mutation (32/41 cases); all TP53 mutated cases had additional mutations in KRAS, NRAS or BRAF. Unexpectedly, two pathogenic APC gene mutations (p.S1460fs, p.E1097X) were detected in one case (CRC liver metastasis). Although the average mutational burden was low for the cohort (average = 8.7/Mb, range 4–16/Mb), 13 cases exhibited >10 mutations/Mb.

**Conclusions:** The majority of gastrointestinal adenocarcinomas driven by an RSP03 fusion exhibit additional pathogenic mutations in genes that include TP53, KRAS, NRAS and BRAF. APC may also rarely be mutated. Immune checkpoint inhibition may be an option for rare cases with PD-L1 overexpression and high tumor mutational burden.

**Conflict of Interest:** Ownership: Caris Life Sciences. OncoMed Pharmaceuticals.

---

**Table 1**

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Cell line</th>
<th>Drug</th>
<th>CIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>MCF-7</td>
<td>palbociclib</td>
<td>0.45 ± 0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>abemaciclib</td>
<td>0.57 ± 0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ribociclib</td>
<td>0.39 ± 0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>paclitaxel</td>
<td>0.80 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>docetaxel</td>
<td>0.37 ± 0.02</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>HCT-116</td>
<td>S-FU</td>
<td>0.07 ± 0.03</td>
</tr>
<tr>
<td>Melanoma</td>
<td>A375</td>
<td>trametinib</td>
<td>0.41 ± 0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dabrafenib</td>
<td>0.33 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>MEL-JUSO</td>
<td>binimetinib</td>
<td>0.32 ± 0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pimatinib</td>
<td>0.39 ± 0.08</td>
</tr>
<tr>
<td>T cell Lymphoma</td>
<td>MOLT-3</td>
<td>romidepsin</td>
<td>0.38 ± 0.00</td>
</tr>
<tr>
<td>B cell Lymphoma</td>
<td>DOHH-2</td>
<td>vincristine</td>
<td>0.09 ± 0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cyclophosphamide</td>
<td>0.11 ± 0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rituximab</td>
<td>0.40 ± 0.07</td>
</tr>
<tr>
<td>Acute Leukemia</td>
<td>MV-4-11</td>
<td>ibritinib</td>
<td>0.59 ± 0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cytarabine-Ara-C</td>
<td>0.68 ± 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>azacitidine</td>
<td>0.49 ± 0.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>decitabine</td>
<td>1.08 ± 0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>midostaurin</td>
<td>0.93 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>venetoclax</td>
<td>0.20 ± 0.03</td>
</tr>
</tbody>
</table>

* In vitro CI value at IC50 or IC100, average ± SD of ≥2 experiments. Values <0.9 are synergistic, 0.9–1.1 additive, >1.1 antagonistic. 1 Single experiment.
changes in the percentage and number of different T-cell subsets and suppressor cells after the administration of tarzoxitinib. Further studies showed increased antigen-release to the lymph nodes, increased T-cell proliferation in the spleen and changes in cytokine production profiles after tarzoxitinib administration.

Conclusion: Tarzoxitinib can be combined effectively with anti-PD1 or anti-CTLA4 to greatly improve their anti-tumour activity in vivo.

Conflict of interest: Ownership: Rain Therapeutics Inc holds worldwide development rights to tarzoxitinib. Advisory Board: Dr Adam Patterson is a member of the Scientific Advisory Board of Rain Therapeutics Inc. Dr Jeff Smail is a member of the Scientific Advisory Board at Rain Therapeutics Inc. Board of Directors: No conflicts. Corporate-sponsored Research: No conflicts. Other Substantive Relationships: No conflicts.

Study goals were to determine expression of TSC1 in esophageal cancer samples and to explore the interaction between miR-141-3p and TSC1, and characterize the functional implications of this interaction.

Background: MIR-141-3p has been shown to be oncocogenic in some malignancies, while functioning as a tumor suppressor in others. Its role in esophageal cancer is not well defined. Our prior studies show marked upregulation of mir-141-3p in the esophageal cancer lines TE7 and TE10 compared to esophageal epithelial cells. MIR-target sequence analysis predicts miR-141-3p to bind TSC1 mRNA with high affinity. TSC1 has been demonstrated to promote autophagy and function as a tumor suppressor. Study goals were to determine expression of TSC1 in esophageal cancer cells, explore the interaction between miR-141-3p and TSC1, and characterize the functional implications of this interaction.

Methods: Studies were performed in human esophageal cancer lines TE7, TE10, OE33, and FLO-1. MiR-141-3p and TSC1 mRNA expression levels were measured by real-time PCR. TSC1 protein expression levels were examined by Western blot. MiR-141-3p function was tested by its overexpression and silencing. Nascent protein synthesis was analyzed using biotinylated RNA pull-down followed by digital droplet PCR to quantify newly synthesized protein. MiR-141-3p function was tested by its overexpression and silencing. Nascent protein synthesis was analyzed using biotinylated RNA pull-down followed by digital droplet PCR to quantify newly synthesized protein.

Results: MiR-141-3p levels are markedly increased in TE7, TE10, and OE33 esophageal cancer cells compared to FLO-1 cells. TSC1 protein expression is reduced in these cells compared to FLO-1 cells. Silencing mirR-141-3p in TE7 and OE33 esophageal cancer cells reduced TSC1 protein levels, with no change in TSC1 mRNA levels. In reciprocal experiments, TSC1 protein expression levels decreased following miR-141-3p overexpression in FLO-1 cells, with no change in TSC1 mRNA levels. TSC1 mRNA translation decreased after ectopic expression of miR-141-3p in FLO1 cells. MiR-141-3p and TSC1 mRNA interaction was confirmed by enrichment of biotinylated TSC1 mRNA levels and with luciferase reporter constructs. Inhibition of miR-141-3p in TE7 cells led to increased autophagy, with a rise in LC3A/B expression and decreased SQSTM1 (p62) expression, confirmed by live cell fluorescence assay. Finally, silencing miR-141-3p resulted decreased migration and invasiveness in TE7 cells.

Conclusions: Esophageal cancer cell line subsets exhibit differential expression of miR-141-3p. MiR-141-3p binds TSC1 mRNA, resulting in decreased TSC1 translation and protein expression. In cells exhibiting elevated miR-141-3p expression, silencing miR-141-3p results in increased TSC1 protein expression, leading to up-regulation of autophagy and reduction in migration and invasion.

No conflict of interest

Poster Session (Friday, 16 November 2018)

European Journal of Cancer 103S1 (2018) e21–e148
e145

Friday, 16 November 2018

POSTER SESSION

Paediatric Oncology

447 (PB-110) Poster

Anaplastic Lymphoma Kinase as an ADC target for the treatment of neuroblastoma

S. Leonard1, R. Forfar1, D. Moore1, S. Large2, C. Fernando3, R. Brown3, C. Southern1, D. Matthews3, L. Friis4, D. Taylor1. LifeArc, Biology, Stevenage, United Kingdom; LifeArc, Chemistry, Stevenage, United Kingdom; BioTherapeutics, Stevenage, United Kingdom

Anaplastic Lymphoma Kinase (ALK) is a recognised neuroblastoma tumour antigen associated with the failure of developing neural crest tissue to completely differentiate. While ALK tyrosine kinase inhibitors have been successfully employed for the treatment of ALK-rearranged tumours, drug-resistance can limit use. Novel methods are therefore urgently needed to treat this rare paediatric cancer. As ALK is aberrantly expressed on neuroblastoma tumours and largely undetectable in normal tissue, an antibody drug conjugate (ADC) targeting this receptor may be a promising approach.

Through an immunisation campaign we identified a panel of monoclonal antibodies that can bind to immobilised antigen with high affinity. However, ALK proved to be a challenging target, particularly if it undergoes proteolytic cleavage at a major site in the extracellular domain where some candidates bind. Nevertheless, we have identified a lead antibody which effectively kills neuroblastoma cell lines when conjugated to a cytotoxic payload, progressing the development of an innovative therapeutic for this devastating disease.

No conflict of interest

448 (PB-111) Poster

Transcriptome analysis in childhood medulloblastoma identifies novel transcripts associated with survival

M. Mateos1, D. Williamson1, S. Bailey1, S.C. Clifford1. Wolfson Childhood Cancer Research Centre, Northern Institute for Cancer Research, Newcastle upon Tyne, United Kingdom

Background: Medulloblastoma is the commonest malignant brain tumour in childhood, with a progression-free survival (PFS) <45% for children with high-risk (HR) features. We sought to evaluate known (associated with curated genes) and novel transcripts in primary medulloblastoma samples, to identify prognostic biomarkers that outperformed current clinical risk-stratification schemes.

Materials and Methods: Our cohort consisted of 217 patients with clinical and RNA sequencing data (Illumina, 90 million paired-end reads). Functional and pathway analysis of transcripts significantly associated with overall survival (OS) and progression in univariate analysis was conducted. Survival analyses were performed for children aged 3–16 years, treated with curative intent. Multivariable survival analyses were adjusted for risk criteria (large cell anaplasia, metastases, MYC/MYCN amplification, resection, sex, molecular subgroup, TP53 status, q13 loss) for (i) overall cohort (n = 145) (ii) Group3/Group4 (G3G4) (n = 95). Statistical analyses were performed in R, for categorical (<median, ≥median) and continuous expression data, and were corrected for multiple testing.

Results: The median age of the cohort was 7.5 years (range 0.24–43 years). From total >60,000 known transcripts, 9693 known transcripts that met criteria related to variability of expression were further assessed. Transcripts (n = 547) associated with OS in univariate cox regression were significantly enriched in cell junction, membrane and oxidoreductase functions. Transcripts associated with PFS in G34 showed enrichment in metabolic pathways (putrescine biosynthesis P = 4.01E−03, 4-hydroxyproline degradation P = 6E−03). Top pathways included the targetable P38 MAPK – ERK1/2 pathway (featuring NQO2).

Three transcripts were independently significant for OS for the entire cohort (categorical data). CXXC4 is a negative regulator of the canonical WNT/beta-catenin signalling pathway and alters DNA methylation. For G34, multivariable PFS analysis (adjusting for G34 HR status, MYC, q13 loss, sex), categorical and continuous expression data revealed four and ten transcripts respectively that were independently prognostic. These included transcripts related to oxidoreductases (NQO2, SELENOW), neuronal toxicity (STKL) and an oncogenic kinase (MELK).

Abstracts, 30th EORTC-NCI-AACR Symposium
From 3150 novel transcripts, 217 were further assessed. Within G34G, there were two novel transcripts that were independently significant in each of OS (continuous), PFS (continuous) and PFS (categorical expression).

**Conclusion:** This study demonstrates that the transcriptome provides additional prognostic information in childhood medulloblastoma, beyond known clinicopathological risk factors. The next step is to validate these markers in further cohorts and to exploit their use as biomarkers and/or therapeutic targets, particularly in G34G medulloblastoma.

**No conflict of interest**

**449 (PB-112)**

*Poster*

**Pharmacological monitoring of asparaginase (ASP) activity during Erwinia C. Asparaginase (ERW-ASP) treatment in pediatric patients with acute lymphoblastic leukemia (ALL), after an hypersensitivity reaction (HSR) to E. Coli PEG Asparaginase (PEG-ASP)**

M. Zucchetti1, T. Ceruti1, C. Matteo1, A. Colombini2, D. Silvestri2, V. Conti2, F. Falcetti1, L. Lo Nigro1, C. Micalizzi2, R.M. Mura2, F. Petruzziello1, M. Lebù1, E. Brivio1, E. Barisone3, M. Rabusin3, L. Vinti10, M. Uggeri1, M.G. Valsecchi1, C. Rizzani1. 1IRCCS – Istituto di Ricerche Farmacologiche Mario Negri, Oncology, Milano, Italy; 2Clinica Pediatrica Universita di Milano-Boicca, Ospedale S. Gerardo, Pediatra, Monza, Italy; 3 Università di Milano-Bicocca, Health Science, Monza, Italy; 4 Ospedale ADU Policlinico Vittorio Emanuele, UOC Ematologia Oncologia Pediatrica/TMO, Catania, Italy; 5 IRCCS-Istituto G. Gaslini, Dipartimento Funzionale Ematologia, Genova, Italy; 6 Azienda Ospedaliera Brotzu, SC Oncologia Pediatrica e Patologia della Coagulazione, Cagliari, Italy; 7 AORN Sanitobono-Fausipilion, Oncologia Pediatrica, Napoli, Italy; 8 ACU Presidio Istituto Regina Margherita, Oncematologia Pediatrica e CTO, Torino, Italy; 9 IRCCS Burlo Garofolo, SC Emasto Oncologia Pediatrica, Trieste, Italy; 10 Ospedale Bambin Gesù, Ematologia Oncologia e medicina Transfusionale, Roma, Italy

**Background:** PEG-ASP is a cornerstone drug in the treatment of childhood ALL. However, 2–18% of the patients cannot successfully complete the PEG-ASP planned treatment due to the occurrence of a clinically evident HSR. ERW-ASP is an alternative formulation of ASP which is given to patients as a single dose of PEG or SI in order to maintain the ASP plasma activity >100 IU/L (target value). A pharmacological monitoring of the ERW-ASP activity is performed in the patients enrolled in the AIEOP-BFM ALL protocols. The preliminary results of this study are here reported.

**Patients and Methods:** Forty-six patients received seven doses of ERW-ASP (20,000 IU/m2 i.v. in 2 hours) every 48/72 hours to replace a single dose of PEG-ASP (2,500 IU/m2) after an HSR. We used an enzymatic test (MAAT test, MEDAC) to evaluate the ERW-ASP serum activity in affected patients. Serum samples were classified in three different classes according to their ASP activity values: optimal (ASP activity ≥100 IU/L), border-line (ASP activity 50–100 IU/L) and inadequate (ASP activity <50 IU/L). We also compared the serum ASP activity in samples taken at 48 hours or 72 hours post ERW-ASP dose.

**Results:** The ASP activity levels were analyzed in 331 samples of 46 patients who were characterized by a marked inter-patient and intra-patient variability. It was optimal in the 53%, borderline in 21% and inadequate in 26% of samples. In particular, ASP activity measured 48-hours post-dose was ≥100 IU/L in 62% (123/199; mean 335 ± 283 IU/L), between 50 and 100 IU/L in 24% (79 ± 13 IU/L), and <50 IU/L in 14% (29 ± 19 IU/L) of the samples, respectively. Conversely the asparaginase activity detected in the samples withdrawn 72 hours after ERW-ASP administration were inadequate in 16% only of samples, while it was inadequate in the vast majority of these.

**Conclusions:** ERW-ASP, administered instead of PEG-ASP in patients showing HSR, determines levels of adequate or border-line enzymatic activity in 86% of the samples when administered every 48 hours according to the timing suggested by the treatment protocol. Conversely, inadequate activity was detected 72-hours post-dose in 6% of the samples analyzed. These results highlight the need to follow more stringently the indication of administer ERW-ASP every 48 hours to ensure a better exposure of patients to the drug and therefore a continuous elimination of asparaginase at systemic level.

**No conflict of interest**

**450 (PB-113)**

*Poster*

**RNA sequencing identifies differences in immune profiles of tumors from pediatric neuroblastoma patients with Ospoclonus myoclonus syndrome**

M. Buchkovich1, W. Jones2, V. Weigman1, M. Rosenberg3, J. Maris1. 1 Q2 Solutions – EA Genomics, Translational Genomics, Morrisville, USA; 2 Q2 Solutions – EA Genomics, Bioinformatics, Morrisville, USA; 3 Hebrew University of Jerusalem, Alexander Silberman Institute of Life Sciences, Jerusalem, Israel; 4 Children’s Hospital of Philadelphia, Department of Pediatrics, Oncology Division, Philadelphia, USA

**Background:** Pediatric Ospoclonus myoclonus syndrome (OMS) is a rare, paraneoplastic neurological disorder that presents in a small portion of pediatric neuroblastoma (NBL) cases, OMS causes ataxia, myoclonic jerks, opsoclonus, and disordered mood. Patients with OMS and NBL have improved tumor outcomes compared to children with NBL alone. Little is known about the molecular basis of OMS disease or the relationship between OMS disease and NBL restriction. Understanding this disorder may improve disease monitoring, offer prognostic indicators for OMS, and lead to improved immune therapy design for NBL. We used RNA sequencing (RNA-seq) data to identity HLA types and examine immune signatures in pediatric NBL tumors to better understand differences between patients with and without OMS.

**Materials and Methods:** We sequenced RNA from 38 patients with OMS from the Children’s Oncology Group ANBL003 FMS OMS clinical trial, as well as 6 high risk (HR) and 5 low risk (LR) NBL patients without OMS. We identified HLA types in these data as well as RNA-seq data from 164 NBL patients previously sequenced by the NCI TARGET initiative. Using Fisher’s exact tests, we tested for associations of HLA alleles with OMS.

**Results:** We aligned sequence reads from the 38 OMS and 10 non-OMS samples, and compared gene counts to the Immune Landscape Signature (ILS) Portfolio. ILS contains 11 distinct but related groups of genes with continuous scores reflecting activity of immune system subcomponents in the tumor microenvironment. The signatures were derived initially from Newman et al. (2015), refined using TCGA tumor expression profiles, and then validated for their predictive and prognostic value in multiple tumor cohorts and cancer types. We used H-tests to identify significant differences between ILS scores of OMS and non-OMS tumors.

**Conflict of interest:** Advisory Board: V. Weigman is on the advisory board for Illumina.

**451 (PB-114)**

*Poster*

**The XPO1 inhibitor selinexor reverses aberrant NF-κB pathway activation in RELA-fusion positive ependymoma cells**

M. Connelly1, M. Mellado-Lagarde1, A. Shelat1. 1 St Jude Children’s Research Hospital, Chemical Biology and Therapeutics, Memphis, TN, USA

**Background:** Supratentorial RELA-fusion positive ependymoma (ST-EPN-RELA) is an aggressive pediatric brain tumor that is marked by the presence of a gene fusion involving RELA, the principal effector of canonical NF-κB signaling, and C11orf95, a less studied gene of unknown function. The translocation induces constitutive, aberrant NF-κB pathway activation, resulting in the increased expression of MDM2 and pro-survival members of the Bcl-2 family, and subsequent p53 inactivation and resistance to apoptosis. Exportin 1 (XPO1/CRM1) is the sole nuclear exporter of over 200 protein cargos bearing leucine-rich nuclear export sequences, including major tumor suppressor proteins. Recent reports suggest that the CNS...
characterization of the genetic landscape of low mutation burden malignancies through multi-parallel genomic and immunologic analysis

T. Trippett 1, M. Berger 2, A. Narendran 3, D. Lyden 4, O. Kovalchuk 5, T. Frawley 1, R. Conlon 1, C. Curtin 2, F. O’brien 2, R. Stallings 1, M. Berger 2, A. Narendran 3, D. Lyden 4, O. Kovalchuk 5, T. Frawley 1, R. Conlon 1, C. Curtin 2, F. O’brien 2, R. Stallings 1.

Background: A comprehensive simultaneous approach encompassing genomic sequencing, whole exome, exosomal, methylation, transcriptome and proteomic analyses coupled with immunogenic profiling has the potential to unravel the genetic landscape in pediatric tumors and inform insightful therapeutic strategies for children.

Materials and Methods: Biospecimens obtained from 41 patients with low mutation pediatric solid tumor malignancies were analyzed using comprehensive, simultaneous multi-parallel molecular analysis of the genomic landscape. The types of pediatric malignancies included osteosarcoma, rhabdomyosarcoma, hepatoblastoma, hepatocellular carcinoma, and neuroblastoma. Molecular analysis was performed on tumor tissue, adjacent normal tissue and blood. Genomic sequencing on tissue and cell free DNA were conducted using MSK-IMPACTTM (Integrated Mutation Profiling of Actionable Cancer Targets), a custom hybridization-capture based assay developed at Memorial Sloan Kettering Cancer Center for targeted deep sequencing of all exons and selected introns as well as whole exome sequencing, where appropriate. Epigenomic analysis was conducted for methylation abnormalities using whole genome methyl bisulfite sequencing and assessment of transcriptomic, small RNAome, signalome and interactome analyses using RNA-Seq. Proteomic analysis was conducted by mass spectrometry. Tumor and exosome exomes were sequenced as potential biomarkers and analyzed for DNA, RNA, miRNA and protein content to determine their influence on cellular response and trafficking of different cell types in the tumor microenvironment including immune cells and fibroblasts.

Results: Preliminary data has demonstrated the ability to detect certain alterations missed on targeted genomic sequencing alone. Prominent, distinct methylation abnormalities in solid tumors were detected using whole genome bisulfite sequencing and distinct pathway activation using RNA-Seq. Evidence has been demonstrated that genetic alterations in oncogenes or tumor suppressor genes may not be functionally relevant due to loss in translation, transcription or post translational modifications. Tumor DNA was detected in the tumor exosomes and the characteristic genetic alterations present in the tumors were identified in the plasma exosomes. Flow cytometry and immunofluorescence for immune profiling of tumors were performed in order to validate the immune cell signatures derived from the DNA sequencing of whole genome or exome.

Conclusion: Multi-parallel genomic and immunologic analysis is feasible and has the potential to characterize the genetic landscape of low mutation burden tumors and inform therapeutic strategies for this population of patients.

No conflict of interest

453 (PB-116) Poster Characterization of the genetic landscape of low mutation burden malignancies through multi-parallel genomic and immunologic analysis

T. Trippett 1, M. Berger 2, A. Narendran 3, D. Lyden 4, O. Kovalchuk 5, T. Frawley 1, R. Conlon 1, C. Curtin 2, F. O’brien 2, R. Stallings 1, M. Berger 2, A. Narendran 3, D. Lyden 4, O. Kovalchuk 5, T. Frawley 1, R. Conlon 1, C. Curtin 2, F. O’brien 2, R. Stallings 1.

Background: A comprehensive simultaneous approach encompassing genomic sequencing, whole exome, exosomal, methylation, transcriptome and proteomic analyses coupled with immunogenic profiling has the potential to unravel the genetic landscape in pediatric tumors and inform insightful therapeutic strategies for children.

Materials and Methods: Biospecimens obtained from 41 patients with low mutation pediatric solid tumor malignancies were analyzed using comprehensive, simultaneous multi-parallel molecular analysis of the genomic landscape. The types of pediatric malignancies included osteosarcoma, rhabdomyosarcoma, hepatocellular carcinoma, hepatoblastoma, and neuroblastoma. Molecular analysis was performed on tumor tissue, adjacent normal tissue and blood. Genomic sequencing on tissue and cell free DNA were conducted using MSK-IMPACT™ (Integrated Mutation Profiling of Actionable Cancer Targets), a custom hybridization-capture based assay developed at Memorial Sloan Kettering Cancer Center for targeted deep sequencing of all exons and selected introns as well as whole exome sequencing, where appropriate. Epigenomic analysis was conducted for methylation abnormalities using whole genome methyl bisulfite sequencing and assessment of transcriptomic, small RNAome, signalome and interactome analyses using RNA-Seq. Proteomic analysis was conducted by mass spectrometry. Tumor and exosome exomes were sequenced as potential biomarkers and analyzed for DNA, RNA, miRNA and protein content to determine their influence on cellular response and trafficking of different cell types in the tumor microenvironment including immune cells and fibroblasts.

Results: Preliminary data has demonstrated the ability to detect certain alterations missed on targeted genomic sequencing alone. Prominent, distinct methylation abnormalities in solid tumors were detected using whole genome bisulfite sequencing and distinct pathway activation using RNA-Seq. Evidence has been demonstrated that genetic alterations in oncogenes or tumor suppressor genes may not be functionally relevant due to loss in translation, transcription or post translational modifications. Tumor DNA was detected in the tumor exosomes and the characteristic genetic alterations present in the tumors were identified in the plasma exosomes. Flow cytometry and immunofluorescence for immune profiling of tumors were performed in order to validate the immune cell signatures derived from the DNA sequencing of whole genome or exome.

Conclusion: Multi-parallel genomic and immunologic analysis is feasible and has the potential to characterize the genetic landscape of low mutation burden tumors and inform therapeutic strategies for this population of patients.

No conflict of interest
Author index

A
Abbattista M., S62 (168)
Abe H., e50 (131)
Abe N., e137 (423)
Abel A., e4 (4)
Aboubakar F., e75 (211)
Abrams T., e24 (21)
Acosta J., e123 (380)
Acs K., e31 (70)
Adachi Y., e127 (390)
Adam J., e26 (52), e100 (297), e118 (358)
Adami V., e9 (19)
Adams L., e64 (176)
Adamson K., e59 (160)
Adebayo A., e88 (256)
Adler N., e142 (438)
Adrada B., e129 (398)
Adurthi S., e80 (230)
Aebersold D.M., e27 (54), e29 (62)
Aebersold R., e29 (62)
Aftimos P., e16 (4)
Agostini M., e10 (24)
Aguilar A., e94 (279)
Ahmad A., e95 (283)
Ahmed S., e57 (153)
Ahrum M., e33 (75)
Ah-See M.L., e12 (28), e118 (360)
Aiello R., e30 (63)
Aithal K., e134 (411)
Akee R., e69 (190)
Al Hasani H., e37 (88)
Al Rawashdeh S., e93 (276)
Al Sorkhy M., e93 (276)
Al-Ahmadie H., e95 (283)
Alamoudi M., e81 (232)
Albain K., e59 (160)
Albertella M., e31 (70), e74 (208)
Albinsen T., e56 (140), e90 (263)
Albrecht F., e26 (51)
Alcaro S., e132 (407)
Alcoser S.Y., e6 (9)
Alcource C., e52 (137)
Alderson R., e76 (216)
Aldrich L., e82 (235)
Alessiani M., e55 (146)
Alewine C., e89 (261)
Alfattal R., e128 (395)
Alhaidar A.A., e138 (425)
Ali F., e46 (119)
Alimbetov D., e37 (99)
Allan S., e25 (24)
Allavena P., e108 (324)
Almanna K., e135 (414)
Alnemy S., e25 (50), e39 (96)
Alonso A., e26 (51)
Alravashdeh S., e68 (188)
Alishawa A., e74 (209), e75 (210)
Allura R., e77 (220)
Alero A., e7 (12), e107 (320)
Amant P., e79 (225)
Ambati S., e147 (453)
Amlber M., e122 (374), e122 (375)
Amemiyi M., e50 (131), e121 (372)
An A., e79 (226), e84 (241), e86 (250)
An H., e34 (81)
An H.J., e64 (175)
An X., e83 (240), e104 (313)
Anania M.C., e108 (324)
Anas G., e80 (229), e120 (367)
Anders R.A., e24 (23)
Anderson A., e9 (18)
Anderson C., e114 (347)
Anderson R.J., e116 (353)
Anderson E., e114 (346)
Anderson L., e16 (5)
Andrada Z.P., e35 (83)
Andre F., e118 (358)
Andre A., e80 (229), e120 (367)
Angelica F., e3 (2LBA)
Anglevin E., e118 (358)
Angioli S.V., e24 (23)
Anjum R., e114 (346)
Annais A., e143 (443)
Annuzziata C., e110 (333)
Anopa J., e112 (337)
Ansell K., e122 (374), e122 (375)
Anslay E.V., e51 (136)
Anthony S., e135 (413)
Anthony T., e8 (17)
Antignani A., e89 (261)
Antignani D., e69 (194)
Antoine H., e80 (229), e120 (367)
Antonia S., e18 (8)
Aono H., e66 (180), e70 (195)
Aparo S., e26 (51)
Apollo A.B., e50 (132)
Applebaum A., e81 (232)
Arapi V., e97 (287)
Argast G., e129 (399)
Argentiero A., e117 (356)
Arhona P., e42 (106)
Arienti C., e11 (27)
Armstrong C., e5 (7)
Armstrong P., e142 (439)
Arora N., e64 (176)
Arora S., e87 (254)
Arribas A., e68 (187)
Arribas J., e11 (25), e46 (120), e128 (393)
Arteaga C.L., e15 (1)
Aruketty S., e96 (285)
Asai A., e38 (93), e124 (381)
Asako N., e51 (135)
Asare S., e15 (2), e15 (Ch01), e59 (160)
Ashkenazi A., e42 (106)
Ashok Kumar V., e79 (224)
Aslifiantzadeh A., e62 (168)
Ashworth A., e62 (52), e29 (58), e100 (297)
Askarova S., e37 (89)
Asrani K., e145 (446)
Astorgues-Xerri L., e40 (98)
Atatreh N., e68 (188), e93 (276)
Athissayamani J., e79 (224)
Atlas D., e98 (290), e103 (308)
Attanasio N., e47 (123)
Atwell B., e137 (421)
Audeh W., e15 (Ch01)
Auger N., e118 (358)
Augustin E., e60 (163), e92 (271)
Auric S., e55 (146)
Aureliani M., e80 (229), e120 (367)
Avigni R., e108 (324)
Avni D., e73 (205)
Awad M., e133 (410)
Awad M.M., e131 (403)
Ayers L., e118 (360)
*Page numbers are followed by the abstract numbers in parentheses.
Ayub M., e96 (285)
Azaro A., e23 (20)
Aziz O., e67 (182)
Azorsa P., e9 (20)
Azuma Y., e55 (147), e107 (322)
Azzaniti A., e117 (356)
Bardia A., e129 (396)
Baris A., e32 (74)
Bardelli A., e140 (435)
Barbacid M., e24 (22)
Barardi R., e23 (20)
Baquero C., e45 (115)
Barbosa Marques R., e100 (296)
Baptista Moreno Martin A.C., e11 (26)
Baquero C., e45 (115)
Barajas J.M., e131 (404)
Barardi R., e23 (20)
Barbacid M., e24 (22)
Barbosa Marques R., e100 (296)
Bardelli A., e140 (435)
Bardia A., e129 (396)
Baris A., e32 (74)
Barisone E., e146 (449)
Barker H., e98 (291)
Barlebo Ahlborn L., e142 (440)
Baren J.M., e16 (8)
Bariggs S., e45 (115)
Berry E., e114 (346)
Barsis S., e78 (222)
Bartelink I., e76 (215)
Barth M., e112 (340)
Barrett B.R., e24 (23)
Basilico P., e80 (228)
Baskar S., e9 (20)
Basilico P., e80 (228)
Barisone E., e146 (449)
Bestgen B., e140 (431)
Bethany B., e55 (148)
Bever K., e102 (306), e103 (307)
Bever J., e69 (192)
Bedingfield S., e91 (269)
Beedie S., e83 (239)
Beeler K., e44 (110)
Beearm M., e17 (6)
Beever H., e135 (413)
Beg A.A., e18 (8)
Beg M.S., e135 (413)
Behenna D., e51 (135)
Behnkke A., e99 (294)
Behrens D., e101 (301)
Beisel H.G., e74 (208)
Bell J., e10 (22)
Bellarosa D., e37 (90)
Belle I., e104 (310)
Bendell J., e17 (6)
Benedetti D., e115 (351)
Benes E., e49 (129)
Benjamin B., e120 (367)
Bennett G., e61 (164), e65 (178)
Bensauid C., e23 (20)
Bereznyhov A., e76 (216)
Berger M., e147 (453)
Berger R., e98 (290)
Berges R., e61 (166)
Berman N., e35 (83)
Bernoth K., e98 (292)
Bermoulli J., e75 (213), e101 (302)
Bernstein S., e10 (22)
Berry D., e15 (2), e59 (160)
Berry J., e47 (123)
Berry S., e59 (160)
Bertels B., e135 (414)
Bertelsen B., e8 (15)
Bertens B., e142 (440)
Bertin S., e9 (18)
Bertoni F., e65 (179), e68 (187), e132 (407)
Beshay J., e86 (249)
Besse B., e75 (211), e118 (358)
Besse B., e75 (211), e118 (358)
Bestgen B., e140 (431)
Bethany B., e55 (148)
Bethell R., e31 (70), e74 (208)
Bettlott L., e146 (449)
Bever K., e102 (306), e103 (307)
Bever J., e69 (192)
Bever J., e69 (192)
Bever J., e69 (192)
Bever J., e69 (192)
Bexon A., e55 (148)
Bhang C., e23 (19)
Bhat M.A., e71 (199)
Bhattacharya A., e106 (318)
Bhavkar D., e114 (346)
Bialis A., e143 (441)
Biesma B., e130 (401)
Bigot L., e100 (297), e118 (358)
Bigot L., e100 (297), e118 (358)
Bigot L., e100 (297), e118 (358)
Bigot L., e100 (297), e118 (358)
Bigot L., e100 (297), e118 (358)
Bigot L., e100 (297), e118 (358)
Bigot L., e100 (297), e118 (358)
Bigot L., e100 (297), e118 (358)
Bigot L., e100 (297), e118 (358)
Bijelic G., e28 (10)
Birudukota S., e79 (224), e120 (368)
Bishi A., e47 (123)
Bishi J., e47 (124)
Biswas M., e4 (5)
Bjerk R., e122 (373)
Blum Murphy M., e17 (6)
Blumenschein G., e75 (210)
Bodenhof M., e109 (328)
Bodour S., e147 (453)
Bodyak N., e63 (173), e92 (272)
Boesler C., e136 (417)
Bohm K., e31 (70)
Boiko S., e114 (347)
Bollig G., e24 (22)
Bonafé M., e11 (27)
Bonifacio L., e48 (125)
Bonnin P., e85 (245)
Bononno S.M., e110 (332)
Bonomo S., e10 (24)
Bonvini E., e76 (216)
Boranel D., e3 (2LB)
Boras B., e51 (135)
Borgel S., e6 (9)
Borgman M., e119 (366)
Borrello M.G., e108 (324)
Bosari S., e10 (24)
Bose N., e82 (234)
Bosso S., e11 (27)
Bottaro D., e35 (84)
Bottaro D.P., e50 (132), e95 (281)
Botticella A., e75 (211)
Bouchlaiker M.N., e76 (214)
Bouillaud F., e100 (297)
Bourassa P., e30 (63)
Bouyguess A., e54 (145)
Bowtell D., e98 (291)
Boyd R., e47 (123), e65 (179)
Boyd-Kirkup J., e36 (87)
Boyle T.A., e18 (8)
Bradbury A., e32 (73)
Bradbury P., e73 (207)
Bradley B., e120 (369)
Bradley M., e39 (96)
Brady G., e96 (285)
Braquer D., e61 (166)
Brandsma I., e29 (58)
Breakstone R., e16 (5)
Brenndorfer E., e31 (70)
Brenton J.D., e28 (57)
Bressan A., e37 (90)
Bretz A.C., e119 (366)
Breznik E., e102 (306), e103 (307)
Brill E., e53 (110)
Britt J., e69 (190)
Brivio E., e146 (449)
Broaddus R., e29 (58), e141 (437)
Brown Swigart L., e15 (Ch01)
Brown Swigart L., e15 (Ch01)
Brown Swigart L., e15 (Ch01)
Brown Swigart L., e15 (Ch01)
Brown Swigart L., e15 (Ch01)
Brown Swigart L., e15 (Ch01)
Brown Swigart L., e15 (Ch01)
Brown Swigart L., e15 (Ch01)
Brown Swigart L., e15 (Ch01)
Brown Swigart L., e15 (Ch01)
Brown Swigart L., e15 (Ch01)
Brown Swigart L., e15 (Ch01)
Brown Swigart L., e15 (Ch01)
Abstracts, 30th EORTC-NCI-AACR Symposium

C

Cabriló G., e57 (153)
Cai J., e104 (312), e109 (327)
Cai M., e34 (81)
Cai S., e129 (399)
Caims L., e42 (104), e43 (109)
Calabro V., e78 (222)
Çalişkan B., e71 (200)
Cambilin A., e81 (231)
Cambria R., e112 (337)
Campbell J., e135 (415), e141 (437)
Campbell M., e74 (209)
Campone M., e12 (28)
Cano M., e51 (136)
Canonici A., e45 (118), e117 (357), e133 (408)
Cantwell J., e23 (19)
Canzoneri V., e10 (24)
Capano S., e37 (90)
Capoccia B., e76 (214)
Caponigro G., e24 (21)
Capucine B., e60 (229), e120 (367)
Caramella C., e75 (211)
Carland C., e81 (232)
Carles J., e128 (393)
Carlson J., e9 (18)
Caroline B., e10 (22)
Carr A., e142 (439)
Carnil S., e123 (380)
Carter A.J., e76 (214)
Carter J., e8 (9)
Carter L., e96 (285)
Caruña J., e5 (110), e9 (96), e130 (400)
Carvalhal L.A., e143 (443)
Casalottie J., e133 (409)
Casanovas O., e113 (343)
Cascione L., e68 (187), e132 (407)
Cash T., e19 (10)
Cassier P., e8 (15)
Castro J., e18 (8)
Catcott K., e63 (173), e92 (272)
Cauhaj N., e84 (244), e103 (309)
Cavalli F., e132 (407)
Cazzaniga M.E., e110 (332)
Celotti F.M., e35 (82)
Cemazar M., e90 (265)
Cemona M., e133 (408)
Cerrito M.G., e10 (24), e110 (332)
Cerut J., e146 (449)
Cervera A., e127 (391)
Chabanon R., e26 (52)
Chabanon R.M., e100 (297)
Chai F., e128 (395)
Chakrabarti A., e40 (97)
Chakraborty P., e76 (214)
Chakraborty D., e67 (183)
Chambers S.K., e28 (57)
Champiot S., e75 (211)
Chan A., e82 (234)
Chandarlapaty S., e42 (103)
Chandler B., e112 (336)
Chang J., e129 (398)
Chang J.M., e67 (185)
Chang G., e24 (22)
Chang S.C., e76 (215)
Chang S.M., e94 (277)
Chao R., e133 (410)
Charamannka K., e94 (280)
Chatta G., e119 (364)
Chau C., e83 (239)
Chau K.M., e125 (385)
Chelur S., e94 (280)
Chen A., e58 (157), e93 (274), e93 (275)
Chen A.P., e15 (1)
Chen B., e9 (18), e50 (133), e59 (160)
Chen D.T., e18 (8)
Chen F., e83 (240)
Chen G., e4 (3)
Chen J., e132 (406), e135 (415)
Chen L., e6 (9), e31 (67), e34 (81)
Chen M., e43 (108)
Chen S., e123 (379)
Chen T.L., e92 (273), e94 (277)
Chen W.W., e67 (185)
Chen Y., e77 (220), e137 (420)
Chen Y.F., e59 (160)
Chenail G., e23 (19)
Cheng W.C., e118 (360)
Chewinski H., e77 (220)
Chesnick I.E., e145 (446)
Chemuk A.T.C., e9 (20)
Chi K.N., e116 (353)
Chiappori A., e18 (8)
Chibaudel B., e54 (145)
Chinnaiyan A., e112 (336)
Chiosis J., e51 (135)
Chimpan M., e81 (232)
Chirade F., e112 (340)
Chisamore M., e82 (234)
Cho B.C., e133 (410)
Cho S.H., e125 (384)
Choi H.J., e77 (217)
Choi S.U., e119 (365)
Chojnowski G., e18 (9)
Choyney V., e60 (161)
Christensen J., e59 (158), e133 (410)
Christodoulidou A., e74 (208)
Christophe M., e80 (229), e120 (367)
Chu L., e31 (66)
Chuaqui C., e39 (96)
Chugh R., e30 (64)
Cialdella A., e10 (24)
Ciccarone V., e76 (216)
Cidado J., e114 (347)
Cillius Nielsen F., e142 (440)
Cipriani T., e123 (378)
Cireillo A., e123 (378)
Ciznadjia D., e85 (246)
Clarke R.B., e111 (334)
Clarkknowles K., e10 (22)
Clifford S.C., e145 (448)
Clynnes M., e108 (326)
Coates S., e6 (10)
Coleman R.L., e28 (57)
Colen R., e57 (153), e74 (209), e75 (210)
Colizzi F., e115 (351)
Collins A., e109 (327)
Collins C., e130 (400)
Collins D., e45 (118), e117 (357)
Collins J., e16 (51)
Collins K., e64 (174)
Collins L., e140 (435)
Collins S., e63 (173), e92 (272)
Collins T., e114 (346)
Cologna S., e82 (235)
Comlombi A., e146 (449)
Comarco E., e115 (351)
Combik M., e143 (441)
Comfort C.P., e35 (83)
Cominetti M.R., e11 (26)
Comitani F., e52 (137)
Concor G., e10 (24)
Condeelis J., e51 (134)
Conrey A., e120 (369)
Conley A., e53 (143)
Conley B.A., e15 (1)
Conlon K., e108 (326)
Conlon N., e108 (326), e117 (357), e133 (408)
Conlon P., e92 (272)
Conlon R., e147 (452)
Connelly M., e146 (451)
Conroy M., e7 (13)
Contal S., e112 (340)
Conter V., e146 (449)
Curvino E., e91 (269)
Curtin N.J., e31 (67)
Custar D., e63 (173)
Dann S., e51 (135)
Costello R., e35 (84), e50 (132)
Costermans J., e16 (4)
Cozzo A., e59 (159)
Crafton S., e132 (406)
Crawford N., e5 (7)
Creelan B., e18 (8)
Cremona M., e117 (357), e142 (439)
Crespo-Eugenio M., e6 (9)
Cripe T., e55 (148)
Crisicione S., e114 (347)
Cristofanilli M., e129 (396)
Crompton C., e122 (374)
Croshiere M., e69 (192)
Crosignani S., e80 (228)
Crowe M., e52 (138)
Crowley K.S., e76 (214)
Crowell S., e100 (296)
Crown J., e41 (100), e45 (118), e48 (127), e108 (325), e113 (345), e117 (357), e133 (408)
Cruciani V., e122 (373)
Crupi M., e10 (22)
Cruz R., e88 (257)
Csengery J., e30 (63)
Cua D., e77 (220)
Cultiva S., e26 (51)
Curigliano G., e3 (2LBA)
Curtin C., e147 (452)
Curtin N., e32 (73), e34 (80)
Curtin N.J., e31 (67)
Curvino E., e91 (269)
Custard D., e63 (173)
Cuthbertson A., e122 (373)
Cutillas P., e143 (442)
Cyr M., e136 (417)

D

Dabrowska M., e71 (198)
Daginakatte G., e94 (280), e134 (411)
Dai D., e40 (99)
Dairiki R., e127 (390)
Dalby K.N., e51 (136)
Daly C., e4 (3)
D’Ambrosio L., e58 (154)
Damelin M., e63 (173), e92 (272)
Darnia G., e10 (24)
D’andrea G., e42 (103)
Daneberg Z., e31 (69)
Daniel W., e6 (10)
Dann S., e51 (135)
Daphne M., e120 (367)
Das B., e6 (9)
Das R., e79 (224), e120 (368)
Das S., e5 (6), e86 (248)
Dasilva J., e4 (3)
Dason S., e95 (283)
Dasseux J.L., e56 (149), e90 (263)
Daszkiewicz L., e105 (314)

Datnow B., e59 (160)
Datta V., e6 (9)
Daven M., e7 (13)
David J., e132 (406)
David K., e86 (292)
David M., e90 (229)
David P., e75 (211)
Davies A., e85 (246), e99 (293)
Davies J., e109 (327), e109 (329)
Davies M., e53 (143)
Davis K.L., e131 (403)
Davis S.L., e142 (348)
Davis T., e37 (89)
Darison C., e27 (55)
Dawson J., e116 (352)
de Araújo Naves M., e11 (26)
de Boer R., e130 (401)
De Braud F., e3 (2LBA)
de Bruyn M., e78 (223)
de Craene B., e79 (225)
de Dios A., e45 (115), e45 (117)
de Giorgi M., e110 (332)
de Gramont A., e54 (145), e85 (245)
de Groot J., e53 (143)
de Jong S., e78 (223), e106 (318), e111 (334)
de Jonge M., e23 (20)
de Man J., e78 (221)
de Michele A., e59 (160)
de Regt B., e84 (244)
de Ridder C., e97 (289), e100 (296)
de Roos J.A.D.M., e53 (140)
de Schmidt A.Y., e142 (440)
de Silva D., e35 (84)
de Silva D.M., e50 (132), e95 (281)
de Stanchina E., e24 (22)
de Vries E., e78 (223)
Dean E., e27 (53)
Deban L., e47 (123)
Debets M.E.F., e45 (117)
Debets R., e100 (296)
Deighan J., e112 (337)
Delaporte S., e112 (340)
Dellera E., e55 (146)
Delmonte A., e23 (20)
Delord J.P., e8 (15)
Demady D., e63 (173), e92 (272)
Demanse D., e122 (376)
Demichele A., e15 (2), e15 (Ch01)
Demichielis F., e9 (19)
Demoret B., e132 (406)
Dempey J., e4 (4)
Deng X., e50 (133)
Denis J., e54 (145)
Denney C., e49 (128)
Denny W., e72 (203)
Denny W.A., e95 (282)
Denroche R., e96 (290)
Denroche R.E., e103 (308)
Depis F., e81 (231)
Deregnaurt Court T., e80 (228)
Deroo S., e44 (113)
Der-Torossian H., e133 (410)
Deschoemaeker S., e62 (168)
Desmet J., e44 (113)

De Theux M., e80 (228)
Deutsch E., e75 (211)
Deverchere J., e140 (431)
Dharran B., e72 (204)
Dhawan M., e28 (56)
Dheeraj A., e89 (260)
Dhudasha A., e80 (230)
Dhudashiy A., e94 (280)
Di Fonte R., e117 (356)
Di Nicolantonio F., e140 (435)
di Tomaso E., e19 (11), e25 (50), e130 (400)
Diamond J., e142 (438)
Diaz J.P., e26 (51)
Diaz L.A.Jr., e24 (23)
Diaz R., e55 (148)
Diaz Z., e26 (51)
Diaz-Rodriguez E., e11 (25)
Dichiara J., e76 (216)
Diedrich G., e76 (216)
Dimasi N., e65 (177)
Dimmock D., e41 (102)
D'incalci M., e58 (154)
Dinesh C., e8 (17)
Ding J., e5 (8)
Dipietro L., e133 (409)
Dittamore R., e31 (66), e127 (389)
Dive C., e96 (285)
Divelbiss R., e6 (9)
Do K.T., e19 (11)
Doak A., e117 (355)
Doak A.E., e45 (114)
Dobosz P., e73 (205)
Dodds M., e48 (125)
Dodheri S., e80 (230)
Dobele R., e117 (355)
Dobele R.C., e45 (114)
Dokunumu T., e88 (256)
Dole-Gleizes F., e52 (137)
Donahue J.M., e145 (446)
Dong O., e50 (133)
Dong-Wan K., e133 (410)
Donio M., e76 (214)
Donoho G., e4 (4)
Doroshow J.H., e6 (9)
Dorvat N., e62 (52), e100 (297)
Douglas R., e42 (104), e43 (109)
Dovrtilova G., e58 (154)
Dowlati A., e133 (410)
Doyle L.A., e15 (1)
Draetta G., e129 (398)
Dreas A., e71 (198)
Dredge K., e18 (9)
Drew L., e114 (347)
Drew S., e93 (275)
Drew Y., e27 (53), e32 (73)
Driessens G., e80 (228)
Drummond D., e81 (231)
Ds S., e8 (17)
Du B., e92 (272)
Du J., e45 (115), e45 (117)
Du X., e77 (220)
Duan W., e26 (51)
Dubiel K., e38 (91), e41 (101)
Dubois L., e62 (168)
Garcia Gomez J.J., e68 (186)
Garcia Valverde L.A., e128 (393)
Garcia-Jaramillo R., e71 (201), e72 (202)
Garcia-Manero G., e7 (12)
Garcia-Martinez J.M., e136 (418)
Garcia-Valverde A., e46 (120)
Garg A., e56 (150)
Gargano M., e82 (234)
Garmanchuk L., e69 (191)
Garrett A., e124 (382)
Garrido M., e26 (52), e100 (297)
Gasparetto C., e124 (382)
Gasperini P., e9 (19)
Gatalica Z., e144 (444)
Gaude E., e118 (360)
Gaudio E., e65 (179), e68 (187), e132 (407)
Gauthier I., e73 (207)
Gavande N., e33 (77)
Gavini J., e29 (62)
Gavory G., e43 (109)
Gaynor N., e45 (118)
Gazdha L.H., e35 (83)
Gazzah A., e75 (211)
Geberhard M., e59 (158)
Gebreyohannes Y., e127 (391)
Gehl J., e90 (265)
Gelman K., e73 (207)
Geng M., e5 (8)
Gentles L., e34 (80)
Geoeerber B., e30 (64)
Georgiadis A., e24 (23)
Gerami-Moayed N., e133 (409)
Gertje H., e4 (5)
Gervasio F.L., e52 (137)
Ghanta S., e79 (224)
Gharbi S., e45 (117)
Ghassemifar S., e81 (231)
Ghattas M., e68 (188), e93 (276)
Ghebremariam S., e23 (20)
Ghim J.L., e125 (384)
Ghose R., e51 (136)
Ghoshal K., e131 (404)
Giaccia A., e48 (125)
Giavazzi R., e10 (24)
Gibbs B.K., e132 (405)
Gibbs W., e122 (374), e122 (375)
Gibson A., e43 (108)
Gibson A.A., e60 (162)
Giddens A.C., e95 (282)
Gierke B., e109 (328)
Giesen B., e86 (249)
Gilard M., e137 (420)
Gilbert T., e23 (19)
Gilcrease M., e129 (398)
Gilissen J., e62 (168)
Gilmore E., e79 (227)
Giordano F., e10 (24)
Giordano H., e28 (57)
Giorgi T., e91 (269)
Giovanni M., e23 (20), e131 (403)
Giragossian C., e136 (418)
Girardi T., e44 (113)
Girgis-gabardo A., e70 (196)
Girish S., e56 (150)
Giurato G., e115 (351)
Giussani M., e35 (82)
Given B., e9 (18)
Glas A., e15 (2), e15 (Ch01)
Glazer E., e30 (63)
Glesson F., e118 (360)
Glenanne A., e130 (401)
Glick-Gorman Y., e98 (290), e102 (308)
Gliison B., e75 (210)
Glod J.W., e30 (64)
Glode A., e142 (438)
Godellass C., e59 (160)
Godfrey L., e65 (178)
Goghi H., e79 (224)
Golan T., e98 (290), e103 (308)
Golas A., e143 (441)
Goldfarb S., e42 (103)
Goldman A., e4 (5)
Goldoni S., e24 (21)
Goldstein D., e18 (9)
Golino G., e68 (187), e132 (407)
Gomez H., e88 (258)
Gomez J., e116 (352)
Gomez-Roca C., e8 (15)
Gong C., e125 (385)
Goodrich D., e119 (364)
Goodwin N., e82 (233), e99 (293)
Gopinath K., e4 (5)
Gopinath K.S., e130 (402)
Gopinath S., e134 (411)
Gordon K., e82 (234)
Gore S., e94 (280)
Gornicka A., e41 (101)
Gornyia S., e7 (14), e103 (309)
Goss G., e73 (207)
Gottfried M., e23 (20)
Gottlohr-Ahalt M., e6 (9)
Goverse G., e105 (314)
Gowda N., e8 (17), e80 (230)
Goy A., e17 (7)
Goyal M., e8 (17)
Gózdz S., e34 (79)
Grabinski N., e98 (292)
Graf R., e31 (66), e127 (389)
Graf J., e82 (234)
Graham D., e96 (285)
Grant D., e122 (373)
Grasselli E., e10 (24), e110 (332)
Graus Porta D., e122 (376)
Gray J.E., e18 (8)
Gray R.J., e15 (1)
Grayson M., e140 (435)
Greco A., e108 (324)
Greene J., e49 (128)
Greenfield J., e112 (337)
Gregg S., e118 (361)
Greil R., e3 (2LBA)
Grein J., e77 (220)
Griffiths T., e121 (370)
Grignani G., e58 (154)
Grigor’eva Y.V., e89 (262)
Grigoriu S., e52 (139)
Grkovic T., e69 (190)
Grobben Y., e78 (221)
Gronding M., e114 (346)
Groenenbacher N., e132 (406)
Grothaus P., e69 (190)
Grobbs C., e88 (258)
Gu Y., e122 (376)
Guala M., e102 (407)
Gualberto A., e137 (420)
Guan S., e17 (7), e36 (87)
Gu X., e57 (152)
Guardiolia V., e26 (51)
Gudkov A., e25 (24)
Gueguen-Dorbes G., e52 (137)
Guelen L., e105 (314)
Guerviaiavis V., e143 (443)
Guichard S., e114 (346)
Guidetti F., e65 (179)
Guinot V., e108 (326)
Guise C., e82 (188), e137 (422), e144 (445)
Gulati A., e141 (437)
Gumerov D., e92 (272)
Gundala C., e80 (230)
Guo S., e84 (241), e102 (304), e102 (305), e104 (312)
Gupta A., e46 (120)
Gupta N., e71 (199)
Gupta S., e7 (12), e121 (370)
Gurova K., e25 (24)
Guru S.A., e71 (199)
Gustafsson D., e64 (174), e86 (248)
Gustin P., e75 (211)
Gutierrez J., e45 (117)
Gutierrez S.M., e45 (117)
Gutkind S., e137 (420)
Gwynne W., e70 (196)

H

Haaq P., e97 (287)
Haass N., e30 (65)
Haber M., e25 (24)
Habra M., e74 (209)
Hagemann U., e122 (373)
Hagiwara S., e90 (264), e91 (267)
Haider S., e26 (52), e118 (360), e141 (437)
Hailman E., e136 (417)
Hainaud P., e85 (245)
Hakanen M., e67 (183)
Halbert G., e65 (178)
Halima S., e47 (122)
Hall M.D., e69 (194)
Hall S., e27 (53)
Hall T., e121 (371)
Hallen J.M., e75 (213), e101 (302)
Hallett R., e70 (196)
Halperin S., e98 (290), e103 (308)
Hamburger A.W., e145 (446)
Hamilton A., e98 (291)
Hamilton E., e17 (6)
Hammond K., e39 (96)
Hammes P., e24 (21)
Hammes E., e18 (9)
Han J.Y., e23 (20)
Han S., e125 (384)
Han X., e36 (85), e120 (369)
Handerson Comes Teles R., e11 (26)
Hanna D., e17 (6)
Hannus S., e69 (192)
Hansen K., e69 (192)
Hansen R., e43 (107), e98 (291)
Hao D., e73 (207)
Hao H., e24 (21)
Hara S., e62 (170), e63 (171)
Hara T., e90 (264), e91 (267)
Harding T.C., e28 (57)
Hari Dass P., e140 (435)
Harker A.R., e114 (346)
Harris A., e118 (360)
Harris D., e104 (310)
Harris E., e83 (239)
Harris L.N., e15 (1)
Harris M., e69 (190)
Harrison B., e82 (234)
Harrison T., e42 (104), e43 (109)
Hartley J., e64 (176), e68 (186)
Haruma T., e137 (423)
Hassako S., e137 (423)
Hasegawa Y., e94 (278)
Hashimoto A., e38 (92), e62 (170)
Hassell J.A., e70 (196)
Hassig C., e43 (107), e98 (291)
Hatton C., e120 (369)
Haugen M.H., e66 (181)
Hauri E., e18 (8)
Hausman D., e17 (6)
Hautaniemi S., e127 (391)
Hawkings D.S., e30 (64)
Hayashida M., e66 (180)
Haynes B., e116 (354)
Hay S., e132 (406)
He D., e79 (226)
Hedrick M., e43 (107), e98 (291)
Heeschen C., e101 (301)
Hegge J., e9 (18)
Heidemann-Dinger C., e40 (97)
Heins M., e7 (14), e87 (253)
Heist R.S., e131 (403)
Heling D., e41 (102)
Hellman L.J., e30 (64)
Heilsund P., e30 (63)
Heiman E., e28 (57)
Henderson I., e31 (70), e74 (208)
Henderson M., e25 (24)
Hendriks H.R., e37 (88)
Hendriks L., e75 (211)
Hennessy B., e117 (357), e133 (408)
Hennessy B.T., e142 (439)
Hénon C., e26 (52)
Henry M., e49 (130)
Heong V., e98 (291)
Herbert C., e52 (137)
Hermans I., e144 (445)
Hermant A., e80 (228)
Herpers B., e39 (85), e104 (311)
Herrera A., e17 (7)
Hess K., e74 (209), e75 (210)
Hetland G., e66 (181)
Hewitt S., e89 (261)
Heyrick A., e62 (168)
Heymach J., e53 (143)
Hideaki K., e91 (270)
Hiebsch R., e76 (214)
Higgs B., e76 (215)
Hill C., e5 (6)
Hill J., e17 (7)
Hiricza K., e34 (79)
Hirata G., e38 (93)
Hirst G., e15 (2), e15 (Ch01)
Ho G.Y., e98 (291)
Ho J., e136 (418)
Hobelka C., e85 (245)
Hochhauser D., e68 (186)
Hodgson G., e19 (11), e25 (50)
Hoffman R., e51 (135)
Hoffman S., e6 (9)
Hoffmann J., e101 (300), e101 (301), e106 (319)
Hollingshead M., e6 (9)
Holohan C., e5 (7), e142 (439)
Holtzer L., e84 (244), e105 (315)
Hom B., e127 (389)
Hong D., e23 (20), e57 (153), e74 (209), e75 (210), e128 (395), e133 (410)
Hong F., e15 (1)
Hong H., e25 (25)
Hong T., e134 (412)
Hoogenboezem E., e91 (269)
Hopkins A., e88 (257)
Hori Y., e126 (388), e138 (427)
Hoshino T., e62 (170), e63 (171)
Hotson A., e5 (6)
Hotte S., e73 (207)
Houlden J., e140 (435)
House C., e110 (333)
Houthuys E., e80 (228)
Howard P., e64 (176), e65 (177)
Hsieh E., e77 (220)
Hsu W.H., e140 (433)
Hu P., e131 (404)
Hu S., e25 (50), e96 (96), e43 (108), e60 (162)
Hu W., e95 (283)
Huang H.J., e57 (153)
Huang L., e36 (85), e76 (216)
Huang P., e83 (239)
Huang T.T., e53 (141), e116 (354)
Huang X., e5 (8)
Huang Y., e44 (112)
Huczk-Kallitowska J., e41 (101)
Hudson C., e82 (233)
Hudson L., e88 (257)
Huertas A., e123 (380)
Huet F., e23 (19)
Huh M., e10 (22)
Huhltala T., e105 (316)
Huins C., e27 (53)
Hung J.J., e140 (433)
Huntsman A., e82 (235)
Hu L., e129 (398)
Hurtado F., e18 (8)
Huser N., e51 (135)
Hussain E.H., e47 (122)
Hussein M., e133 (410)
Huyvaert N., e16 (4)
Hydbring P., e97 (287)
Hylton N., e15 (2), e59 (160)
Hyman D., e112 (337)
I
Iacobazzi R.M., e117 (356)
Ianlano L., e10 (24)
Ibrahim M., e133 (408)
Ibrahim T., e75 (211)
Icay K., e127 (391)
Ichikawa K., e62 (170), e63 (171), e126 (388), e138 (427), e139 (430)
Idate R., e86 (248)
Ideker T., e33 (76)
Iijima T., e38 (93), e124 (381)
Ikeda H., e126 (387)
Im H.S., e58 (155)
Imaizumi A., e83 (237)
Imoto M., e67 (184), e126 (387)
Impagnatiello M.A., e136 (418)
Ingram P., e36 (87)
Inomata A., e91 (267)
Investigators I., e15 (2), e15 (Ch01)
Iorio T., e90 (264), e91 (267)
Iratni R., e47 (122)
Irie H., e124 (383)
Irwin G.W., e79 (227)
Isaacs C., e59 (160)
Isaacsnow J., e28 (57)
Isaka M., e96 (284)
Ishiguro T., e55 (147)
Ishii S., e38 (93), e124 (381)
Issaeva N., e25 (24)
Italiano A., e3 (2LBA)
Ito J., e39 (94), e126 (388), e138 (427)
Ito K., e124 (383)
Ito S., e63 (171), e139 (430)
Iversen P., e4 (4)
Iwasawa Y., e62 (170), e63 (171), e124 (383), e137 (423), e139 (430)
Iweala E., e88 (256)
Izumi Y., e90 (264)
J
Jackson M., e91 (269)
Jacob S.T., e131 (404)
Jadhav T., e79 (224)
Jagani Z., e23 (19)
Jahns H., e42 (106), e88 (257)
Ja-Hyun J., e58 (155)
Jain D., e75 (210)
Jain S.K., e97 (288)
Jalving H., e106 (318)
Jamieson S.M.F., e95 (282)
Janc J., e5 (6)
Janes M., e137 (420)
Jang M., e77 (217)
Jang S., e32 (74)
Janku F., e53 (143), e57 (153), e75 (210), e128 (395)
Janne P., e114 (347), e133 (410)
M
Macdonald D., e4 (3)
Macek P., e34 (79)
Machado-Pichardo L., e58 (157)
Machida T., e139 (430)
Machiels J.P., e16 (4)
Maciej P., e131 (404)
Maciejko L., e4 (5)
Madriaga A., e5 (6)
Madwani K., e57 (153)
Maelandsmo G.M., e111 (334)
Maertens G., e79 (225)
Magnano S., e115 (350)
Magne N., e123 (380)
Mahajan V., e130 (344), e127 (390), e138 (427)
Matsuki M., e127 (390)
Matsumoto T., e90 (264)
Matsumura Y., e135 (413)
Matsuo K., e62 (170), e63 (171), e124 (383), e139 (430)
Matsuzaki T., e38 (93), e124 (381)
Mayo C., e58 (154), e146 (449)
Mayhoffer M.T., e111 (334)
Mazan A., e71 (198)
Mazan M., e143 (441)
Mazan-Mamczarz K., e110 (333)
Mazerska Z., e92 (271)
Mazzoli Z., e108 (324)
Mau-Sorensen M., e142 (440)
Mawji N.R., e116 (353)
Maurer P., e5 (7)
May E., e52 (139)
Mayo H., e25 (24 )
Mayorga D., e123 (380)
Mayrhofer M.T., e111 (334)
Mazan A., e71 (198)
Mazan M., e143 (441)
Mazan-Mamczarz K., e110 (333)
Mazerska Z., e92 (271)
Mazzoli Z., e108 (324)
Mc Nulty A., e4 (4)
Mceachern D., e36 (85), e94 (279)
Mcloughlin D., e136 (417)
Megersa A., e53 (7)
Mai E., e52 (139)
Manning P.T., e76 (214)
Mansfield A.S., e131 (403)
Mao B., e84 (241), e102 (304)
Maqbool S., e46 (119)
Marabell L., e26 (52), e75 (211)
Marapin S., e94 (280)
Marchante J., e80 (228)
Margarita L., e35 (82)
Mambili C., e80 (228)
Marin Margheri L., e39 (96)
Marin J., e146 (450)
Mark W., e135 (413)
Markel G., e98 (290)
Marlow R., e26 (52), e39 (95)
Marshall D., e30 (63)
Marsen D., e29 (61)
Marfell G., e35 (82)
Martin Romano P., e80 (229), e120 (367)
Martin A., e71 (201), e72 (202)
Martin A.M., e72 (204)
Martin E., e27 (53)
Martinet M., e40 (98)
Martinez R., e4 (4)
Martinez-Lopez A., e113 (343)
Martinioli C., e80 (228)
Mascarenhas L., e30 (64)
Masiejczyk M., e143 (441)
Massard C., e26 (52), e118 (358)
Masters L., e65 (177)
Mateos M., e145 (448)
Matsuda S., e91 (267)
Matsui J., e39 (94), e126 (388), e127 (390), e138 (427)
Matsuki M., e127 (390)
Matsumoto T., e90 (264)
Matsumura Y., e135 (413)
Matsuo K., e62 (170), e63 (171), e124 (383), e139 (430)
Matsuzaki T., e38 (93), e124 (381)
Matta C., e58 (154), e146 (449)
Mayhoffer M.T., e111 (334)
Mazan A., e71 (198)
Mazan M., e143 (441)
Mazan-Mamczarz K., e110 (333)
Mazerska Z., e92 (271)
Mazzoli Z., e108 (324)
Mc Nulty A., e4 (4)
Mceachern D., e36 (85), e94 (279)
Mcloughlin D., e136 (417)
Megersa A., e53 (7)
Mai E., e52 (139)
Manning P.T., e76 (214)
Mansfield A.S., e131 (403)
Mao B., e84 (241), e102 (304)
Maqbool S., e46 (119)
Marabell L., e26 (52), e75 (211)
Marapin S., e94 (280)
Marchante J., e80 (228)
Margarita L., e35 (82)
Mambili C., e80 (228)
Marin Margheri L., e39 (96)
Marin J., e146 (450)
Mark W., e135 (413)
Markel G., e98 (290)
Marlow R., e26 (52), e39 (95)
Marshall D., e30 (63)
Marsen D., e29 (61)
Marfell G., e35 (82)
Martin Romano P., e80 (229), e120 (367)
Martin A., e71 (201), e72 (202)
Martin A.M., e72 (204)
Martin E., e27 (53)
Martinet M., e40 (98)
Martinez R., e4 (4)
Martinez-Lopez A., e113 (343)
Martinioli C., e80 (228)
Mascarenhas L., e30 (64)
Masiejczyk M., e143 (441)
Massard C., e26 (52), e118 (358)
Masters L., e65 (177)
Mateos M., e145 (448)
Matsuda S., e91 (267)
Matsui J., e39 (94), e126 (388), e127 (390), e138 (427)
Matsuki M., e127 (390)
Matsumoto T., e90 (264)
Matsumura Y., e135 (413)
Matsuo K., e62 (170), e63 (171), e124 (383), e139 (430)
Matsuzaki T., e38 (93), e124 (381)
Matta C., e58 (154), e146 (449)
Mayhoffer M.T., e111 (334)
Mazan A., e71 (198)
Mazan M., e143 (441)
Mazan-Mamczarz K., e110 (333)
Mazerska Z., e92 (271)
Mazzoli Z., e108 (324)
Mc Nulty A., e4 (4)
Mceachern D., e36 (85), e94 (279)
Mcloughlin D., e136 (417)
Megersa A., e53 (7)
Mai E., e52 (139)
Manning P.T., e76 (214)
Mansfield A.S., e131 (403)
Mao B., e84 (241), e102 (304)
Maqbool S., e46 (119)
Marabell L., e26 (52), e75 (211)
Marapin S., e94 (280)
Marchante J., e80 (228)
Margarita L., e35 (82)
Mambili C., e80 (228)
Marin Margheri L., e39 (96)
Marin J., e146 (450)
Mark W., e135 (413)
Markel G., e98 (290)
Marlow R., e26 (52), e39 (95)
Marshall D., e30 (63)
Marsen D., e29 (61)
Marfell G., e35 (82)
Martin Romano P., e80 (229), e120 (367)
Martin A., e71 (201), e72 (202)
Martin A.M., e72 (204)
Martin E., e27 (53)
Abstracts, 30th EORTC-NCI-AACR Symposium

Author Index

Pidorugü H.B., e94 (277)
Piezczkoiał J., e38 (91), e41 (101)
Pierce L., e112 (336)
Pierre V., e76 (215)
Pierron G., e100 (297)
Pietz E.J., e95 (283)
Pignatta S., e11 (27)
Pignochino Y., e58 (154)
Piha-Paul S., e75 (210), e128 (395)
Piha-Paul S.A., e57 (153)
Pikor L., e10 (22)
Pilch J., e60 (163)
Piller M., e98 (292)
Pinto C., e134 (412)
Pinto J., e113 (343)
Piorkowska N., e38 (91)
Piotrowska K., e130 (400)
Piris-Gimenez A., e111 (334)
Pinson R., e80 (228)
Pisercchio A., e51 (136)
Piskareva O., e147 (452)
Pitruzzello M., e107 (320)
Pittman M., e65 (178)
Pivetta E., e115 (351)
Płonica-Worms H., e129 (398)
Planchard D., e118 (358)
Plaase T., e124 (382)
Plyte S., e78 (222)
Pocard M., e85 (245)
Podutdooor R., e94 (280)
Podergajs N., e103 (307)
Polak A., e143 (441)
Politz O., e128 (393)
Pollik M., e118 (360)
Pollock R., e52 (139)
Popov A., e140 (431)
Porat Z., e41 (102)
Porcelli E., e117 (356)
Portnik A., e102 (306)
pot M., e24 (244)
Posch M., e84 (244)
Postel-Vinay S., e26 (52), e100 (297)
Pothuganti M.K., e134 (411)
Poutlainei P., e105 (316)
Powell S., e55 (148)
Powers J., e93 (275)
Powers J.P., e93 (274)
Prager G., e3 (LBA)
Prakash C., e40 (99)
Prandi D., e9 (19)
Prasad S., e80 (228)
Prehln J., e42 (106)
Prellion J., e80 (228)
Principe M., e48 (127)
Prescott M.F., e72 (204)
Press M., e17 (6)
Preuss Hasselby J., e142 (440)
Price L., e39 (95), e104 (311), e105 (314), e111 (334)
Pricht S., e29 (61), e55 (146)
Prief L., e45 (115), e45 (117)
Prinsen M.B.W., e63 (140)
Print C., e37 (422)
Prise V., e113 (344)
Przyadasherini G., e8 (17)
Proctor M., e30 (65)
Prohaska D., e48 (125)
Proia T., e114 (347)
Prost J.F., e8 (15)
Protopopova M., e63 (173)
Protosenko V., e60 (161)
Przybrowski S., e36 (85), e94 (279)
Pulkka O.P., e127 (391)
Punnonen J., e77 (220)
Percei C., e142 (438)
Purkalne G., e31 (69)
Puro R., e76 (214)
Putta R., e79 (224)
Pzyika K., e71 (198)

Q
Qeriq B., e24 (22)
Qi S., e83 (240)
Qian W., e104 (312), e109 (327)
Qiao M., e83 (240)
Qin C., e36 (85)
Qin L., e63 (173), e92 (272)
Qin Z., e51 (135)
Qiu X., e82 (234)
Quinn J., e123 (378)
Quinn M., e84 (243), e85 (247)
Quintin A., e29 (62)
Quinton A., e140 (435)

R
Rabe D.C., e95 (281)
Rabinovich S., e41 (102)
Rabusin M., e146 (449)
Rack S., e96 (285)
Radhakrishnan P., e4 (5)
Radke M.R., e28 (57)
Rafee S., e142 (439)
Rainbolt E., e104 (310)
Rafties Gurevitch M., e98 (290)
Rafties M., e103 (308)
Rajagopal N., e25 (50)
Rajagopal S., e79 (224), e120 (368)
Rajagopalan S., e94 (280)
Rakiec D., e23 (19)
Ramachandra M., e6 (17), e80 (230), e94 (290), e134 (411)
Ramachandra R., e80 (230)
Ramdaswamy B., e87 (252)
Ramchandren R., e17 (7)
Rangwala F., e3 (LBA)
Ranjit B., e30 (63)
Rasco D., e84 (243), e85 (247)
Rassoulpor A., e7 (14), e87 (253)
Rastilav B., e80 (229), e120 (367)
Rathnavayake R., e82 (235)
Rau B., e110 (331)
Rauch G., e129 (398)
Rau A., e91 (231)
Raval A., e12 (28)
Ravenstein S.V., e51 (136)
Ravinda S., e94 (280)
Ray-Coquard I., e7 (13)
Recnard E., e55 (146)
Rich S., e138 (426)
Richards J., e81 (231)
Richardson R., e31 (66)
Rickett C.J., e132 (405)
Ridker P.M., e27 (204)
Rigamonti N., e78 (222)
Rigby A., e52 (139)
Rigby M., e65 (178)
Riley D., e24 (23)
Rinnenthal J., e136 (418)
Rios-Lucí C., e11 (25)
Ritter C., e112 (336)
Rivero E., e132 (407)
Rivera Sanchez L., e51 (134)
Rivoltini L., e35 (82)
Rix U., e117 (355)
Rizzardi C., e146 (449)
Rizzetti M.G., e108 (324)
Rizzotto L., e111 (334)
Roberto G., e110 (332)
Roberts C., e109 (327), e140 (435)
Roberts P., e47 (124)
Robinson B., e30 (63)
Robinson H., e34 (80)
Rocca R., e132 (407)
Roche E., e28 (56)
Roche S., e108 (325), e108 (326), e113 (345), e133 (408)
Rodgers A., e49 (129)
Rodon Ahtner J., e74 (209), e75 (210)
Rodon J., e128 (395)
Rodrigues Simoes da Costa J., e68 (186)
Rodrigues A., e85 (245)
Roehrl C., e147 (453)
Roesser J., e87 (253)
Rogado A., e42 (103)
Rohll C., e47 (123), e65 (179)
Rolf C., e140 (435)
Roman-Roman S., e111 (334)
Roméo A., e11 (27)
Roméo P., e108 (324)
Romero-Rojas A., e123 (380)
Rosell J., e128 (393)
Rosen B., e93 (274)
Rosen N., e24 (22)
Rosenberg M., e146 (450)
Weitman S., e135 (413)
Weishaar K., e5 (6), e64 (174)
Weiner R., e49 (129)
Wengner A., e101 (301)
Westmose Yde C., e142 (440)
Weichert L., e7 (14)
Wang C., e36 (85)
Wang H., e24 (21), e114 (346)
Wang J., e86 (250), e116 (353)
Wang M., e129 (399)
Wang S., e36 (85), e94 (279), e136 (418)
Wang X., e72 (204)
Wang Y., e5 (8), e31 (66), e51 (134), e127 (389)
Wang Y.A., e72 (204)
Wang Z., e137 (420)
Wani A.E., e79 (224)
Wani N., e131 (404)
Wapinsky G., e135 (414)
Ward G., e38 (92)
Ware M., e49 (129)
Warner S., e135 (413)
Wardhaka M., e51 (136)
Watanabe Miyano S., e39 (94)
Watanabe H., e39 (94), e127 (390)
Watanabe T., e50 (131), e121 (372)
Watson W., e48 (127)
Watters J., e133 (409)
Waugh D., e118 (361)
Waugh D.J., e5 (7)
Weaver V., e3 (1LBA)
Weber H., e86 (249)
Weber T., e70 (197)
Wei D., e132 (405)
Wei P., e51 (135)
Wei S., e59 (160)
Wei Y., e7 (12)
Weichert L., e7 (14)
Weigel B., e19 (10)
Weigman V., e146 (450)
Weiner R., e49 (129)
Weinnich S., e51 (135)
Weishaar K., e5 (6), e64 (174)
Weishauep C., e136 (418)
Weisz A., e115 (351)
Weitman S., e125 (413)
Welsh E., e108 (326)
Wen P.Y., e3 (2LBA)
Wengner A., e101 (301)
Wennerberg K., e127 (391)
Wernitzing A., e136 (418)
Wesa A., e99 (293)
Westmose Yde C., e142 (440)
Wetmore C., e19 (10)
Whalen K., e123 (378)
Whatcott C., e135 (413)
Wheatley-Price P., e73 (207)
Wheelan J., e30 (84)
White B., e123 (378)
White J., e88 (258)
White J.R., e24 (23)
White M., e51 (135)
Whitt J., e69 (190)
Whittaker M., e52 (137)
Wiatrowska K., e71 (198)
Wick M.J., e84 (243), e85 (247)
Widemann B.C., e30 (84)
Wielopole M., e38 (91), e41 (101)
Wiersma C., e140 (434)
Wilboux M., e122 (376)
Wilbur J., e133 (409)
Wilder-Romans K., e112 (336)
Willemsen-Seegers N., e53 (140), e78 (221)
Willemen R., e59 (159)
Williams B., e133 (409)
Williams D., e116 (353)
Williams J., e24 (21)
Williams P.M., e6 (9), e15 (1)
Williamson D., e145 (448)
Wilson D., e77 (220)
Wilson J.M., e103 (308)
Wilson L., e34 (80)
Wilson M.L., e113 (344)
Wilson P., e27 (55), e113 (344)
Wilson R., e27 (55), e112 (337), e140 (435)
Wilson W.C., e76 (214)
Winkler W., e72 (204)
Wondrak R., e143 (441)
Wise H.C., e95 (283)
Woldemichael G.M., e95 (281)
Wolf D., e15 (2), e15 (Ch01)
Wolf D.J., e35 (83)
Wolf J., e131 (403)
Wolfe B., e5 (6)
Wolfgang L., e134 (412)
Wong H.S., e64 (175)
Wong C., e72 (204), e81 (231)
Wong S., e39 (18), e42 (103), e72 (204)
Woozer R., e123 (378)
Wozniak A., e127 (391)
Wrigley J., e109 (327), e109 (329)
Wrolab A., e71 (198)
Wrobel A., e84 (244)
Wu H., e83 (240), e84 (241)
Wu H.C., e94 (277)
Wu J., e70 (196)
Wu M.H., e94 (277)
Wu W., e4 (4)
Wulf-Goldenberg A., e101 (300), e106 (319)
Wurth L., e44 (113)
X
Xiang K., e23 (19)
Xiang W., e36 (85)
Xiao L., e25 (24)
Xiu J., e144 (444)
Xu J., e92 (272)
Xu L., e63 (173), e81 (231)
Xu M., e51 (135)
Xu T., e36 (85)
Xue J., e104 (312)
Xue L., e52 (139)
Y
Yadav P., e71 (199), e97 (288)
Yaege R., e24 (22)
Yakubo O., e88 (256)
Yamada K., e126 (388), e138 (427)
Yamada M., e139 (430)
Yamada S., e106 (317)
Yamani A., e38 (91)
Yan J., e5 (8)
Yan K., e39 (95), e104 (311), e105 (314)
Yan X., e93 (275)
Yang C.Y., e94 (279)
Yang D., e135 (415)
Yang H., e40 (99)
Yang J., e94 (278)
Yang K., e4 (3)
Yang L., e63 (173)
Yang S., e122 (376)
Yang W., e83 (240), e84 (241)
Yang Y., e104 (312)
Yang Y.C., e116 (353)
Yano W., e62 (170), e63 (171)
Yao Z., e24 (22)
Yap T., e128 (395)
Yaromina A., e62 (168)
Yau C., e15 (2), e15 (Ch01), e59 (160)
Ye F., e34 (81)
Yee A., e81 (232)
Yee D., e15 (2), e15 (Ch01), e59 (160)
Yin M., e63 (173), e92 (272)
Yin Y., e57 (151), e109 (327), e109 (329)
Ying M., e44 (112)
Yokota Y., e48 (125)
Yolla E.D., e80 (229), e120 (367)
Yonekura T., e63 (171)
Yoo M., e117 (355)
Yosef L., e69 (192)
Yoshida J., e50 (131)
Yoshida T., e94 (278)
Yoshimura C., e38 (92)
Yoshinaga A., e124 (381)
Yoshino Y., e90 (264)
Young A., e82 (235)
Young L., e42 (106)
Young S., e93 (274), e93 (275)
Yu F., e91 (269)
Yu J., e99 (295)
Yu J.C., e50 (133)
Yu L.B., e131 (404)
Yu S., e125 (384)
Yu Y., e121 (371), e128 (395), e143 (442)
Yuan C.C., e120 (369)
Yung W.A., e53 (143)
Yunxia H., e63 (172)
Yurkovetskiy A., e63 (173), e92 (272)
Yusra A.D., e47 (122)
Yuzhakov A., e52 (139)
Z
Zakharia Y., e49 (130)
Zaki K., e30 (64)
Zalewski K., e34 (79), e48 (126)
Zamagni A., e11 (27)
Zaman G.J.R., e53 (140), e78 (221)
Zamboni W., e19 (11), e56 (150)
Zambrina S., e56 (150)
Zamora-Olivares D., e51 (136)
Zanella N., e105 (316)
Zang D.Y., e139 (429)
Zanoni M., e11 (27)
Zavorotskaya T., e52 (138)
Zanoni M., e11 (27)
Zhao F., e120 (369)
Zhao H., e24 (22), e79 (225)
Zhao L., e83 (240)
Zhao Q., e104 (312)
Zhao Y., e16 (4)
Zheng L., e79 (226)
Zheng Y., e76 (215)
Zhou D., e135 (415)
Zhu L., e130 (401)
Zhu R., e9 (18)
Zhu Z., e15 (Ch01)
Zhuang Y.J., e88 (256)
Zhu L., e130 (401)
Zhu R., e9 (18)
Zhu Z., e15 (Ch01)
Zhuang Y.J., e88 (256)
Zhu L., e130 (401)
Zhu R., e9 (18)