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PLENARY SESSION 3

Exopoling Genomic Instability as a Target

1LBA Late Breaking Abstract

Phase I modular study of AZD6738, a novel oral, potent and selective ataxia telangiectasia Rad3-related (ATR) inhibitor in combination (combo) with carboplatin, olaparib or durvalumab in patients (pts) with advanced cancers


1 Royal Marsden Hospital and The Institute of Cancer Research, Drug Development Unit, London, United Kingdom; 2 The Christie NHS Foundation Trust and The University of Manchester, Faculty of Biology-Medicine and Health, Manchester, United Kingdom; 3 Institut Gustave Roussy, DITEP Département d’Innovation Therapeutique et Essais Précoces, Paris, France; 4 Seoul National University Hospital, Department of Internal Medicine, Seoul, Korea; 5 University of Southern California, Department of Medicine, Los Angeles, USA; 6 Memorial Sloan Kettering Cancer Center, Developmental Therapeutics Center, New York, USA; 7 Royal Marsden Hospital and The Institute of Cancer Research, Division of Radiotherapy and Imaging, London, United Kingdom; 8 Royal Marsden Hospital, Drug Development Unit, London, United Kingdom; 9 AstraZeneca, Quantitative Clinical Pharmacology, Melbourn, United Kingdom; 10 AstraZeneca, Translational Medicines Unit, Melbourn, United Kingdom

This abstract is embargoed until the day of presentation.

2LBA Late Breaking Abstract

Opposing therapeutic efficacy of BET inhibitors is determined by cancer type-specific SPOP mutants


1 Institute of Oncology Research IOR, Department of Oncology Southern Switzerland, Bellinzona, Switzerland; 2 Broad Institute, MIT & Harvard, Cambridge, USA; 3 Institute of Biochemistry, Eidgenössische Technische Hochschule ETH Zurich, Zurich, Switzerland; 4 Dana Farber Cancer Institute, Medical Oncology, Boston, USA

This abstract is embargoed until the day of presentation.

3LBA Late Breaking Abstract

A phase Ib trial with MK-8628/OTX015, a small molecule inhibitor of bromodomain (BRD) and extra-terminal (BET) proteins, in patients with selected advanced solid tumors

C. Massard1, J.C. Soria1, A. Stathis2, J.P. Delord3, A. Awada4, S. Peters5, J. Lewin6, M. Bekradda7, K. Reza8, Z. Zeng9, H. Azher10, S. Perez11.

1 Gustave Roussy, Dept of Medical Oncology, Le Kremlin-Bicêtre, France; 2 Oncology Institute of Southern Switzerland, Lymphoma Unit, Bellinzona, Switzerland; 3 Institut Claudius Regaud, Dept of Medical Oncology, Toulouse, France; 4 Institut Jules Bordet, Dept of Medical Oncology, Brussels, Belgium; 5 Centre Hospitalier Universitaire Vaudois and Lausanne University Hospital, Dept of Medical Oncology, Lausanne, Switzerland; 6 Princess Margaret Cancer Center, Clinical Cancer Research Unit, Toronto, Canada; 7 OTD, Medical Oncology, Clichy, France; 8 Institut Curie-Hôpital René Huguenin, Dept of Medical Oncology, Saint-Cloud, France; 9 MRL- 126 East Lincoln Avenue, Dept of Statistics, Rahway, USA; 10 MRL- 126 East Lincoln Avenue, Dept of Pharmacokinetics, Rahway, USA; 11 MRL- 126 East Lincoln Avenue, Dept of Oncology, Rahway, USA

This abstract is embargoed until the day of presentation.
A systematic liquid biopsy program identifies novel and heterogeneous mechanisms of acquired resistance in gastrointestinal (GI) cancer patients

A. Parikh, E. Kwak, L. Goyal, L. Blaszkowsky, M. Hazar-Rethinam, G. Siravegna, M. Russo, E. Van Sevenet, B. Nadres, H. Shahzade, J. Clark, J. Allen, A. Iafrate, A. Bardelli, D. Ryan, J. Murphy, A. Zhu, T. Hong, R. Corcoran, 1Massachusetts General Hospital, Cancer Center, Boston- Massachusetts, USA; 2University of Torino, Candiolo Cancer Center, Torino, Italy

5LBA Late Breaking Abstract
Plasma EGFR T790M mutation detection in NSCLC patients using a combined exosomal RNA and circulating tumor DNA qPCR assay


This abstract is embargoed until the day of presentation.
Preliminary safety and activity in a first-in-human phase 1 study of BLU-285, a potent, highly-selective inhibitor of KIT and PDGFRα activation loop mutants in advanced gastrointestinal stromal tumor (GIST)


1OHSU Knight Cancer Institute, Hematology and Medical Oncology, Portland- OR, USA; 2Royal Marsden Hospital/Institute of Cancer Research, Sarcoma, London, United Kingdom; 3University Hospitals Leuven- Leuven Cancer Institute, Department of General Medical Oncology, Leuven, Belgium; 4University of Essen, Medical Oncology, Essen, Germany; 5Fox Chase Cancer Center, Hematology and Medical Oncology, Philadelphia- PA, USA; 6Erasmus MC Cancer Institute, Medical Oncology, Rotterdam, Netherlands; 7Centre Leon Berard, Medical Oncology, Lyon, France; 8Institut Gustave Roussy, Cancer Medicine and Early Drug Development, Paris, France; 9Blueprint Medicines, Clinical Development, Cambridge- MA, USA; 10Dana-Farber Cancer Institute, Medical Oncology, Boston- MA, USA

This abstract is embargoed until the day of presentation.
Clinical remissions and limited toxicity in a first-in-human multicenter study of bb2121, a novel anti-BCMA CAR T cell therapy for relapsed/refractory multiple myeloma

J.G. Berdeja1, Y. Lin2, N. Raje3, D. Siegel4, N. Munshi5, A. Turka6, L.P. Lam6, M.T. Quigley6, J.N. Kochenderfer7. 1Sarah Cannon Research Institute and Tennessee Oncology, Nashville, TN, USA; 2Mayo Clinic, Rochester, MN, USA; 3Massachusetts General Hospital Cancer Center, Boston, MA, USA; 4Hackensack University Medical Center, Hackensack, NJ, USA; 5Dana Farber Cancer Institute, Boston, MA, USA; 6bluebird bio, Inc., Cambridge, MA, USA; 7Experimental Transplantation and Immunology Branch, National Cancer Institute/National Institutes of Health, Bethesda, MD, USA

This abstract is embargoed until the day of presentation.

Overcoming drug resistance to Trk inhibition by rational combination of entrectinib and trametinib: from bench to bedside

A. Ho1, G. Wei2, E.C. Maneval3, V. Esquibel3, M.F. Berger4, S. Haque5, R. Patel2, C. Walsh2, Z. Hornby5, P. Multani3, G. Li2, A. Drilon1. 1Memorial Sloan Kettering Cancer Center, Department of Medicine, New York, USA; 2Ignyta- Inc., Translational Research, San Diego, USA; 3Ignyta- Inc., Clinical Development, San Diego, USA; 4Memorial Sloan Kettering Cancer Center, Department of Molecular Oncology, New York, USA; 5Memorial Sloan Kettering Cancer Center, Radiology, New York, USA; 6Ignyta- Inc., General Management, San Diego, USA

This abstract is embargoed until the day of presentation.
9LBA Late Breaking Abstract

CHK1 targeting as a therapeutic strategy in soft-tissue sarcomas


1Institut Bergonié, Early Phase Trials Unit, Bordeaux, France; 2Institut Bergonié, INSERM 1218, Bordeaux, France; 3Institut Bergonié, Bioinformatics Unit, Bordeaux, France; 4Institut Bergonié, Department of Pathology, Bordeaux, France; 5Genentech, Genentech, San Francisco, USA

This abstract is embargoed until the day of presentation.

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10LBA Late Breaking Poster

Development and pre-clinical assessment of a first-in-class small molecule inhibitor of FLIP for treatment of NSCLC and CRC

C.A. Higgins1, J. Majkut1, L. Humphreys1, J. Fox1, M. Espona-Fiedler1, A. Malik1, Z. Nemeth1, P. Burton2, R.J. Boffey2, D. Haigh1, T. Harrison1, D.B. Longley1.

1Queens University Belfast, Centre for Cancer Research and Cell Biology, Belfast, United Kingdom; 2Domainex Ltd., Chemistry, Cambridge, United Kingdom

This abstract is embargoed until the day of presentation.

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11LBA Late Breaking Poster

Functional mutational analysis to assess the oncogenic activity of variant of uncertain significance (VUS) detected in patients included in the SHIVA trial

G. Tarcic1, M. Kamal2, O. Edelheit1, Z. Barbash1, M. Vidne1, B. Miron1, C. Callens3, N. Servant4, I. Bilche2, C. Le Tourneau2.

1NovellusDX, Research & Development, Jerusalem, Israel; 2Institut Curie, Department of Medical Oncology, Paris, France; 3Institut Curie, Department of Genomics, Paris, France; 4Institut Curie/INSERM U900, Paris, France

This abstract is embargoed until the day of presentation.
Late Breaking Poster

Ribociclib + letrozole for first-line treatment of HR+, HER2− ABC: efficacy, safety, and pharmacokinetics

F. André1, S.M. Stemmer2, G.N. Hortobagyi3, H.A. Burris4, S. Paluch-Shimon5, M. Campone6, C. Villanueva7, A. Chan8, E. Wist9, N. Marschner10, T. Bachet11, S. Blau12, W. Janni13, S. Verma14, P. Conte15, S. Dhuria16, S. Yang17, M. Slegert17, C. Germa18, C.L. Arteaga18, 1Institut Gustave Roussy- Université Paris Sud, Department of Medical Oncology, Villejuif, France; 2Davidoff Center-Rabin Medical Center- Tel Aviv University, Oncology, Tel Aviv, Israel; 3The University of Texas MD Anderson Cancer Center, Department of Breast Medical Oncology, Houston, USA; 4Sarah Cannon Research Institute, Medical Oncology, Nashville, USA; 5Sheba Medical Centre, Oncology, Ramat Gan, Israel; 6Institut de Cancérologie de l’Ouest/ René Gauducheau, Medical Oncology, Saint-Herblain, France; 7University Hospital of Besançon, Medical Oncology, Besançon, France; 8Breast Cancer Research Centre – Western Australia and Curtin University, Medical Oncology, Perth, Australia; 9Oslo University Hospital, Department of Oncology, Oslo, Norway; 10Joint Practice for Interdisciplinary Oncology and Hematology, Department of Oncology and Hematology, Freiburg im Breisgau, Germany; 11Centre Léon Bérard, Medical Oncology, Lyon, France; 12Rainier Hematology-Oncology- Northwest Medical Specialties, Medical Oncology and Hematology, Puyallup, USA; 13University of Ulm, Department of Gynecology and Obstetrics, Ulm, Germany; 14Tom Baker Cancer Centre, Department of Oncology, Calgary, Canada; 15University of Padova and Istituto Oncologico Veneto- IRCCS, Division of Medical Oncology, Padova, Italy; 16Novartis Pharmaceuticals Corporation, Novartis Pharmaceuticals, East Hanover, USA; 17Novartis Pharma AG, Novartis Pharmaceuticals, Basel, Switzerland; 18Vanderbilt-Ingram Cancer Center, Department of Medicine, Nashville, USA

This abstract is embargoed until the day of presentation.

First-in-human phase I dose escalation study of the Bromodomain and Extra-Terminal motif (BET) inhibitor BAY 1238097 in subjects with advanced malignancies

S. Postel-Vinay1, K. Herbschleb2, C. Massard1, V. Woodcock2, M. Ocker3, G. Wilkinson3, A. Walter3, F. Ewerton3, M. Poelman4, M. Middleton1, J.C. Soria1, 1Institute Gustave Roussy, Department of Drug Development DITEP, Villejuif, France; 2Churchill Hospital- University of Oxford, Department of Oncology, Oxford, United Kingdom; 3Bayer AG, Pharmaceuticals Division, Berlin, Germany; 4Covance, Translational Medicine, Brussels, Belgium

This abstract is embargoed until the day of presentation.
28th EORTC–NCI–AACR Symposium on Molecular Targets and Cancer Therapeutics

Oral abstracts
PROTAC BET degraders are more broadly effective than BET inhibitors

J. Winkler, K. Raina, M. Attieri, H. Dong, J. Wang, X. Chen, A. Crew, C. Crews, Y. Qian, R. Kleinfield, K. Coleman. 1 Arvinas, Discovery, New Haven, USA; 2 Yale University, Molecular, Cellular, and Developmental Biology, Chemistry, and Pharmacology, New Haven, USA; 3 Arvinas, Development, New Haven, USA

This abstract is embargoed until the day of presentation.
A first-in-human phase I study of sEphB4-HSA in patients with advanced solid tumors with expansion at the maximum tolerated dose (MTD) or recommended phase II dose (RP2D)

A. El-Khoueiry¹, B. Gitlitz¹, S. Cole¹, D. Tsao-Wei¹, A. Goldkorn¹, D. Quinn¹, H.J. Lenz¹, J. Nieva¹, T. Dorff¹, M. Oswald¹, J. Berg¹, X. Menendez¹, K. Kankozian¹, V. Krasnoperov², R. Liu¹, J. Thomas¹, S. Groshen¹, P. Gill¹. ¹USC Norris Comprehensive Cancer Center, Oncology, Los Angeles, USA; ²Vasgene, Vasgene operations, Los Angeles, USA

This abstract is embargoed until the day of presentation.

A first-in-human phase I study to evaluate the ERK1/2 inhibitor GDC-0993 in patients with advanced solid tumors

A. Varga³, J.C. Soria³, A. Hollebecque¹, P. LoRusso³, U. Vaishampayan³, K. Okrah³, S.M.A. Huang³, E. Murray³, S. Sanabria-Bohorquez⁴, M. Tagen⁴, H. Dokainish⁵, L. Mueller⁵, H. Burris⁵. ¹Gustave Roussy Cancer Centre, Department of Medicine, Villejuif, France; ²Yale University, Cancer Center, New Haven, USA; ³Karmanos Cancer Center, Solid Tumor Oncology, Detroit, USA; ⁴Genentech, Inc., Research and Early Development, South San Francisco, USA; ⁵Sarah Cannon Research Institute, Oncology, Nashville, USA

This abstract is embargoed until the day of presentation.
Phase 1 study of CB-839, a small molecule inhibitor of glutaminase, in combination with everolimus in patients (pts) with clear cell and papillary renal cell cancer (RCC)

F. Meric-Bernstam1, N. Tannir2, J. Harding3, M. Voss1, J. Mier4, A. DeMichele5, P. Munster6, M. Patel7, O. Ilipoulos8, T. Owonikoko9, S. Whiting10, K. Orford10, M. Bennett11, R. Carvajal12, R. McKay13, A. Fan14, M. Teil14, J. Infante15. 1University of Texas MD Anderson Cancer Center, Department of Investigational Cancer Therapeutics, Houston, USA; 2University of Texas MD Anderson Cancer Center, Department of Genitourinary Medical Oncology, Houston, USA; 3Memorial Sloan Kettering Cancer Center, Medical Oncology, New York City, USA; 4Dana Farber/Harvard Cancer Center, Boston, USA; 5University of Pennsylvania Perelman School of Medicine, Division of Hematology and Oncology, Philadelphia, USA; 6University of California San Francisco Helen Diller Family Comprehensive Cancer Center, Department of Medicine Hematology/Oncology, San Francisco, USA; 7Florida Cancer Specialist & Research Institute, Hematology/Oncology, Sarasota, USA; 8Dana Farber/Harvard Cancer Center, Massachusetts General Hospital, Boston, USA; 9Emory University School of Medicine, Department of Hematology & Medical Oncology, Atlanta, USA; 10Calithera Biosciences, Clinical Development, South San Francisco, USA; 11Calithera Biosciences, Research, South San Francisco, USA; 12Columbia University Medical Center, Division of Hematology/Oncology, New York City, USA; 13Dana Farber Cancer Institute, Department of Breast Oncology, Boston, USA; 14Stanford University Medical Center, Medicine – Oncology, Stanford, USA; 15Tennessee Oncology Oncology, Nashville, USA

This abstract is embargoed until the day of presentation.
Secondary mutations in RAD51C and RAD51D are associated with acquired resistance to the PARP inhibitor rucaparib in patients with high-grade ovarian cancer.

K. Lin1, A.V. Tinker2, O. Kondrashova3, G. Ho3, M. Wakefield3, R.L. Coleman4, D.M. O'Malley5, A. Floquet6, J. Sun1, L. Maloney8, H. Giordano7, T. Harding10, M. Raponi1, I.A. McNeish11, E. Swisher12, C.L. Scott1,13. 1 Clovis Oncology, Inc., Molecular Diagnostics, Boulder, USA; 2 British Columbia Cancer Agency, Department of Medical Oncology, Vancouver, Canada; 3 Walter and Eliza Hall Institute of Medical Research, Department of Medical Oncology, Parkville, Australia; 4 The University of Texas MD Anderson Cancer Center, Department of Gynecologic Oncology and Reproductive Medicine, Houston, USA; 5 The Ohio State University, James Cancer Center, Department of Obstetrics and Gynecology, Columbus, USA; 6 Institut Bergonié, Department of Medical Oncology, Bordeaux, France; 7 Foundation Medicine, Inc., Biomarker Development and Analysis, Cambridge, USA; 8 Clovis Oncology, Inc., Clinical Development, Boulder, USA; 9 Clovis Oncology, Inc., Clinical Science, Boulder, USA; 10 Clovis Oncology, Inc., Translational Medicine, Boulder, USA; 11 Institute of Cancer Sciences, University of Glasgow, Department of Gynecologic Oncology, Glasgow, United Kingdom; 12 University of Washington, Department of Medicine, Seattle, USA; 13 Royal Melbourne Hospital, Department of Oncology, Parkville, Australia.
**Oral abstracts**

**Plenary Session 6, Thursday 1 December 2016**

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**Oral**

**Comparison of PD-L1 expression between tumor tissues and circulating tumor cells in patients with lung cancer**

Y. Koh1, S. Yagi2, H. Akamatsu1, A. Tanaka1, K. Kanai1, A. Hayata1, N. Tokudome1, K. Akamatsu1, M. Higuchi2, H. Kanbara2, H. Ueda1, M. Nakanishi1, N. Yamamoto1. 1Wakayama Medical University, Third Department of Internal Medicine, Wakayama, Japan; 2Hitachi Chemical Co., Ltd, Medical Business Unit, Chikusei, Japan

This abstract is embargoed until the day of presentation.

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**Oral**

**First-in-human phase I trial of the anti-CEACAM5 antibody–drug conjugate SAR408701 in patients with advanced solid tumors (NCT02187848)**

A. Gazzah1, N. Stjepanovic2, M.H. Ryu3, J. Tabernero4, J.C. Soria5, P. Bedard6, Y.K. Kang7, R. Bahleda1, H. Guillemin-Paveau6, C. Henry6, L. Hatteville8, C. Zilocchi7, B. Demers6, C. Hierro9. 1Institut Gustave Roussy, Drug Development Department, Villejuif Paris-Sud, France; 2Princess Margaret Cancer Center, Division of Medical Oncology & Hematology, Toronto, Canada; 3Asan Medical Center, Department of Oncology, Seoul, Korea; 4Vall d’Hebron Institute of Oncology, Medical Oncology Department, Barcelona, Spain; 5Sanofi, Oncology, Alfortville, France; 6Sanofi, Oncology, Vitry-sur-Seine, France; 7Sanofi, Oncology, Milan, Italy

This abstract is embargoed until the day of presentation.

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**Thursday 1 December 2016 15:30–16:30**

**PLENARY SESSION 6**

**Proffered Paper Session**

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**Oral**

**First-in-human phase I trial of the anti-CEACAM5 antibody–drug conjugate SAR408701 in patients with advanced solid tumors (NCT02187848)**

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This abstract is embargoed until the day of presentation.
A phase II study of rapid cycling high dose testosterone (Bipolar Androgen Therapy) in men with metastatic castrate-resistant prostate cancer (mCRPC) resistant to abiraterone and/or enzalutamide

S. Denmeade1, E. Antonarakis1, C. Paller1, H. Wang1, T. Benjamin1, C. Drake1, M. Carducci1, M. Eisenberger1. 1Johns Hopkins University School of Medicine, Oncology, Baltimore, USA

This abstract is embargoed until the day of presentation.

First-in-human study of LY3039478, an oral Notch signaling inhibitor in advanced or metastatic cancer

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This abstract is embargoed until the day of presentation.
28th EORTC–NCI–AACR Symposium on Molecular Targets and Cancer Therapeutics

Poster abstracts
Chemoprevention

Minor modifications to ceritinib enhance the activity against G1202R mutation, in vivo activity, and BBB penetration

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Background: Ceritinib, an ALK inhibitor, was hurriedly approved by the US FDA last year, and demonstrates impressive results in EML4-ALK positive patients. To get a superior ALK inhibitor, we synthesized several ceritinib derivatives with minor modifications to the phenylpiperidin moiety.

Materials and Methods: We made several ceritinib derivatives and conducted the in vitro enzyme assay with ALK mutants, in vivo efficacy assay, and BBB penetration assay.

Results: Biochemical and cellular assays demonstrated the improved activity of KRCA-386 over that of ceritinib. KRCA-386 has superior inhibitory activity against ALK mutants commonly found in crizotinib-resistant patients. Particularly, KRCA-386 has considerably greater activity than ceritinib against the G1202R mutant, one of the most challenging mutations to overcome. The cell cycle analysis indicates that ALK inhibitors induce G1/S arrest results in apoptosis. The in vivo xenograft data also demonstrate that KRCA-386 is significantly better than ceritinib. KRCA-386 dosed at 25 mpk caused 105% tumor growth inhibition (TGI) compared to 72% TGI with ceritinib dosed at 25 mpk. (n=8, P=0.010). The kinase profiling assay revealed that several kinases, which are known to be critical for tumor growth, are inhibited by KRCA-386, but not by ceritinib. We anticipate that this characteristic of KRCA-386 enhances its in vivo efficacy. In addition, KRCA-386 shows excellent blood brain barrier penetration compared to ceritinib.

Conclusion: These results suggest that KRCA-386 could be useful for crizotinib-resistant patients with brain metastases.

No conflict of interest.

Molecular targeted agents I

Inhibition of the oncoprotein FUBP1 by SN-38 represents a novel therapeutic option for the treatment of hepatocellular carcinoma

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Background: Hepatocellular carcinoma (HCC) is a severe complication of advanced liver disease with a worldwide incidence of more than 600,000 patients per year. Standard of care for intermediate and late stage HCC is TACE. However, this therapeutic option lacks consistency of treatment protocols and studies on effective chemo agent choices are missing.

Materials and Methods: Drug-screening was performed using AlphaScreen technology in a medium-throughput manner, the hits were verified using SPR. Cellular effects were investigated by analysis of FUBP1-target gene expression, apoptosis and expansion assays. Mouse models included xenograft transplantation models and orthotopic injection of Hepa129 cells into the liver. Patients were treated using TACE.

Results: In previous studies, we detected significant overexpression of the transcriptional regulator FUSE Binding Protein 1 (FUBP1) in more than 90% of HCCs compared to healthy liver tissue. We could demonstrate that FUBP1 acts as an anti-apoptotic oncoprotein that supports tumor growth regulation of cell cycle inhibitors and pro-apoptotic target genes. We hypothesize that the inhibition of FUBP1 by small molecules may result in a therapeutic benefit for HCC patients. For this purpose, recombinantly expressed FUBP1 was used for an AlphaScreen interaction-displacement assay to identify small molecules that were able to disrupt or prevent the binding of FUBP1 to its single stranded target DNA FUSE. One of the identified inhibitors was camptothecin (CPT). Upon treatment of several HCC cell lines with CPT, a sensitizing effect on apoptosis, as well as reduced cell expansion and cell viability were observed. Furthermore, when treated with CPT, mRNA expression of the cell cycle inhibitor p21 and the pro-apoptotic BCL2 family member BIK increased significantly. Studies with human HCC cell lines transplanted into immunosuppressed mice demonstrated that treatment of HCC with the clinically used CPT derivative irinotecan in combination with mitomycin c lead to complete tumor remission in 100% of the treated mice. Upon retrospective analysis of 6 intermediate stage HCC patients, treated with irinotecan and mitomycin c using transarterial chemoembolisation (TACE), we could show a favourable and beneficial safety profile. We observed one complete remission and three partial responses. To address the efficacy of this treatment, we are planning to start a randomized, multi-center clinical trial.

Conclusion: We propose that camptothecin, in addition to its known function as a topoisomerase I inhibitor, exerts at least parts of its cytotoxic activity via inhibition of FUBP1. Taken together, our data suggest FUBP1 as a potential therapeutic target in primary HCC, and we are continuing to test and optimize further potential FUBP1 inhibitors that are structurally unrelated to CPT for future therapy.

No conflict of interest.
of NSCLC exposure, BGB324 restricts cellular plasticity and prevents the development of resistance to Epithelial Growth Factor Receptor (EGFR) inhibitors through mesenchymal transformation.

**Patients and Methods:** BGB324 was administered at an oral loading dose (600 mg) on days one and two followed by a daily maintenance dose (200 mg) to eight patients with previously treated NSCLC (EGFR mutant or wildtype). The same dose of BGB324 was then administered in combination with erlotinib to patients who had previously experienced disease progression on erlotinib with EGFR mutated NSCLC (non-T790M).

**Results:** Two of eight patients treated with BGB324 monotherapy achieved at least six months of stable disease (ongoing). The most common treatment related adverse events were increased serum creatinine, diarrrhea and nausea. No Grade 3 or 4 adverse events were reported and no patients discontinued treatment as a result of a toxicity. Treatment with BGB324 was accompanied by increases in patient serum levels of soluble AXL receptor consistent with receptor inhibition. Additional biomarkers that are being explored as potential predictive markers include the presence or absence of a transcriptional EMT signature (previously associated with increased AXL expression and erlotinib resistance in cell lines and patient tumors) and/or AXL protein expression.

**Conclusion:** BGB324 can be safely administered to patients with advanced NSCLC for prolonged periods at doses that abrogate AXL signalling. A proportion of patients achieve durable disease stabilisation following treatment with BGB324 alone. Further data is being collected exploring BGB324 in combination with erlotinib in the EGFR mutant population. The results of combination therapy will be presented at the meeting.

**No conflict of interest.**
Clinical responses to ERK inhibitor (GDC-0994) treatment combinations predicted using a Quantitative Systems Pharmacology model of MAPK signaling in BRAF/V600E-mutant colorectal cancer

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Background: Approximately 10% of colorectal cancers (CRC) harbor BRAFV600E mutations, driving constitutive activation of the MAPK signaling pathway and a poor prognosis. However, these patients do not respond to BRAF and MEK inhibitor treatments as in BRAFV600E-mutant melanoma. Here we describe a mechanism-based translational model which predicts sensitivity of BRAFV600E-CRC tumors to MAPK inhibitor combinations, including the ERK inhibitor GDC-0994, and the prospective benefit of such treatments to patients.

Material and Methods: A mechanism-based computational model, linking cell surface receptor (EGFR) engagement, the MAPK cascade, feedback mechanisms (i.e. DUPI SPRY phosphatases) and tumor growth regulation was constructed from published literature and model validation was performed in a step-wise manner. First, signal transduction parameters were estimated using mutation-response data and a system of logic-based and ordinary differential equations in MATLAB SimBiology™. Parameter estimation and model validation was performed in a step-wise manner. First, signal transduction parameters were estimated using mutation-response data taken from published literature (i.e. cell viability in BRAFV600E-CRC lines ± treatment). Next, drug-target IC50 values were estimated from dose- viability curves in panels of BRAFV600E-CRC lines treated with BRAF (vemurafenib), MEK (cobimetinib) and ERK (GDC-0994) inhibitors. In vivo tumor proliferation and turnover rates were estimated from tumor growth kinetics in cell line and patient-derived xenografts treated with EGFR (cetuximab), BRAF (vemurafenib), MEK (cobimetinib), and ERK (GDC-0994) inhibitors. To evaluate the effects of BCKD inhibition in vivo, a virtual population of BRAFV600E-CRC patients, using tumor response data from three published Phase 1 clinical trials testing combinations of EGFR, BRAF and/or MEK inhibitors along with population-pharmacokinetics.

Results: GDC-0994 treatment (400 mg, daily) was predicted to be the most effective monotherapy, with an estimated 16% ORR. Synergistic activity was predicted for the combination with cobimetinib (60 mg, daily), increasing to 30% ORR. While significant, this falls far below the 80% ORR achievable in BRAFV600E-melanoma. Loss of cellular dependence on continued MAPK signaling was predicted as the main driver of resistance. That is, approximately 2/3 BRAFV600E-CRC tumors harbor clones capable of activating other oncogenic pathways (e.g. PI3K/AKT, JAK/STAT, or epithelial–mesenchymal transition), and thus survive and proliferate despite MEK/ERK inhibition.

Conclusion: We predict that increasing response rates to MAPK inhibition in BRAFV600E-CRC will necessitate either the use of predictive biomarkers to pre-select patients with increased MAPK-dependence, or combination with agents targeting orthogonal oncogenic pathways or survival mechanisms.

Conflict of interest: Ownership: All authors are employees of Genentech Inc. and shareholders of Roche Holdings Inc.

Inhibition of BCKDK increases the sensitivity of ovarian cancer cells to paclitaxel

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Introduction: Ovarian cancer is inadequately treated because many tumors become resistant to chemotherapy. A siRNA screen to identify genes which regulate the sensitivity of ovarian cancer cells to chemotherapy identified BCKDK as a potential determinant of sensitivity to paclitaxel. The metabolism of branched-chain amino acids (BCAA) has been associated with multiple cellular functions comprising gene transcription, translation and autophagy, amongst others as well as hormone secretion and signalling. Cancer and some other clinical conditions lead to substantial fluctuations in plasma levels of BCAA. The branched-chain α-ketoadic dehydrogenase (BCKD) complex is a mitochondrial multi-enzyme complex responsible for oxidative decarboxylation of the branched-chain α-ketoadic derived from the BCAA.

Regulation of BCKD activity occurs through the action of a Branch-chain α-ketoadic dehydrogenase kinase (BCKDK), which inhibits it by phosphorylation. Overexpression of BCKDK in cancer cells inhibits the BCKD complex leading to high BCAA, activation of mammalian target of rapamycin (mTORC1) and inhibition of autophagy.

Materials and Methods: the study was designed to evaluate the contribution of BCKDK to the sensitivity of ovarian cancer cells to paclitaxel. The activity of BCKDK was inhibited by using siRNA targeted to BCKDK, and also by using BCKDK inhibitor (S-(2-Chloro-4-methylpyridyl-2-amino)-3,4-Dichloro-1-Benzenophene-2-Carboxylic acid (DBCA)). The activity of paclitaxel was evaluated using SRB cell proliferation assay and the caspase 3/7 activity was used to quantify the level of apoptosis. A combination index was calculated in order to evaluate the efficacy of drug combinations.

Results: siRNA knockdown of BCKDK increased the sensitivity of Ovar-4 cells to paclitaxel in cell growth assays (paclitaxel IC50 6.8±2.2 μM (non-targeting siRNA), 1.9±0.2 μM (siRNA #1), 1.3±0.5 μM (siRNA #2), and 2.3±0.6 μM (siRNA #3). Knockdown of BCKDK also potentiated the activation of caspase 3/7 by paclitaxel more than the combined effect expected from applying the Bliss independence criterion to the effects of the single agents. CMVA and DBCA, reduced the phosphorylation of BCKD in ovacr-4 and COV-318 cells but not in COV-362 cells. Reflecting this, CMVA and DBCA were synergistic (Combination index values are <0.8) with paclitaxel in cell growth assays using Ovar-4 and COV-318 cells, but additivity was observed in COV-362 cells (CI > 1). Hap1 cells gene-edited to lack BCKDK also showed altered sensitivity to paclitaxel.

Conclusion: These results suggest that BCKDK is a novel target to sensitize ovarian cells to paclitaxel.

No conflict of interest.

B-701, a selective and potent inhibitor of fibroblast growth factor receptor 3 (FGFR3), may enhance the activity of bladder cancer therapies

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Background: B-701 is a fully human monoclonal antibody that selectively binds to and inhibits Fibroblast Growth Factor Receptor 3 (FGFR3), a protein that is highly expressed in a large subset of advanced urothelial cancers. Studies described explore whether B-701, by blocking signaling through FGFR3, can enhance the effects of either traditional chemotherapy or immune checkpoint blockade.

Materials and Methods: In separate subcutaneous mouse tumor models, B-701 was combined with either i) paclitaxel or gemcitabine or ii) antibodies to PD-1. To evaluate the effects of B-701 in combination with paclitaxel or gemcitabine, the human UM-UC-1 bladder cancer cell line, which expresses wildtype FGFR3, was tested. Efficacy was assessed using tumor growth delay in a conditional survival study.

To evaluate the effects of B-701 in combination with PD-1 antibodies, a syngeneic colorectal carcinoma model, MC38, that was shown to express FGFR3 was used, as no syngeneic bladder cancer models were available at the time of the study. Tumor growth inhibition was assessed throughout the study. To further explore the effects of B-701 in the MC38 model, an additional study was performed in which tumors were harvested after treatment with single-agent B-701 and analyzed by flow cytometry to assess immune cell infiltrate.

Results: In the UM-UC-1 xenograft model, when used as single agents, each of the agents tested (B-701, paclitaxel and gemcitabine) significantly extended survival of tumor bearing mice. When B-701 was combined with either paclitaxel or gemcitabine, effects of single agents were greatly enhanced and a significant increase in conditional survival was observed. While tumor growth was highly variable in the MC38 model, it appears that combining agents may enhance tumor growth inhibition. This was particularly apparent in tumors that were large at study initiation. In a subsequent study in which the immune infiltrate in tumors was evaluated using flow cytometry, we found that as tumors establish, treatment with B-701 alone may improve the immunological status of the tumor.

Conclusions: Even with the advent of immune checkpoint blockade, advanced urothelial cancer continues to be a deadly condition. B-701, by blocking signaling through FGFR3, represents a novel and selective agent that can enhance the efficacy of both traditional and novel drugs currently being used to treat urothelial cancer. Preclinical models described here show that combining B-701 with chemotherapy leads to greatly enhanced efficacy. In addition, B-701 treatment can enhance the immune environment of tumors leading to improved clinical activity of immune checkpoint inhibitors.

Conflict of interest: Ownership: All authors are employees of BioClin Therapeutics, Inc.
Development of BT1718, a Bicycle Drug Conjugate (BDC) targeting MT1-MMP for treatment of solid tumours

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Background: BT1718 is a Bicycle drug conjugate (BDC) comprising a constrained bicyclic peptide that binds with high affinity and specificity to membrane type 1-matrix metalloprotease (MT1-MMP). MT1-MMP is overexpressed in highly invasive breast cancer, and the inhibition of MT1-MMP has been shown to inhibit cancer cell invasion and promote apoptosis. To expand on our previous finding, we used publicly available gene expression data from a large cell line panel to identify four cell lines of interest for the development of BT1718.

Results: We identified four cell lines that were sensitive to Phosphodiesterase inhibitors. A phase I study of the MDM2 antagonist RO6839921, a pegylated prodrug of idasanutlin, for intravenous (IV) administration in patients with advanced solid tumours

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Background: p53 is a tumor suppressor regulated by MDM2. Idasanutlin (idas) is an oral MDM2 antagonist in development. R06839921, an oral MDM2 antagonist in development.
inactivate pegylated prodrug of ida, was developed for IV administration with the goal of reducing exposure variability, and thus increasing safety and efficacy. The active principle (AP = ida) is released upon cleavage by plasma esterases. We report the safety, pharmacokinetic (PK) and pharmacodynamic results in a Phase 1 study in solid tumor patients (pts).

Methods: Based on experience with ida, a 5 day (d) (d) dosing schedule q28d (QD 5/28d) was evaluated. An initial cohort was treated with 14 mg AP to confirm preclinical predictions regarding cleavage in humans. Following this cohort, dose escalation proceeded using the Bayesian New Continual Reassessment Model (nCRM) with limits on maximum dose increments. Accelerated dose titration was permitted until Grade 2 or higher drug related adverse events (AEs) were observed. Subsequent cohorts were enrolled at least 3 pts. The target toxicity interval used to determine the maximum tolerated dose (MTD) was defined as a 16–25% dose limiting toxicity (DLT) rate. p53 activation was assessed by measuring serum levels of a transcriptional target macrophage inhibitory cytokine-1 (MIC-1).

Results: 41 solid tumor pts were enrolled at doses between 14–120 mg AP as summarized in the table; 39 were DLT-evaluable. Using the nCRM model, the dose was escalated to 120 mg AP. 4/9 pts at 120 mg AP had DLT events and the dose was de-escalated. 110 mg AP was the MTD; a total of 14 patients were enrolled to confirm safety/ PK. The most common related AEs (>30%) at MTD were nausea (11/14, 79%), fatigue (8/14, 57%), and abdominal pain (5/14, 36%). The most common related AEs > grade 3 were thrombocytopenia (TCP) and neutropenia (ANC) (both 3/14, 21%). PK analyses showed rapid and near complete conversion of prodrug to AP and dose-proportional exposure across the dose range tested. There was ~40% decrease in exposure with a similar overall safety profile compared to historical ida data. TCP and ANC were the main DLTs, while gastrointestinal events, primarily grade 1–2, were the most frequently observed AEs. MIC-1 increase was exposure dependent. Stable disease was seen in 14/41 (34%) pts; median number of treatment cycles in these patients was 5 (range 1–11 cycles).

Conclusions: ROC838929 shows reduced PK exposure variability compared to ida and an acceptable safety profile at the MTD of 110 mg AP QD 5/28d. MIC-1 increase was seen at doses < MTD suggesting that lower doses may be considered when combining with agents with overlapping toxicities. NCT02098697.

Conflict of interest: None.

46 Poster (Board P017)

An open-label, multi-center, phase III, dose escalation study of IV TKM-080301 in subjects with advanced hepatocellular carcinoma

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Background: Polo-like kinase 1 (PLK1) is a serine/threonine kinase which regulates multiple critical aspects of cell cycle progression and mitosis. It is highly expressed in many human tumors including hepatocellular carcinoma (HCC), and its expression correlates negatively with patient outcomes. TKM-080301 is a lipid nanoparticle (LNP) formulation of a small interfering RNA (siRNA) directed against PLK1. This open-label study is conducted in HCC patients (NCT02191876) sponsored by Arbutus Biopharma Corporation.

Methods: Subjects had metastatic or locally advanced inoperable HCC refractory to or ineligible for standard therapy; prior exposure to local or systemic therapy; Child Pugh A liver function; and adequate organ function. All subjects gave informed consent and regulatory approvals were obtained. After premedication, TKM-080301 was administered as a 2-hour intravenous infusion on Days 1, 8, and 15 of a 28-day cycle. Objectives included evaluation of anti-tumor activity per RECIST 1.1 criteria and exploratory evaluation by Choi criteria.

Results: Forty-three subjects (36 males, 7 females) with an age range of 40–80 years (mean 62.6 years) were treated. Baseline risk factors for HCC included HBV (58.1%), HCV (14.0%), excess alcohol consumption (7.0%), none (20.9%) and other (2.3%). Sequential cohorts reached needed doses of 0.3 (n = 3), 0.6 (n = 5) and 0.75 mg/kg (n = 3) TKM-080301. An expansion cohort (n = 32) was initiated at 0.75 mg/kg then reduced to 0.6 mg/kg due to Grade 4 thrombocytopenia (n = 1) and Grade 3 hypotension (n = 1). The median (range) number of cycles in the cohort was 2 (1–14). Grade 3 or 4 AEs related to TKM-080301 (>5%) were: AST increase (16.3%), ALT increased (9.3%), and thrombocytopenia (7%). Treatment-related thrombocytopenia led to discontinuation in 3 subjects. Of 39 subjects evaluated locally for tumor response, 51% (20/39) had an overall tumor response (n = 8). (95% CI 4.4–12.4) and 2 mos. (95% CI 1.6–3.4) respectively.

Conclusions: The tolerable TKM-080301 dose was 0.6 mg/kg without significant hepatotoxicity of LNP in a population with prevalent underlying liver disease. Radiographic tumor density changes suggested tumor necrosis in a significant proportion of subjects and warrants further study.


47 Poster (Board P018)

Combination targets for the treatment of ovarian cancers with phosphatidylinositol 3-kinase inhibitors

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Background: The phosphatidylinositol 3-kinase (PI3K) pathway is frequently hyper-activated in cancers, including ovarian carcinomas. However, PI3K inhibitors have generally not given the therapeutic benefit hoped for as single agents in solid tumours. The aim of this project is to identify proteins that affect the response to PI3K inhibitors in clear cell ovarian carcinoma. Such proteins may serve as targets for agents to be used in combination with PI3K inhibitors.

Material and Methods: We have taken an unbiased screening approach, to identify potential targets for combination with PI3K inhibition in human clear cell ovarian carcinoma cell lines. We examined the effects of PI3K inhibition combined with 485 known cancer drugs or tool compounds. Those compounds, which acted synergistically with PI3K inhibition to reduce carcinoma cell viability, were identified using the syngenic index score.

Results: The compound screen identified a number of synergistic interactions with inhibitors of several classes of targets. We identified MEK inhibitors as one therapeutic combination (n = 1). The median (range) number of cycles for combination of PI3K inhibitor with MEK inhibitor using combination cell proliferation studies and obtained a combination index using the Chou Talalay method. We also found additional classes such as HDAC and bromodomain inhibitors.

Conclusions: The screen we performed showed that in principle synergistic interactions between PI3K inhibition and compounds with known protein targets, such as MEK, could be identified using this method. Based on these results, we plan to use a pooled lentiviral shRNA whole-genome library to identify novel protein targets that show synthetically lethal interactions with PI3K inhibitors.


48 Poster (Board P019)

Characterisation of TYK2 inhibitors as potential T-cell acute lymphoblastic leukaemia therapeutics

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Background: TYK2, a member of the JAK family, is believed to play an important role in the signalling of type I interferons, as well as IL-12 and
IL-23, via phosphorylation of STATs. The TYK2-STAT1-BCL2 pathway is implicated in the survival of leukemic cells in many T-ALL cases, with validation by gene knockdown and non-selective JAK inhibitors which induced T-ALL cell death. We have previously reported on SAR-20347, a potent and selective inhibitor of TYK2, showing a striking reduction in clinical score in a disease model of psoriasis. Here we report the effects of SAR-20347 and analogs on proliferation and TYK2-STAT1 signalling in in vitro and in vivo models of T-ALL.

Material and Methods: Proliferation assays were performed against 5 T-ALL cell lines with ATLPiThe readout. Kinetics of apoptosis were measured using the CellPlayer™ Caspase 3/7 reagent and an IncuCyte kinetic kinetic endpoint. The effect of compounds on the phosphorylation of TYK2, JAK1, STAT1, STAT3 and STAT5 was assessed by Western blot in Jurkat cells following stimulation with IFNα. The effect of compound exposure on cell death was assessed by Western blot analysis of PARP cleaved and cleaved Caspase 3 in T-ALL patient MOLT4 cells. For in vivo PD studies, MOLT-4 cells were implanted into SCID mice and tumors allowed to grow to 200–250 mm³, compounds dosed p.o., and apoptosis induction and pathway inhibition determined by Western blotting. For in vivo efficacy studies, MOLT-4 cells were implanted into SCID mice and allowed to grow to 150–200 mm³. Compounds were dosed p.o., BID for 21 days and bodyweight and tumour volume were measured 3x per week.

Results: Compounds inhibited proliferation and induced apoptosis of T-acute cell lines with IC₅₀ values in the low μM range. In common with other JAK inhibitors of JAKs, activation loop phosphorylation of TYK2 increased, but pSTAT1, pSTAT3 and pSTAT5 were decreased in the presence of compounds following IFNα stimulation of Jurkat cells. In the absence of IFNα, only CCRF-HSB-2 cells demonstrated STAT phosphorylation sufficient for detection by Western blotting, with levels decreasing following compound treatment and a modest reduction in BCL2 levels observed at optimal concentrations (+/-1μM).

In vivo experiments showed good exposure of compound in plasma & tumor following a single oral dose of SAR-20347, correlating with increased pTYK2, reduced pSTAT1 and induction of cleaved PARP. BID dosing for 21 days in a xenograft model was well tolerated and showed a dose-dependent reduction in tumor.

Conclusions: ATP competitive inhibitors of the kinase domain of TYK2 have an anti-proliferative effect, by inducing apoptosis, on T-ALL cell lines in vitro and in vivo. A robust and dose dependent effect on STAT phosphorylation is observed, accompanied by a modest reduction in BCL2 levels, reinforcing earlier reports that targeting TYK2 could be a therapeutic strategy in T-ALL patients.

Conflict of interest: Ownership: Shareholder of Sareum Holdings plc. Sareum Limited is a wholly-owned subsidiary of Sareum Holdings plc. Board of Directors: Director of Sareum Limited Director of Sareum Holdings plc
51 Preclinical evaluation of anti-CD269 antibody–drug conjugates

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Background: ATACs (antibody-targeted Amanitin conjugates) comprise a new class of antibody–drug conjugates using aminatin as toxic payload. Amanitin binds to the eukaryotic RNA polymer II and thereby inhibits the cellular transcription process at very low concentrations. In the current study, in vitro and in vivo data of new ATACs targeting CD269 are presented. CD269 (also known as B cell maturation antigen; BCMA) is highly expressed on malignant plasma cells like in Multiple Myeloma (MM). Since relapsed and refractory MM is an incurable malignancy of plasma cells, new therapies are urgently needed.

Materials and Methods: MM cell lines: NCI-H929, MM.1S Luc (stable luciferase transfected) and CCRF-CEM (CD269 negative).

Antibody: anti-CD269 J22.9-1S Thiomab (provided by Max Delbrück Centrum, Berlin; derivationization and production at Heidelberg Pharma).

Synthesis of ATACs: Maleimide aminatin compounds HDP 30.1699 and HDP 30.2115 were conjugated to substituted cysteine residues of the anti-CD269 Thiomab.

Cell proliferation assay: Quantitative determination of cytotoxicity was performed by CellTiter Glo 2.0 assay (Promega) or WST-1 assay (Roche).

Animal models: subcutaneous and metastatic Mouse xenograft tumor models with MM cell lines NCI-H929 and MM.1S Luc were performed in single-dose and multiple-dosing experiments. Tolerability was assessed in mice and non-human primates.

Results: Anti-CD269-ATACs showed in vitro cytotoxicity on CD269+ cell lines in picomolar range, whereas no cytotoxic activity on CD269- cells was observed.

Table 1: EC50 [M] values of anti-CD269-ATACs on NCI-H929, MM.1S Luc and CCRF-CEM cells after incubation for 96h

<table>
<thead>
<tr>
<th>Cell line</th>
<th>J22.9-1S-Thiomab-30.1699</th>
<th>J22.9-1S-Thiomab-30.2115</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI-H929</td>
<td>7.0 × 10^-11</td>
<td>9.7 × 10^-11</td>
</tr>
<tr>
<td>MM.1S Luc</td>
<td>6.5 × 10^-11</td>
<td>2.4 × 10^-10</td>
</tr>
<tr>
<td>CCRF-CEM</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

In mouse xenograft models, the anti-CD269-ATACs showed dose-dependent tumor regression and complete remission after single dose i.v. of 2.0 mg/kg and 4.0 mg/kg and multiple doses i.v. between 0.1 mg/kg and 2.0 mg/kg. Safety profiling in Cynomolgus monkey revealed a good tolerability index and therapeutic index as well as multiple doses applied doses of 0.3, 1.0, 3.0 mg/kg and multiple dose application of 3x 3.0 mg/kg.

Conclusions: Targeted cytotoxic drug delivery to CD269 positive MM cell lines was achieved by using anti-CD269-ATACs. The mode of action of the payload aminatin led to an efficient anti-tumorigenic potential in vitro and in vivo. Using ADCs for the treatment of MM could be a promising approach, especially by using a cytotoxic agent whose mode of action was not applied before, like ATACs. The preclinical findings warrant the clinical development of anti-CD269-ATACs.

No conflict of interest.

52 Cell panel profiling of pre-clinical and clinical anti-cancer agents reveals conserved therapeutic clusters and differentiates the mechanism of action of different PI3K/mTOR, Aurora kinase and EZH2 inhibitors

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Background: Cancer cell line panels are important tools to characterize the in vitro activity of new investigational drugs.

Materials and Methods: Here we present the inhibition profiles of 122 anti-cancer agents in proliferation assays with 44 or 66 genetically characterized cancer cell lines from diverse tumor tissue origin (Oncolines®). The library includes 29 cytotoxicites, 68 kinase inhibitors and 11 epigenetic modulators. For 55 compounds this study provides the first profile in a major comparative cancer cell line panel. By strictly maintaining optimized assay protocols, biological variation was kept to a minimum.

Results: Replicate profiles of 16 agents over three years show a high average Pearson correlation of 0.9. Good correlations were observed with other panels. Curve fitting appears the largest source of variation. Hierarchical clustering revealed 44 basic clusters, of which 27 contain compounds with common mechanisms of action, of which 14 were not reported before, including TTK, BET and two clusters of EZH2 inhibitors. To investigate unexpected clusterings, sets of BTX, Aurora and PI3K inhibitors were profiled in biochemical enzyme activity assays and surface plasmon resonance binding assays.

Conclusions: The BTX inhibitor brinzolamide clusters with EGFR inhibitors, because it crosses-reacts with EGFR. Aurora kinase inhibitors separate into two clusters, related to Aurora A or pan-Aurora selectivity. Similarly, twelve inhibitors in the PI3K/AKT/mTOR pathway separated into different clusters, reflecting biochemical selectivity (pan-PI3K, PI3K(α)-isoform selective or mTOR-selective). Of these, only allosteric mTOR inhibitors preferentially targeted PTEN-mutated cell lines. Cell line profiling is therefore a good tool for the unbiased classification of antiproliferative compounds.

53 Overcoming anti-VEGF therapy resistance through use of PMN-MDSC-derived PyNPase

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Background: Although angiogenesis inhibitors, including bevacizumab, have been providing substantial clinical benefits to various malignancies, clinical resistance to their therapeutic effects has been a critical issue on their extensive use. Some preclinical studies suggested that myeloid-derived suppressor cells (MDSCs) would regulate the tumor refractoriness to anti-vascular endothelial growth factor (VEGF) treatment via immune-related and escaping anti-angiogenic mechanisms; however, clinical approach to MDSC-mediated resistance has not been determined yet.

Methods: Murine Lewis lung carcinoma (LLC, resistant to anti-VEGF Ab) was s.c. inoculated into C57BL/6 WT mice or thymidine phosphorylase (TP)−/−/uridine phosphorylase (UP)−/− knockout mice. Mice were randomly allocated to control and treatment groups after tumor formation. Anti-VEGF Ab (5mg/kg, weekly), anti-G-CSF Ab (4mg/kg, daily) and 5-fluourouracil (50mg/kg, twice a week) were i.p. administrated, and capectabine (Cape) (718mg/kg, daily) was p.o. administered for 18 days. Cytokine levels were analyzed by ELISA and cytometric bead array. Polymorphonuclear (PMN)-MDSCs were identified as CD11b+Gr−/− knockout mice. Mice were assessed by immunobiochemistry by CD31-immunostaining.

Results: Anti-VEGF Ab accelerated the intratumor recruitment of CD11b+Gr−/− PMN-MDSCs specifically in LLC tumor. This process was mediated by enhanced expression of intratumor G-CSF as previously identified; however, neither IL-17 nor Bv8 was likely to be involved in this model. We found that CD11b+Gr−/− PMN-MDSCs expressed TP and capectabine, a pro-drug converted to 5-fluorouracil via TP or UP, effectively eliminated such type of MDSCs from both tumor and peripheral blood. Importantly, capectabine-treatment restored inhibition both of tumor angiogenesis and tumor growth under treatment of anti-VEGF antibody, and thus effect was partly canceled in tumors implanted in mice deficient both with TP and UP.

Conclusion: These results not only further confirmed the essential role of G-CSF and the recruitment of CD11b+Gr−/− PMN-MDSCs as a cause of tumor resistance to anti-VEGF, but also revealed that capectabine overcome this resistance through elimination of PyNPase-expressing PMN-MDSCs.

No conflict of interest.
A novel anti-androgen candidate galetinone targets by using USP12, a deubiquitinating enzyme that controls prostate cancer growth and survival

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Background: Galetinone is a highly selective, orally bioavailable small molecule drug candidate that disrupts androgen receptor (AR) signalling through AR degradation. Galetinone is also a potent CYP17 inhibitor and AR antagonist. We previously demonstrated that USP12 functions as an AR co-activator by deubiquitinating the AR and stabilizing its protein levels. USP12 protein targets the PHLPPh phosphatases and thereby reduces the level of activated AKT (pAKT) and as such indirectly stabilizes the AR. Galetinone has previously been reported to induce pAKT and prostateational inhibition abrogates galetinone’s ability to induce AR degradation consistent with the involvement of the ubiquitination pathway. Our project focused on determining the main enzymes involved in prostate cancer AR degradation.

Materials and Methods: We utilised a series of biochemical, biophysical and cell-based in vitro methodologies to elucidate the enzymes responsible for the proteasomal-dependent mechanism of galetinone-induced AR degradation. We screened a panel of 22 deubiquitinating enzymes (DUBs) in vitro to test whether galetinone inhibited enzymatic activity. Surface plasmon resonance (SPR) was applied to confirm direct binding to DUBs which were inhibited by galetinone. We then used next-generation RNA sequencing and immunobiochemistry to determine the role of USP12 in prostate cancer (PCa).

Results: We discovered that galetinone acts on AR by selectively inhibiting USP12, a DUB that relies on UAF1, for its enzymatic activity. Galetinone dose-dependently bound to USP12, alone or when pre-complexed with UAF1. Global transcriptome profiling identified that in addition to regulating the AR signalling cascade, USP12 controls the p53 signalling pathway. USP12 directly targets the E3 MDM2, stabilising MDM2 protein and consequently controlling p53 protein levels. The clinical importance of USP12 was confirmed in PCa samples where increased USP12 was found to be a marker of poor prognosis that correlated with shorter relapse-free survival and reduced overall survival. Additionally, USP12 may play a role in the development of castration-resistant PCa (CRPC) as USP12 protein levels were significantly increased in CRPC patients from two independent clinical cohorts.

Conclusions: We report that galetinone, a candidate small molecule drug currently in a phase 3 registration trial in metastatic CRPC inhibits AR activity in prostate cancer by inhibiting the AR, namely USP12. We determined that USP12 additionally controls the p53 pathway with USP12 protein levels elevated in PCa and associated with decreased relapse-free survival and overall survival. Our results indicate that galetinone could offer a therapeutic alternative in PCa treatment as no other anti-androgens to date have been shown to inhibit the DUBs that regulate AR levels in PCa.

No conflict of interest.

Poster Session – Molecular targeted agents I, Wednesday 29 November 2016

Poster abstracts

Poster(BoardP025)

Poster(boardP026)

Poster(boardP027)
58 Poster (Board P029)

TPC-107, a next generation, HER2 selective covalent inhibitor demonstrates potent and sustained inhibition against the HER2–HER3 signaling while sparing EGFR activity, leading to a large therapeutic window

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Background: HER2 (human epidermal growth receptor 2) is a member of ERBB family kinases. The amplification of HER2 is a well-established therapeutic target in breast and gastric cancer. Although there are several reports of pan-ERBB covalent inhibitors in development, a selective covalent HER2 inhibitor is yet to be reported. Diarrhea is one of the most common adverse events for EGFR and HER2 TKIs, and the most efficacious dose for these inhibitors needs to be found. However, the high structural similarity between EGFR and HER2, the development of a selective HER2 inhibitor remains very difficult. Here we report on a highly selective, covalent HER2 inhibitor that blocks HER2 and the downstream substrate HER3, with reduced activity against wild type EGFR.

Materials and Methods: Selectivity for kinases by TPC-107 was determined in a panel of 396 kinases. For growth inhibition studies, cell lines were treated with TPC-107 for 3 days, and the number of living cells was determined. To evaluate the effect of TPC-107 and lapatinib (a reversible HER2/EGFR dual inhibitor) on HER2-HER3 signaling, the phosphorylation level of HER2, HER3 and the downstream pathway molecules in HER2 positive breast cancer SKBR3 cells were examined after 3 or 48 hours treatment by Western blot analysis. For evaluation of efficacy of TPC-107 and lapatinib in vivo, we dosed the compounds orally in a N87 (HER2 positive gastric cancer cell line) peritoneal dissemination xenograft model.

Results: TPC-107 was a potent inhibitor of HER2 with an IC50 value of 13 nM, and demonstrated little activity against the majority of other kinases, including EGFR. TPC-107 exhibited antiproliferative activity only against HER2 activated cells across various cell lines. In SKBR3 cells, although lapatinib initially inhibited pHER2, pHER3, and the downstream pathway at 100 nM after 3 hours incubation, reactivation of those molecules was observed after 48 hours of continuous exposure. In contrast, TPC-107 exhibited a sustained and robust inhibition of pHER2, pHER3, and the downstream signaling pathway at 100 nM during 48 hours treatment. Consistent with these results, TPC-107 showed superior efficacy compared to lapatinib in N87 peritoneal dissemination xenograft model without any evident toxicities including diarrhea.

Conclusion: TPC-107 is a highly potent, selective inhibitor of HER2. TPC-107 demonstrated sustained and robust inhibition of HER2-HER3 in vivo and a survival benefit in a HER2-positive xenograft model without diarrhea. These data suggest that TPC-107 may be a promising therapeutic option for HER2 amplified cancers. Further clinical development of TPC-107 may have an improved therapeutic window compared to current HER2 inhibitors.

No conflict of interest.

59 Poster (Board P030)

Combination treatment with novel GLUT1 inhibitors and sorafenib in hepatocellular carcinoma

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Background: Hepatocellular carcinomas (HCC) are characterised by having increased glycolysis and reduced oxidative phosphorylation relative to normal hepatocytes, supporting that a therapeutic index in HCC could be obtained through inhibition of glucose transport. This study aimed to characterise the effects of combining novel GLUT1 inhibitors with sorafenib, the standard of care drug in HCC.

Materials and Methods: The IC50 values and combination indices of combination treatment of 6 HCC cell lines with sorafenib and four GLUT1 inhibitors (OMI1–4) were assessed by MTS assay and the method of Chou and Talalay, where CI < 1 denotes antagonism, > 1 an inhibition and CI = 1 synergy. Phenotypic analysis was carried out using standard techniques.

Results: Combination of GLUT1 inhibitors and sorafenib led to at least additivity or synergy (Table 1), at least a 3-fold reduction in the IC50s of both GLUT1 inhibitors and sorafenib was noted when combined together. However, the combination of IOM4 and sorafenib was selected for further analysis as a result of desirable pharmacokinetic properties of IOM4 for future in vivo evaluation. Glucose consumption was decreased at 3h.

No conflict of interest.
or 24 h by IC50 concentration of IOM4 but no observable effects were observed post-sorafenib exposure. However, the combination of sorafenib and IOM4 led to significant decrease in glucose consumption, irrespective of synergy or additivity. Levels of ATP were also significantly reduced by the combination in all HCC cells which was associated with a decrease in viability. Synergy or additivity was not associated with an increase in early (3 h or 24 h) apoptotic cell death, but with significant increase in G1 cell cycle arrest in response to the combination compared to single agents. Furthermore, the combination significantly reduced levels of extracellular lactate at 24 h but not reactive oxygen species (ROS) levels.

**Conclusion:** Combination treatment with GLUT1 inhibitors and sorafenib in HCC can be synergistic or additive, and can be a strategy to reduce dosage requirements and glucose metabolism in HCC.

### Table 1. Combination Indices @ f0.5 of GLUT1 inhibitor and Sorafenib in HCC cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>IOM1 + Sorafenib</th>
<th>IOM2 + Sorafenib</th>
<th>IOM3 + Sorafenib</th>
<th>IOM4 + Sorafenib</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3A</td>
<td>0.89 ± 0.05</td>
<td>0.65 ± 0.19</td>
<td>0.58 ± 0.09</td>
<td>1.26 ± 0.42</td>
</tr>
<tr>
<td>PI330</td>
<td>1.00 ± 0.01</td>
<td>0.81 ± 0.07</td>
<td>0.81 ± 0.07</td>
<td>1.26 ± 0.13</td>
</tr>
<tr>
<td>SNUR449</td>
<td>0.45 ± 0.18</td>
<td>0.61 ± 0.11</td>
<td>0.57 ± 0.13</td>
<td>1.26 ± 0.13</td>
</tr>
<tr>
<td>SKHEP</td>
<td>0.61 ± 0.13</td>
<td>0.49 ± 0.05</td>
<td>0.91 ± 0.17</td>
<td>1.00 ± 0.02</td>
</tr>
<tr>
<td>HUH7</td>
<td>0.62 ± 0.04</td>
<td>0.54 ± 0.09</td>
<td>0.30 ± 0.15</td>
<td>0.68 ± 0.09</td>
</tr>
<tr>
<td>HEP3B</td>
<td>0.51 ± 0.09</td>
<td>1.74 ± 0.37</td>
<td>1.53 ± 0.74</td>
<td>0.86 ± 0.15</td>
</tr>
</tbody>
</table>

**Conflict of interest:** Other Substantive Relationships: Dr Alan Wise is the CEO of IOMET Pharma (Merck and Company Subsidiary).

**Poster Session – Molecular targeted agents I, Wednesday 29 November 2016 Poster abstracts S27**

**Material and Methods:** Through a high-throughput virtual screen of a natural-product based library, we have identified a series of MDM2–NFAT1 inhibitors, including desisquenoterpins (DSTs). Fluorescence polarization (FP)-based binding assay and cellular thermal shift assay were performed to determine the binding of DSTs to MDM2 and NFAT1. Their cytotoxicity was initially evaluated in more than 50 cell lines of various cancer types. The in vitro and in vivo activities of these compounds and the underlying mechanism of action were further demonstrated in breast cancer cell lines with various p53 statuses.

**Results:** DSTs inhibited cell proliferation and induced G2/M phase arrest and apoptosis in breast cancer cells independent of p53 status. They inhibited the tumor growth and lung metastasis in mouse breast cancer xenograft models, without any host toxicity. DSTs directly bound to MDM2 and reduced MDM2 protein in cancer cells in a p53-independent manner. DSTs also promoted MDM2 protein degradation. Furthermore, DSTs inhibited the nuclear localization of NFAT1, disrupted the NFAT1–MDM2 P2 promoter complex, and induced NFAT1 proapoptotic degradation, resulting in the repression of MDM2 transcription.
Conclusions: DSTs are novel dual NFA1 and MD2M inhibitors, repressing MD2M transcription and promoting MD2M degradation. These results pave a new avenue for validating the NFA1−MD2M−p53 pathway as a novel molecular target for cancer therapy.

Conflict of interest: The authors declare that they have no competing financial interests; this work was supported by NIH R01 CA186662 and ACS RSG-15-009-01-CDD.

62 Poster (Board P033)

ADCT-502, a novel pyrrolobenzodiazepine (PBD)-based antibody–drug conjugate (ADC) targeting low HER2-expressing solid cancers

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Background: Human epithelial growth factor receptor 2 (HER2) is a well-established, clinically validated target for cancer therapy. Despite numerous HER2-targeted therapies existing in the market, they achieve good results primarily in HER2-overexpressing malignancies, while they lack efficacy in medium/low HER2-expressing cancer indications.

Methods: ADCT-502 is an ADC composed of the humanized anti-HER2 IgG1 trastuzumab, site-specifically conjugated to a PBD dimer by a protease-cleavable linker. The drug to antibody ratio of ADCT-502 is 1.7. PBD dimers exert their potent anti-tumor activity by forming highly cytotoxic interstrand cross-links in the DNA minor groove.

Results: ADCT-502 showed potent in vitro cytotoxicity in a panel of HER2-expressing human cancer cell lines while its efficacy was strongly reduced in HER2-negative cells. In vivo, ADCT-502 showed potent anti-tumor activity in BT474 (HER2 3+), and in the trastuzumab-resistant JIMT-1 (HER2 2+) breast cancer xenograft models, compared to the vehicle- and isotype control ADC-treated mice. In the BT474 xenograft, a single dose of ADCT-502 at 0.6 mg/kg induced significant anti-tumor activity in all treated animals and resulted in 10/10 tumor-free survivors (TFS) at the end of the study. Moreover, ADCT-502 showed impressive anti-tumor activity in two patient-derived xenograft (PDX) models expressing very low level of HER2 (FISH negative, HER2 1+). In a breast cancer PDX, a single dose of ADCT-502 at 0.2 mg/kg showed remarkable anti-tumor activity resulting in 10/10 TFS at the end of the study. In a second PDX model (esophageal cancer), ADCT-502 demonstrated dose-dependent anti-tumor activity with a single dose at 0.44 mg/kg resulting in 10/10 TFS at the end of the study. ADCT-502 was stable, well tolerated and showed a favorable PK profile both in rats and cynomolgus monkey.

Conclusions: These data demonstrate ADCT-502 is a potent, specific and well-tolerated ADC directed against low HER2-expressing solid cancers which warrants further development into the clinic.

and AXL phosphorylation in vitro at cell IgG0 values of 6 nM and 0.2 nM, re-
spectively. This inhibition of MET and AXL activation in vitro is accompanied
by a decrease in downstream MAPK and PI3K signaling and cell viability.
Consistent with our in vitro findings, treatment with RDXX-106, at doses as
low as 3–10 mg/kg (PO, qd), results in dose-dependent NSCLC tumor
growth inhibition with complete tumor regression of MET-amplified EBC-1
xenografts and MET D exon 14 skipping LU2503 PDX tumors. RDXX-
106 similarly results in robust tumor growth inhibition when administered
on an alternative dosing schedule (q2d, q3d), indicating that these intervals
may be exploited clinically to minimize side effects and/or afford
combination therapies. Importantly, RDXX-106 displays equivalent, if not
superior, in vivo efficacy compared to a late clinical trial candidate in a
preclinical model. RDXX-106 is a selective, pseudo-reversible TAM/MET inhibitor
that is efficacious in the treatment of MET amplified and exon D 14 skipping-dependent human
cancers. These studies support further investigation of RDXX-106 in these
unique molecular subtypes of NSCLC, and other human malignancies, that
Conflict of interest: Ownership: All authors are/were Ignyta employees
and own Ignyta stocks.

66  Poster (Board P037)
Chromosome 18q11.2 loss as a predictive marker for response to
bevacizumab in metastatic colorectal cancer
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1VU University Medical Center, Cancer Center Amsterdam VUMC-CCA, Dept. of Pathology, Amsterdam, Netherlands; 2University Medical Center Utrecht, Dept. of Oncology, Amsterdam, Netherlands;
3Academic Medical Center, University of Amsterdam, Dept. of Oncology, Amsterdam, Netherlands; 4University of California San Francisco, Department of Medicine, San Francisco, USA
Background: Bevacizumab is an angiogenesis inhibitor that is currently
used to treat patients with metastatic colorectal cancer (mCRC). However, treatment response is variable and predictive biomarkers are urgently
needed. The aim of this study was to identify copy number aberrations
within the EU-funded AngioPredict project, formalin fixed and paraffin embedded (FFPE) tumor tissue samples from 182 mCRC patients treated with chemotherapy only (non-Bev) or chemotherapy plus
Bevacizumab (plus-Bev) were retrospectively collected. The overall median
progression-free survival (PFS) was of this cohort was 217 days. Copy
number analysis using a routine pipeline, generating regions called for gain or
loss of chromosomes 18q11.2. This predictive value of this region was confirmed in the CAIRO2 validation set.

Conclusions: NGS copy number analysis revealed that loss of chromo-
somal 18q11.2 is associated with prolonged PFS in plus-Bev patients and may
serve as a predictive marker for Bevacizumab treatment.

Conflict of interest: Funding: The AngioPredict project was funded by the
European Commission Framework Programme Seven (FP7) initiative under contract No. 278981 ‘AngioPredict’ (www.angiopredict.com).

67  Poster (Board P038)
From bench to bedside: Translation of preclinical data led to
a custom tailored first in human clinical trial design of RG7386, a
novel bispecific FAP-DR5 agonist antibody
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Early Development pRED, RICB, Basel, Switzerland; 2Roche Pharma
Research and Early Development pRED, Mülheim, Germany
Introduction: FAP-DR5 (RG7386) is a novel bispecific antibody binding
with high affinity to fibroblast activation protein (FAP) and with low affinity
to death receptor 5 (DR5). Avidity-driven binding of the bispecific antibody
mediates hyper-clustering of DR5, triggering tumor cell death. Here, we
combined preclinical pharmacological and pharmacokinetic (PK) data to
inform a safe and robust first-in-human study design. These data were
condensed into a mathematical model allowing for exploration of a variety of
design options.
Material and Methods: Single-dose PK of RG7386 was investigated in
cynomolgous monkeys over a broad dose range. The dose dependency of
the anti-tumor activity of RG7386 was investigated in vivo in two distinct
xenograft mouse models. In an epithelial cell-line based model, FAP was
expressed on tumor stroma (DDL-1-NIH3T3). In a mesenchymal patient-
derived model, FAP was expressed on tumor cells directly (Sarc4G65). The
effect of differential FAP localization within tumors was also investigated.
Results: The observed target-mediated drug disposition (TMDD) of RG7386 was well-described with a mathematical model extending the usual
framework of published models. Based on the PK/pharmacodynamics (PD)
studied in the mouse xenografts, we observed no plateau in the observed
efficacy and determined the average concentration for tumor regression. The TMDD
model built on the monkey data was scaled to predict human PK and
allowed placing anticipated human exposure predictions in the context
of the concentration-activity relationships seen in mice. This combined
information was used to support planning of the currently ongoing phase I
clinical trial.
Conclusion: We demonstrate how modeling of a single-dose PK study with
RG7386 in cynomolgus monkeys and the subsequent scaling of this model
led to the translation of preclinical information into a custom tailored first
in human clinical trial design. Placing these simulations in relation to pre-
clinical efficacy data from PK/PD studies in rodent tumor models, including
patient derived xenografts, supported an early estimate of the efficacious
dose in humans, enabling the design of the first-in-human (FiH) trial. Three
key aspects were optimized in the clinical phase I design: (i) a safe starting
dose, (ii) rapid escalation to pharmacologically significant exposure levels
and (iii) thorough characterization of PK including TMDD.
Conflict of interest: Other Substantive Relationships: Employee of Roche.

68  Poster (Board P039)
Initial results from a phase 1a/b study of OMP-131R10, a first-in-class
anti-RSPO3 antibody, in advanced solid tumors and previously
untreated metastatic colorectal cancer (mCRC)
J. Bendell1, G.S. Eckhardt2, H.S. Hochster3, V.K. Morris4, J. Strickler4, A.M. Kapoun5, M. Wang6, X. Lu7, K. McGuire8, J. Dupont9, L. Faoro9, P. Munster9, 1Sarah Cannon Research Institute, Drug Development Unit, Nashville, USA; 2University of Colorado School of Medicine, Department of Medicine, Aurora, USA; 3Yale School of Medicine, Yale Cancer Center, New Haven, USA; 4University of Texas, M.D. Anderson Cancer Center, Houston, USA; 5Duke University, Duke Cancer Institute, Durham, USA; 6OncoMed Pharmaceuticals Inc, Translational Medicine, Redwood City, USA; 7OncoMed Pharmaceuticals Inc, Department of Research, Redwood City, USA; 8University of California San Francisco, Department of Medicine, San Francisco, USA
Background: The R-spondin (RSPO) pathway plays a crucial role in
regulating stem cell maintenance and renewal. RSPOs act with WNT ligands
in a redundant manner to activate WNT signaling. RSPO translocations occur in CRC.
OMP-131R10 is a novel IgG1 that targets RSPO3 ligand. Inhibition of
RSPO3 binding to its receptor by OMP-131R10 demonstrates anti-tumor effects in
patient derived xenograft models as a single agent and with combination of CRC.
Here we present initial results from the ongoing Ph 1a/b study of OMP-131R10.
Materials and Methods: this study involves a Ph 1a single-agent portion
(subjects with advanced solid tumors) and a Ph 1b combination portion
(subjects with previously treated CRC, combined with 5-FU, leucovorin,
and irinotecan [FOLFIRI]). Objectives include the determination of the maximum
tolerated dose (MTD), recommended Ph 2 dose, pharmacokinetics, immunogenicity, preliminary efficacy, pharmacodynamic and predictive
biomarkers. Dose escalation follows a modified 3+3 framework. Following dose escalation, a dose expansion cohort is planned for both 1a (RSP03 high CRC) and 1b portions.

Results: 19 subjects were treated with OMP-131R10 (14 in phase 1a, 5 in phase 1b). Subjects were male (83%) and Caucasian (81%). Tumor types included CRC (50%) and ovarian (12%). Subjects received a median of 4 doses of OMP-131R10 (range 1–17), with a median treatment duration of 43 days (range 1–225). Doses ranged from 2.5 to 15 mg/kg every 2 weeks. Most frequent treatment-related AEs included nausea (92%), decreased appetite (31%), diarrhea (12%), vomiting (12%), weight decrease (12%). No dose-limiting toxicities were observed. 7 subjects had stable disease as best response; 3/5 with RSP03 high had SD. OMP-131R10 demonstrates a linear pharmacokinetic profile, with an estimated half-life of approximately 13 days. Bone turnover markers demonstrate change from baseline, consistent with WNT pathway engagement. Other biomarker analyses are ongoing. The MTD has not been reached, and dose escalation continues. (clinicaltrials.gov: NCT02482441, sponsored by Oncomed Pharmaceuticals).

Conclusions: RSP03 is a potential therapeutic target against cancer. OMP-131R10 has been well tolerated in the tested doses. Early signs of efficacy have been observed in subjects with prolonged stable disease. Target engagement was observed. The ongoing phase 1a/b clinical study will establish the safety profile, as well as potential signs of efficacy and predictive biomarkers.


70 Poster (Board P041)
Identification of driver of anti-tumor activity of TAK-931 in human colorectal cancer xenograft model

Background: CDC7, a serine/threonine kinase that triggers DNA replication by phosphorylation of MCM2, is a component of the MCM helicase complex. Given that DNA replication is a key factor for cancer cells to proliferate or survive, CDC7 is an attractive target molecule for a next generation of cancer therapeutic drugs. We have developed a novel oral CDC7-selective inhibitor, TAK-931. TAK-931 has exhibited antitumor activity in various cancer xenograft models, such as the human colorectal adenocarcinoma xenograft model. The results of the preclinical study thus far have determined the pharmacokinetic–pharmacodynamic (PK–PD) relationship for TAK-931 antitumor activity in the COLO205 xenograft model.

Methods: TAK-931 was orally administered to nude female bearing COLO205 xenograft tumors. For dose- and time-dependent PK/PD analyses, plasma and tumor tissue were collected between 0.25 and 72 hours after single administration of TAK-931 at doses of 10–80 mg/kg. PD effect was assessed using evaluating phospho-MCM2 (pMCM2) expression in the tumor tissue by immunohistochemistry and western blot. Antitumor activity at multiple dosing regimens was evaluated by measuring tumor size.

Results: Orally-administered TAK-931 resulted in increased drug exposure in the plasma and in COLO205 xenograft tumor tissue. Dose- and time-dependent decrease in pMCM2 expression was also observed at all doses administered in this experiment. Dose-dependent antitumor activity was observed with continuous and intermittent dosing regimen schedules. Using this PK, PD and antitumor activity data, we built a model to determine which PK and PD parameters are responsible for the antitumor activity of TAK-931. The PD/antitumor activity analysis revealed that the time-averaged PD suppression was correlated with antitumor activity, while the PK/PD analysis revealed that the time-averaged PD suppression was correlated with AUC and plasma exposure above effective concentration of TAK-931. Taken together, the total AUC and the total duration of effective plasma concentration of TAK-931 appear to be responsible for antitumor activity of TAK-931 in these preclinical mouse models.

Conclusion: The novel CDC7-selective inhibitor TAK-931 exhibited a good correlation between PK, PD, and antitumor activity in the COLO205 xenograft mouse model. The results of the preclinical study thus far have determined the pharmacokinetic–pharmacodynamic (PK–PD) relationship for TAK-931 antitumor activity in the COLO205 xenograft model. The results of this study may provide guidance for dosing and scheduling in a future clinical trial.

Conflict of interest: Ownership: All authors are employees of Takeda Pharmaceutical Company, Ltd. Corporate-sponsored Research: This research was supported by Takeda Pharmaceutical Company, Ltd.
PKC and p53-MDM2 or TORC1 inhibitors may provide significant clinical benefit for UM patients.


73 Poster (Board P044) Immuno-oncologic efficacy of RXDX-106, a selective, TAM family small molecule kinase inhibitor

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The TAM family receptor tyrosine kinases (RTKs), including TYRO3, AXL, and MERTK, have been implicated in the pathogenesis and progression of many cancer types. In cancer cells, overexpression of TAM RTKs is associated with mechanisms of resistance and mesenchymal phenotype. In immune cells, however, TAM RTKs play a key homeostatic role as negative regulators of immune responses, contributing to the evasion of cancer cells from immune surveillance.

Here, we evaluated the immuno-oncologic impact and anti-tumor efficacy of RXDX-106, a selective and potent, small molecule TAM/MET inhibitor, both in vitro in TAM expressing primary immune cells and in vivo in a 4T1 breast cancer model. We found that RXDX-106 is a potent inhibitor of TAM activation and function in bone marrow derived macrophages, inhibiting both TAM receptor phosphorylation and TAM-dependent phagocytosis at concentrations as low as 2.5 nM. Most significantly, RXDX-106 exhibited in vivo efficacy in a syngeneic model of 4T1. Briefly, Balb/c mice were inoculated with 4T1 cells in the mammary fat pad and treated with RXDX-106 and anti-CTLA-4 monoclonal antibody (mAb), either as single agent or in combination, for 2 weeks. RXDX-106 dose-dependently inhibited primary tumor growth as a single agent and demonstrated further tumor growth inhibition in combination with anti-CTLA-4 mAb, achieving almost complete regression. Immuno-phenotypic analyses showed that tumor infiltrating T cells were significantly increased in RXDX-106 treated animals in a dose-dependent manner. Interestingly, RXDX-106 decreased suppressive regulatory T cells in the primary tumor. In addition, RXDX-106 significantly increased IFNγ production in tumor infiltrating NK cells relative to splenic NK cells, indicating that suppressed NK cell activity in the tumor environment was recovered by RXDX-106. Finally, analysis of serum from 4T1 tumor bearing mice revealed an increase in soluble Axl with RXDX-106 treatment.

In conclusion, we demonstrate that RXDX-106 is a potent TAM/MET inhibitor that removes the molecular brake on immune activation in macrophages, NK and T cells, resulting in repolarization of the immune response to create an anti-tumor environment. We identified with checkpoint inhibitors such as an anti-CTLA-4 antibody, RXDX-106 could potentiate the antitumor effect to achieve greater efficacy.

Conflict of interest: Ownership: All authors are Ignyta employees and own Ignyta’s stocks.
**Results:** We selected a transcription factor, FOXA2, which was significantly overexpressed than the other genes in K4 cells. And we observed that E-cadherin and Snail mRNA were down-regulated, but N-cadherin and Twist were up-regulated in K4 cells. Results of wound healing assay and transwell assay showed that K4 cells have low migratory and invasiveness than V5 cells. In addition, the protein expression of E-cadherin was markedly reduced in K4 cells. FOXA2 mRNA was down-regulated and N-cadherin was up-regulated in V5 cells when their density was lower. However, FOXA2 protein expression was increased and N-cadherin expression was decreased gradually when they were overgrown with higher density. On the other hands, we confirmed the opposite result in K4 cells compared with V5 cells. The pattern of immunofluorescence staining of V5 and K4 cells were similar to the protein expressions of E-cadherin and FOXA2 in Western blot analysis. Knock down of FOXA2 mRNA in K4 cells showed that Vimentin and Snail mRNA expressions were down-regulated and Twist was up-regulated.

**Conclusion:** Taken together, FOXA2 and E-cadherin were down-regulated and Twist was up-regulated in HEK-293T cells stably expressing the KIF5B-RET. Thus, our data suggest that KIF5B-RET fusion gene may involve in EMT via the regulation of FOXA2.

**No conflict of interest.**

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**Poster (Board P046)**

**75**

**TPX-0005, a novel ALK/ROS1/TRK inhibitor, effectively inhibited a broad spectrum of mutations including solvent front ALK G1202R, ROS1 G2032R and TRKA G595R mutants**

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Drug-resistance has emerged as a major challenge to targeted cancer therapies. An ever increasing number of acquired resistance mutations are being reported from the clinic. In addition to the gatekeeper mutations, the solvent front mutations have been recently recognized as common resistance mutations to many kinase inhibitors. For example, the solvent front ALK G1202R mutant conferred resistance to many clinical ALK inhibitors in lung cancer including crizotinib, ceritinib, and alecrintib. The same position mutations ROS1 G2032R and TRKA G595R rendered resistance to solvent-front inhibitors in lung cancer and TRK inhibitor entrectinib in colon cancer, respectively. A conserved glycine residue at the hinge C-terminal forms a hydrophobic sandwich with the kinase b1 sheet. Kinase inhibitors often use an aromatic ring or a flat motif to fit through this narrow glycine sandwich to the solvent. Alterations at the conserved glycine or the nearby residues, commonly referred to as solvent front mutations, clash with the inhibitor motif and induce clinical resistance.

Here, we designed TPX-0005, a novel three-dimensional macrocycle with a much smaller size (MW <370) than current ALK, ROS1, and TRK inhibitors in the clinic. TPX-0005 resists at the center of the highly conserved ATP site without direct contact with gatekeeper and the solvent front glycine sandwich. As expected, TPX-0005 potently inhibited both wild type and mutant ALK/ROS1/TRKs including gatekeeper and solvent front mutations. TPX-0005 inhibited WT EML4-ALK, gatekeeper EML4-ALK L1196M and solvent front G1202R mutants with similar activities in both enzyme assay (WT Ic50 20.5 nM vs L1196M 0.65 nM) and pEg3 kinase, is a member of the AMP-activated protein kinase (AMPK)-related kinase family. MEK is involved in the regulation of various biological processes, including cell proliferation, spliceosome assembly, hemostasis, stem cell self-renewal and apoptosis. Therefore, the potential of MEK inhibitor as therapeutic agent is currently attracting considerable interest. Previously, OTSSP167 is reported as potent MEK inhibitors, and it showed anti-cancer activities against various cancer cell lines.

**Conflict of interest:** Ownership: TP Therapeutics, Inc.

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**77**

**Structure–activity relationship of pyrrolopyrimidine derivatives as manual embryonic leucine zipper kinase inhibitor**

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**Background:** Maternal embryonic leucine zipper kinase (MELK), which is also known as murine protein serine/threonine kinase 38 (MPTK38) and pEg3 kinase, is a member of the AMP-activated protein kinase (AMPK)-related kinase family. MELK is involved in the regulation of various biological processes, including cell proliferation, spliceosome assembly, hemostasis, stem cell self-renewal and apoptosis. Therefore, the potential of MEK inhibitor as therapeutic agent is currently attracting considerable interest. Previously, OTSSP167 is reported as potent MEK inhibitors, and it showed anti-cancer activities against various cancer cell lines.

**Materials and Methods:** The aim of the present work was to develop a new series of MELK inhibitors by modifying OTSSP167, keeping the structural similarities. We designed, synthesized, and evaluated pyrrolopyrimidine-based compounds, and tested the MELK inhibitory activity by enzyme-based assay. We also tested the cytotoxicity of the compounds against human breast cancer cell lines including MCF7, MEA-MB-231. Then, we analyzed the structure and activity relationship (SAR) by using the computer modeling system.

**Results:** We have synthesized more than 50 compounds, and several pyrrolopyrimidine derivatives represented good MELK inhibitory activity (Ic50 <100 nM) in enzyme-based and cell-base assay with SAR. In the SAR analysis, 3,5-dichloro-4-phenyl at position 3 is important for MELK inhibitory activity of OTSSP167 analogues, and six-membered ring at 4-position will be better than 5-membered ring. In the cell cytotoxicity, the triple-negative breast cancer cell lines such as MIA-MB-231, BT549, were more sensitive than other cell lines such as MCF7 to the compounds tested in general.

**Conclusion:** Based on the enzyme-based assay and crystal structure of the MELK enzyme, we designed, synthesized, and evaluated pyrrolopyrimidine-based compounds. Several pyrrolopyrimidine represented good MELK inhibitory activity (Ic50 <100 nM) in enzyme-based assay with SAR. Pyrrolopyrimidine can be a valuable scaffold for MELK inhibitor.

**Conflict of interest.”**
Entrectinib, a highly potent pan-Trk, ROS1, and ALK inhibitor, has broad-spectrum, histology-agnostic anti-tumor activity in molecularly defined cancers

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Poster Session – Molecular targeted agents I, Wednesday 29 November 2016 Poster abstracts S33

Summary:
The development of a few compounds such as LDK378 (ceritinib), CH5424802 (crizotinib), and DUSZ122 has been significantly validated as therapeutic targets for anticancer drugs. With the technological advancement of detection methods, the identity of gene rearrangements partners, the spectrum of tumor histologies where the gene rearrangements have been found and their overall prevalence have significantly expanded in the past few years. Entrectinib (RXDX-101) is an orally available, brain-penetrant, highly potent and selective inhibitor with low nanomolar potency against kinase activities of Trk/A/B/C, ROS1 and ALK (encoded by NTRK1/2/3, ROS1 and ALK genes, respectively). Gene rearrangements in each of these genes have been detected in many solid and hematological tumors, including lung, colorectal, salivary gland, sarcoma, thyroid, glioblastoma, melanoma, anaplastic large cell lymphoma (ALCL), and other histologies. The significant unmet medical need of these cancer patients and the relatively low frequency of rearrangement events justify a molecularly targeted, histology-agnostic approach to providing maximal benefit to patients.

To test the anti-tumor potency of entrectinib in a broad spectrum of gene rearrangements involving NTRK1/2/3, ROS1 and ALK, we generated a panel of engineered Ba/F3 cells expressing clinically identified gene rearrangements with various fusion partners. In these cells, entrectinib exhibited potent, low nM anti-proliferative activity regardless of the identity of the fusion partners. The clinical relevance of histology-agnostic targeting of NTRK1/2/3, ROS1 and ALK rearrangements by entrectinib was further demonstrated by in vitro and in vivo studies using cancer cell lines, patient-derived tumor cells (PDCs) and patient-derived xenografts (PDXs). These models were derived from various cancer histologies, including non-small cell lung cancer, colorectal cancer, head and neck cancer, anaplastic large-cell lymphoma and other tissues of origin. In these models, entrectinib effectively inhibited target activation and cancer cell proliferation in vitro. Additionally, in vivo tumor growth was significantly inhibited across various fusion partners and cancer histologies at clinically achievable concentrations.

In conclusion, our preclinical data demonstrate the potential of entrectinib as an effective treatment for patients with NTRK1/2/3, ROS1 and ALK rearranged tumors, regardless of the fusion partners or the origin of tissue; and provide the rationale for performing histology-agnostic clinical trials in multiple molecularly defined cancers.

Conflict of Interest:
Ownership: All authors are employees of Ignyta and own Ignyta stocks.

Design, synthesis and evaluation of new pyrimidine derivatives for novel KRO518 inhibitor

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Anaplastic lymphoma kinase (ALK), a receptor tyrosine kinase belonging to the insulin receptor family, has been a attractive target based on its remarkable preclinical and clinical studies. Among various oncogenic fusion genes, ALK has obtained tremendous attention due to ALK-positive tumors in various cancer types such as anaplastic large-cell lymphoma (ALCL), diffuse large B-cell lymphoma (DLBCL), inflammatory myofibroblastic tumors (IMT), and non-small-cell lung cancer (NSCLC). In 2011, Crizotinib (Xalkori) was first approved as an ALK inhibitor drug to treat ALK-positive NSCLC. However, its clinical efficacy is limited by drug-resistance mutations and brain metastases within one to two years. Thus, an extensive efforts for the second-generation ALK inhibitors which could overcome crizotinib-resistant issues have been pursued comprehensively, resulting in development of a few compounds such as LDK378 (ceritinib), CH5424802 (lectinib), AP26113 (brigatinib), and PF06463922 (lorlatinib). LDK378 is known to be three to six-fold more active than crizotinib in cell cytotoxicity assays. RXDX-101, Co to be the clinical most of the resistant mutants, such as L1196M, G1269A, I1171T, and S1206Y, but ineffective against G1202R and F1174C. In this presentation, we have developed a new series of pyrimidine derivatives which are modified at 2-position of pyrimidine ring in LDK378. KRCA-0391 has been identified as a highly potent and selective ALK inhibitor with high potency profiles (ALK wt, IC50 = 6 nM) not only ALK mutants (L1196M, C1156Y, F1174L, R1275Q and G1202R) and BaF3 ALK L1196M cell line. KRCA-0391 showed good pharmacological properties including blood-brain barrier (BBB) penetration ability together with significant inhibitory activities. In vivo xenograft mouse study (H3122 NSCLC), we observed dramatic tumor growth inhibition without significant body weight change. Most importantly, several compounds turned out to be a highly potent inhibitor of the closely cros were oncogene 1 kinase (ROS1). It has excellent activities both ROS1(G2032R) and ROS1 G1202R mutant. In summary, we will discuss a new series of pyrimidine derivatives with good in vitro and in vivo efficacies for ALK/ROS1 dual inhibition.

No conflict of interest.

Towards precision medicine: A cancer molecular subtyping nano-strategy for RNA biomarkers in tumor and urine

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Background: In order to allow individualized cancer detection and therapy, the detection of multiple oncogenic biomarkers is required to characterize cancer molecular subtypes for better diagnosis and treatment (i.e. precision medicine). For molecular cancer subtyping, multiplexed assays for screening multiple mutations simultaneously are essential. Yet, current methodologies for multiplexed biomarker screening are limited in terms of cost, complexity, and sensitivity. Towards precision medicine, we aimed to develop a cost-effective nano-subtyping platform for rapid multiplexed detection of prostate cancer biomarkers, whilst addressing the limitations of current methodologies.

Material and Methods: In this study, we selected a panel of five RNA targets comprising of next-generation biomarkers which are exceptionally promising for prostate cancer subtyping and risk stratification. The targets include two most common TMPRSS2:ERG gene fusion variants: TMPRSS2:ERG exon 1–ERG exon 4 (T1E4) and TMPRSS2:ERG exon 5 (T1E5). PCAG:ARV7; and an endogenously-expressed housekeeping RNA (RN7S1L). Extracted target RNA biomarkers from samples are firstly isothermally amplified concurrently by multiplexed reverse transcription–recombinase polymerase amplification (RT–RPA). By use of modified primers, the generated amplicons are tagged with biotin molecules and target-specific 5’ overhang barcode sequences on either ends to facilitate hybridization with complementary sequences on Surface-enhanced Raman spectroscopy (SERS) nanotags. Next, the amplicon-SERS nanotag complexes are attached to streptavidin (SA)-coated magnetic beads via biotin tags, and magnetically enriched. Finally, the enriched SERS-labeled amplicons are interrogated by Raman spectrometer to both identify (unique spectral peak) and quantify (peak intensity) the target biomarkers present in the sample.

Results: We achieved successful five-plexed screening of promising prostate cancer RNA biomarkers with excellent limit-of-detection from as low as 200 zmol (100 RNA copies) and high target-specificity. The sample-to-answer assay time was 80 min (two times faster than traditional techniques) and the well-resolved SERS spectral peaks allowed for clear and simple data interpretation. Clinical transition potential was demonstrated on clinical samples such as tissue biopsy specimens, and non-invasive urine samples.

Conclusions: This is the first translational application of a RT–RPA/SERS-based platform for multiplexed cancer biomarker detection to address a clinical need. With excellent sensitivity and specificity, we envisaged that this platform methodology could be a useful tool for rapid multiplexed subtyping of cancers, which in turn facilitates precision medicine.

No conflict of interest.

Development and evaluation of a novel MAPK and PI3K inhibitor

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Background: Clinical and preclinical studies have led to identification of primary resistance mechanisms to MEK inhibition (MEKi) in "oncogene-addicted" cancer cells. Intrapathway regulatory loops and signaling pathway cross-talk remain a challenge for the clinical success of MEK inhibitors. PI3K represents a major signaling node activated by MEKi and inhibition of PI3K has been shown to forestall the onset of MEKi resistance. Furthermore, expression of activated AKT by relief of negative feedback mechanisms (MEKi) or by loss of PTEN promote metastasis making PI3K signaling pathway an important target in the management of
A phase I study of the MDM2 inhibitor AMG 232 in patients with advanced p53 wild type (p53WT) solid tumors or multiple myeloma

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Background: Mouse double minute 2 homolog (MDM2) is a negative regulator of the p53 tumor suppressor. In preclinical models p53 activation is an important cellular process to guard against abnormal growth. AMG 232 is an oral, selective MDM2 inhibitor that restores the tumor suppressor function of p53 by blocking the MDM2–p53 interaction.

Material and Methods: This international, multi-center, first in human, 2-part (dose escalation/Part1 and dose expansion/Part2; using 3+3 design) Phase I study investigated the administration of AMG 232 orally once daily (QD) on days 1–7 in 21 day cycles (QD 7/21 days) schedule. Objective was to determine the maximal tolerated dose (MTD), pharmacokinetics and biomarker activity of AMG 232 in patients (pts) with p53WT solid tumors as determined by next generation sequencing in Part I and in Part 2 to evaluate the efficacy of AMG 232 in participants with solid tumors with MDM2 amplification or potential MDM2 overexpression or in patients with multiple myeloma.

Results: Part I: 39 pts (26 men/13 women; median age 64 years; range 41–84) with advanced p53WT solid tumors were treated in escalating dose cohorts from 15 to 480 mg of AMG 232 in QD 7/21 days schedule. Treatment-related adverse events (AEs) in >10% of pts; included nausea, vomiting, diarrhea, fatigue, thrombocytopenia, and neutropenia. In part I, 3 dose limiting toxicities (DLTs) were reported for 3 subjects during the first treatment cycle of AMG 232, consisting of grade 4 thrombocytopenia and failure to recover from AMG 232 related toxicities (Gr 2 thrombocytopenia and Gr 3 neutropenia) in one subject each to grade 1 or baseline severity after delaying next cycle up to 7 days. Delayed cytophenias occurred (cycle 2 and beyond) were also included in dose escalation decisions. The MTD was determined to be 240 mg in the (QD 7/21 days) schedule. Preliminary PK data show that exposure (AUC) increased dose proportionally with doses up to 480 mg QD. Induction of MIC-1 (marker of p53 activation) correlated with drug exposure. In 38 pts who had post-baseline tumor evaluation, 33 showed stable disease (SD). Durable SD was seen in one subject each with NSCLC (11.4 mo), de-differentiated liposarcoma (7.5 mo), chondrosarcoma (10.2 mo), bladder cancer (17 mo) and neuroendocrine tumor (14.9 mo).

Conclusions: AMG 232 is an oral selective MDM2 inhibitor which demonstrates p53 pathway activation with an acceptable PK and tolerability profile when administered in dosages of 240 mg in the (QD 7/21 days) schedule. Durable SD was observed across different tumor types during the dose escalation phase. AMG 232 is currently being evaluated in the Part 2 in patients with solid tumors with potential MDM2 overexpression or in patients with multiple myeloma.


A novel CDC7-selective inhibitor TAK-931 with potent antitumor activity

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Background: Cell division cycle 7 (CDC7) is a serine/threonine kinase, which plays an important role in initiation of DNA replication by phosphorylating MCM2. Kinase activity of CDC7 is controlled by its binding protein DBF4 in a cell-cycle dependent manner. A novel CDC7 inhibitor, TAK-931, was developed as a cancer therapeutic drug candidate based on biochemical screening of CDC7 kinase activity. Here, we report the pharmacological characterization of TAK-931 on enzymatic mode of action, cellular morphology, pharmacodynamics (PD) biomarker, and antiproliferative activity. We also report the in vivo antiproliferative activities demonstrated by TAK-931 in multiple human cancer xenograft mouse models.

Materials and Methods: The CDC7 enzymatic assay for TAK-931 used Transgeneer ADP Assay® with double phosphorylation level of MCM2. Assessment with visualized nuclei was applied for cell proliferation assays. TAK-931 was orally administered to nude mice bearing the xenograft tumors. PD effect was assessed by evaluating phospho-MCM2 expression in tumor by immunoblotting. Antitumor activity in multiple cancer models was evaluated by measuring tumor size.

Results: In the xenograft mouse models, TAK-931 potently inhibited CDC7 kinase activity (IC50 <0.3 mM) with a time-dependent ATP-competitive kinetics to its ATP-binding pocket. The selectivity studies using the 308 kinases revealed >120-fold selectivity of TAK-931 for CDC7 kinase inhibition compared to other kinase inhibitors. Treatment with TAK-931 suppressed the cellular MCM2 phosphorylation at Ser40 (pMCM2) in a dose-dependent manner, resulting in a delayed S phase progression, DNA-damage checkpoint activation, and caspase-3/7 activation. A cellular antiproliferative activity of TAK-931 was also observed in multiple cancer cell lines. In the COLO205-xenograft mouse model, oral administration of TAK-931 inhibited pMCM2 of the xenografted COLO205 in dose- and time-dependent manners. Furthermore, TAK-931 exhibited an significant antitumor activity in multiple xenograft models, which included both cell lines-based xenografts and patient-derived xenografts (PDX) models.

Conclusion: TAK-931 is a highly potent and selective inhibitor of CDC7 kinase and exhibited significant in vitro and in vivo antiproliferative activity in multiple cancer models. These findings suggest the therapeutic potential of TAK-931 as a cancer drug.

Conflict of interest: Ownership: All authors are employees of Takeda Pharmaceutical Company, Ltd. Corporate-sponsored Research: This research was supported by Takeda Pharmaceutical Company, Ltd.
Synergistic effect of combined CDK4/6 inhibitor with docetaxel in lung cancer cell lines harboring KRAS mutations

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Background: LY2835219 (LY), a novel CDK4/6 inhibitor, arrests the G1 phase and inhibits the cell proliferation. Docetaxel (DTX) is a cytotoxic anti-cancer drug which induces G2/M arrest and apoptosis. In present study, we evaluated the activities of CDK4/6 inhibitor alone or combined with docetaxel on the anti-proliferation, cell cycle and apoptosis in lung cancer cell lines harboring KRAS mutations.

Material and Methods: We measured the anti-proliferative activities of LY or DTX single and their combinations (DTX+LY 72h and DTX 24 h → LY 48 h) on cell proliferation in Calu-3(WT), A549(G12S) and H727(G12V) cells using CCK-8 assay. We evaluated the expression of CDK2, CDK4, cPARP and caspase-3 by Western blot. The cell cycle distribution and apoptosis detection were analyzed by flow cytometry.

Results: The IC50 values of the LY and DTX alone were 0.73 ± 0.6 μM and 3.03 ± 1.4 nM in Calu-3 cells, 0.4 ± 0.2 μM and 0.9 ± 0.2 nM in A549 cells and 2.0 ± 0.7 μM and 3.1 ± 0.3 nM in H727 cells, respectively. Both LY and DTX alone inhibited cell proliferation in a dose-dependent manner in 3 NSCLC cell lines. To evaluate the combination effects of LY and DTX, we performed CCK-8 assay in Calu-3, A549 and H727. In all three cell lines, a significant synergistic activity of LY and DTX was observed in the LY–DTX combination (CI<1). In Calu-3 cells, 0.8 and 0.9 in A549 cells and 0.6 and 0.8 in H727 cells, respectively. In three cell lines, a synergistic activity of LY and DTX was observed in the DTX–LY combination in Calu-3, A549 cells and LY–DTX in H727 cells. In Calu-3 cells, the sub-G1 fraction in G0/G1 phase increased by 54.5% relative to control (24.6%). With LY alone, the fraction of A549 cells in G0/G1 phase increased compared to control (78.4% vs. 49.5%). In case of DTX+LY, the G2/M fraction of A549 cells significantly increased by 33% vs 24.6% in a dose-dependent manner. Meanwhile, in DTX–LY, cell fractions in G2/M were increased (44.5% vs 24.6%), especially those in G0/G1 phases were remarkably reduced (14.7% vs 49.5%). In addition, in case of DTX alone, the subG1 fractions of both A549 and H727 cells increased in a dose-dependent manner (0.7% vs. 17.4% and 2.5% vs. 28.1%, respectively). In DTX–LY, the subG1 fraction increased while the relative cell numbers were reduced by 23% vs 15.9%. The DTX–LY induced significant apoptotic cell death in A549 cells relative to control and DTX alone (32.8%, 21.5% and 2.6%, respectively).

Synergistic anti-apoptotic effects of combined CDK4/6 inhibitor with docetaxel in lung cancer cell lines harboring KRAS mutations

85 Poster (Board P056)

RDXX-105 demonstrates anti-tumor efficacy in multiple preclinical cancer models driven by molecular alterations in RET or BRAF oncogenes

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Background: RET mutation is a validated therapeutic target for multiple tumors including medullary thyroid carcinoma (MTC) and RET-rearranged cancers. Herein we evaluated the preclinical efficacy of RXDX-105, an oral, selective, non-covalent RET tyrosine kinase inhibitor (TKI) in multiple tumor models harboring RET rearrangements.

Material and Methods: RXDX-105 was evaluated in 12 xenograft models harboring RET rearrangements (86+ tumors). The compounds were administered orally by gavage for 5 days per week for 6–12 weeks. Tumor volumes were measured as in vivo (IV) tumor volumes for each tumor model. In addition to RET and BRAF, RXDX-105 is believed to potentially drive additional anti-tumor activity, durability and/or therapeutic potential from its MKI properties, such as anti-angiogenesis and immune-modulatory activities.

Results: RXDX-105 potently antagonized constitutively active, rearranged and point-mutated RET proteins. In cell based assays, RXDX-105 demonstrated a dose-dependent inhibition of RET and downstream signaling events, resulting in inhibition of cellular proliferation. In vivo, RXDX-105 achieved dose-dependent anti-tumor activity, including tumor regression at doses achieving exposures in several patient derived xenograft (PDX) models harboring RET rearrangements. Similarly, RXDX-105 demonstrated significant antitumor activity in a panel of CRC PDX models harboring BRAF mutations, including V600E and non-V600E variants. In addition to RET and BRAF, RXDX-105 is believed to potentially drive additional anti-tumor activity, durability and/or therapeutic potential from its MKI properties, such as anti-angiogenesis and immune-modulatory activities.

Conclusions: RXDX-105 demonstrates anti-tumor activity and is well tolerated in preclinical studies for both RET and BRAF targetable tumors. Further studies are needed to determine the clinical utility for each target. The laboratory data will be included as a supplement to the corresponding clinical presentations.

No conflict of interest.
tumor malignancy. Therefore, ROS1 and NTRKs may be promising therapeutic targets. The MET/ALK1/ROS1 inhibitor crizotinib has shown responses in patients with ROS1 fusions, however, acquired resistance to crizotinib has been a concern and potential resistance mechanisms including ROS1 kinase mutations are implicated. DS-6051b is a novel, orally available, small molecule tyrosine kinase inhibitor of ROS1 and NTRKs, and is currently being investigated in phase I clinical trials. In this study, we examined in vitro and in vivo activities of DS-6051b against ROS1 and NTRK, and found ROS1 mutations to be one of the potential resistance mechanisms to crizotinib.

**Material and Methods:** In vitro inhibitory activity against ROS1 and NTRKs was tested in biochemical and cell-based assays. Antitumor efficacy was examined in a mouse subcutaneous xenograft model using cells expressing ROS1 or NTRK fusion gene. In addition, the activity against crizotinib-resistant ROS1 mutant was evaluated using Ba/F3 cells expressing ROS1 fusion with gatekeeper mutation (L2026M).

**Results:** Potent in vitro activity of DS-6051b was shown against ROS1, NTRK1, NTRK2, and NTRK3 kinases with IC50s of 0.2 nM to 2.3 nM. When the compound was administered orally to xenografted mice, the growth of KMr12 cells harboring TPM3-NTRK1 fusion and U-118MG cells harboring Fig-Ros1 fusion was significantly inhibited at 25 mg/kg and above. In Ba/F3-Ros1 allograft mice, tumor regression with inhibition of phospho-ROS1 in the tumor was observed even against the tumor with a ROS1 gatekeeper mutation in which crizotinib has no significant effect.

**Conclusions:** These results indicated potent in vitro and in vivo activities of DS-6051b against ROS1 and NTRKs, suggesting the potential of the compound for the targeted therapy against cancers with ROS1 or NTRK gene aberrations. Moreover, the potential for the effectiveness against acquired ROS1 resistant tumors is also demonstrated. DS-6051b is currently being evaluated in phase I clinical trials.

**Conflict of interest:** Other Substantive Relationships: All authors are employees at Daiichi Sankyo Co., Ltd.
of its broad antiproliferative activity against a panel of murine and human tumor cells (IC50s 0.5–1.6 μM), ABC1183 did not cause microtubule depolymerization in cells, but did inhibit signaling through ERK, AKT and GSK3β and promoted cell cycle arrest and apoptosis. Kinome profiling demonstrated inhibition of GSK3α/GSK3β and cdk9 (IC50 = 0.35 μM and 0.32 μM, respectively), without significant inhibition of more than 400 other kinases, including other cdkks. Oral administration of ABC1183 at doses as low as 2 mg/kg/day inhibited tumor xenograft growth in all models tested (B16, 166 and TRAMP). Additionally, ABC1183 had excellent anti-inflammatory activity in the dextran sulfate sodium model of ulcerative colitis. No hematologic or major organ toxicity was observed in mice treated with ABC1183 at 500 mg/kg/day for 7 days.

**Conclusions:** ABC1183 is a new potential anticancer agent with a unique profile of targeting GSK3α/GSK3β and cdk9. The low toxicity and oral activity of ABC1183 are highly supportive of its development as an anticancer, and paves the way to further studies in vivo.

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**Material and Methods:** In this study, roles of miR-205 in cancer progression, angiogenesis and EMT processes were investigated. Undifferentiated thyroid cancer cells (MB-1 and BHT-101) were permanently transfected by pCMV-MIR-205 expression vector. Expression of VEGF-A and EMT markers, ZEB1, SNAIL and E-Cadherin, were checked using western blotting and enzyme-linked immunosorbent assay (ELISA). Additionally, the aggressiveness and invasiveness of cancer cells were examined by wound healing migration and transwell invasion assays. The ability of human umbilical vein endothelial cells (HUVECs) subjected to pCMV-MIR-205 transfection to form capillary networks was also evaluated using angiogenesis assay tube formation kit.

**Results:** Western blot analysis showed that VEGF-A, ZEB-1 and SNAIL expression was notably downregulated in cancer cells after miR-205 vector transfection while E-cadherin up-regulated (P < 0.05). ELISA assay also significantly confirmed a VEGF drop in cancer cell media (P < 0.05). The migratory and invasive ability of stable cell lines have markedly decreased (P < 0.05). Co-culture of HUVECs and transfected cells has significantly blocked endotho-breast tube formation by HUVEC (P < 0.05).

**Conclusions:** Our findings provide important insights into simultaneous regulatory role of miR-205 and its capability in cancer management through affecting several cellular pathways. Because a single miRNA can target several genes at the same time, it can add advantage to function of miRNA to control a range of cellular processes leading to tumour formation and metastasis. It might open avenues to use miR-205 as a new therapeutic method for undifferentiated cancers in which angiogenesis and EMT are critical steps.

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**Conflict of interest:** No conflict of interest.
We conducted signal sequence trap by retrovirus-mediated expression method and identified coxsackievirus and adenovirus receptor (CXADR) as a new molecular target for cancer treatment. We then developed a novel anti-tumor antibody, anti-CXADR antibody (6G10A) and previously reported it exerted anti-tumor activity in vivo through both antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity. Here we report the selectivity of 6G10A. While 6G10A inhibited the growth of human prostate cancer DU-145 cells expressing CXADR in vivo, it did not affect that of DU-145 cells with CXADR knockdown. On the other hand, 6G10A did not inhibit the growth of human gastric cancer MKN-7 without CXADR, but it did against MKN-7 with CXADR overexpression. These results confirmed the CXADR-selective anti-tumor activity of 6G10A.

**Conflict of interest:** No conflict of interest.
Background: Targeting the signaling downstream of the B cell receptor and/or the Toll like-receptors is one of the main novel therapeutic approaches for lymphoma patients. Different drugs with distinct or overlapping targets are now in the clinical setting. A better understanding of the mechanisms of action could lead to rationally designed combinations and to better patient selection strategies. With this in mind, here, we aimed to study the mechanism of action of idelalisib, duvelisib and ibrutinib. The overlapping among the targets is now in the clinical setting. A better understanding of the mechanism of action could lead to rationally designed combinations and to better patient selection strategies.

Materials and Methods: Gene expression profiling (GEP) was obtained with the Illumina-HumanHT-12 Expression-BeadChips and analyzed with the limma-test (significant: log-ratio with the Illumina-HumanHT-12 Expression-BeadChips and analyzed with limma-test (significant: log-ratio

Results: GEP was obtained in 3 ABC-DLBCL cell lines (TM6, RIVA, U2932) exposed to PQR309 (1 μM), idelalisib (1 μM), duvelisib (1 μM), ibrutinib (500 nM), or DMSO for 4−8−12h. At GSEA, all the drugs appeared to decrease Myc target genes, PERK-regulated genes, genes involved in NFKB, in the signaling of IFNA/G, mTORC1, IL6/JAK/STAT3, IL2/STAT5, cytokine/chemokine, in protein or unfolded protein response. They increased genes involved in mitotic spindle, DNA repair, IL4 signaling and BCR signaling.

With the chosen statistical criteria, PQR309 was the drug that led to the highest number of differentially expressed genes (2033 down; 180p), followed by idelalisib (82.27), idelalisib (77.17) and duvelisib (56.32). A total of 302 and 187 genes were down or upregulated in at least 1 condition: 35 genes (12%) were downregulated by all 4 drugs and 48 (23%) by at least 2; 5 (3%) were upregulated by all 4 drugs and 40 (22%) by at least 2. To gain insight into the overlaps of the effect of the different drugs on ABC DLBCL cells, we performed GSEA using the limma-derived GEP signatures obtained for each drug. PQR309 signature was highly enriched in genes differentially expressed after exposure to idelalisib, duvelisib or ibrutinib treated cells (NES >0.1, P and FDR <0.0001). The same was true also for the other signatures in each treatment group. The overlapping among signatures increased with exposure time (4−6<=12h).

Conclusions: In ABC-DLBCL models, PQR309, idelalisib, duvelisib and ibrutinib all successfully targeted fundamental pathways sustaining lymphoma cell proliferation and survival. Their early effects on the lymphoma cell transcriptome were very similar, although the degree of changes varied among drugs, possibly reflecting their main targets (the dual PI3K/mTOR-induced stronger changes). These data sustain the rational to use combinations aimed to more efficiently block the same pathways but also suggest these agents might be more beneficial when combined with different classes of compounds.

Conflict of interest: Board of Directors: Piqur: Vladimir Cmiljanovic, Corporate-sponsored Research: Francesco Bertoni, Anastasios Stathis has received institutional research funds from Piqur. Other Substantive Relationships: Petra Hillmann, Doriano Fabro are Piqur employees.

98 Poster (Board P069)
Osimertinib (A2D9291), an irreversible 3rd generation TKI, induces tumor growth inhibition in NSCLC pre-clinical models harboring the most prevalent EGFR Ex20ins (in vitro and in vivo)

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Background: Exon 20 insertions (Ex20ins) have been identified in approximately 5% of all epidermal growth factor receptor (EGFR)-mutated lung tumours in non-small cell lung cancer (NSCLC). Although small molecule tyrosine kinase inhibitors (TKIs) of EGFR such as gefitinib, erlotinib and afatinib have been approved for the treatment of NSCLC patients harbouring activating mutations, including L858R and exon 19 deletions (EGFRm), these TKIs are poorly active against EGFR Ex20ins mutation positive NSCLC, leaving few treatment options for these patients. EGFR Ex20ins therefore remains an area of unmet need. Acquired resistance to approved 1st generation TKIs via the additional ‘gatekeeper mutation’ T790M, led to the development of osimertinib in multiple indications. Osimertinib is a potent and selective irreversible inhibitor of the activating and T790M mutant forms of EGFR, whilst importantly, having selectivity over wild-type EGFR. Due to the current unmet need in patients with EGFR Ex20ins positive NSCLC and the recent approval of osimertinib as a 3rd generation TKI we wished to explore, using pre-clinical models, whether osimertinib may provide a more effective treatment option for patients with these insertions.

Material and Methods: Notably, using CRISPR CAS9 approach in H2073 EGFR wild-type NSCLC cell line, we have established for the first time cellular disease models to measure in vitro and in vivo activity against the most prevalent V769-D770insASV (17%) and D770-N771insSV (22%) forms of Ex20ins EGFR. The activity of osimertinib and A2S104 was benchmarked against key clinical compounds including erlotinib and afatinib (at clinically relevant doses), and other disclosed EGFRm/T790M inhibitors.

Results: In our work, osimertinib and its major metabolite (A2S104) demonstrated robust EGFR Exon20ins activity using a variety of biochemical, in vitro cellular phosphorylation and proliferation assays. In these recent studies, osimertinib has shown good anti-tumour activity in both prevalent CRISPR Ex20ins H2073 models at compound exposures consistent with the 80 mg and 160 mg clinical doses. In addition, anti-tumour activity was observed in patent derived xenograft (PDX) models harboring the rarer H7T37-V774insNPH and M766-A76insASV exon 20 insertions.

Conclusions: This pre-clinical data package supports the clinical evaluation of osimertinib in NSCLC patients harboring Ex20ins of EGFR.

No conflict of interest.

99 Poster (Board P070)
The novel BTK and PI3K-delta inhibitors acalabrutinib (ACP-196) and ACP-319 show activity in pre-clinical B-cell lymphoma models

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Background: BTG and PI3K-delta inhibitors are among the most promising classes of anti-lymphoma agents, as demonstrated by the FDA approval of ibrutinib and idelalisib. Acalabrutinib (ACP-196) and ACP-319 are novel BTK and PI3K-delta selective inhibitors, respectively, under clinical evaluation. Here, we assessed their anti-tumor activity in lymphoma pre-clinical models.

Material and Methods: Cell lines derived from activated B-cell like (ABC) diffuse large B-cell lymphoma (DLBCL), (OCI-LY-10, TMD8, SU-DHL-2, U2932), from mantle cell lymphoma (MCL) (Rec1, Jeko1, Z138, Maver1), from splenic marginal zone lymphoma (SMZL) (Karpas1718, VL51, SSK41) and chronic lymphocytic leukemia (MEC1) were exposed to compounds alone and in combination. Synergy was assessed with Chou-Talayai combination index (CI): strong synergism (<0.3), synergism (0.3−0.9), additive (0.9−1.1), antagonism/no benefit (>1.1). For in vivo studies, OCI-LY-10 cells (15 × 10^6) were subcutaneously injected in NOD-Scid (NOD.CB17-Prkdcscid/Ncxid) mice; treatments (15 mg/kg twice per day, Qd ×7×w×2w) were started with 100−150 mm^3 tumors.

Results: ACP-319 showed a potent anti-proliferative activity in ABC-DLBCL TMD8 and OCI-LY-10 (IC50, 150nM and 800nM, respectively), a moderate activity in MEC1 and VS1 (11−31nM). Synergy was assessed with Chou-Talaiya combination index (CI): strong synergism (<0.3), synergism (0.3−0.9), additive (0.9−1.1), antagonism/no benefit (>1.1). For in vivo experiments, OCI-LY-10 cells (15 × 10^6) were sc inoculated in NOD-Scid (NOD.CB17-Prkdcscid/Ncxid) mice; treatments (15 mg/kg twice per day, Qd ×7×w×2w) were started with 100−150 mm^3 tumors.

Conclusions: This pre-clinical data package supports the clinical evaluation of acalabrutinib in ABC-DLBCL patients harboring Ex20ins of EGFR.

No conflict of interest.
only the 3 acalabrutinib sensitive cells responded also to spekrutinib, the anti-proliferative effect observed in Jeko1 with brutinib might be not BTK-mediated.

The acalabrutinib/ACP-319 combination was of benefit in 10/12 cell lines. ABC-DLBCL had a strong synergism in 2 (OCT2LY-10, median CI 1.32; Karpas1718 0.99). Finally, acalabrutinib and ACP-319 were in vivo tested as single agents: treatment ABC-DLBCL xenografts achieved a 2.5-fold volume decrease when compared with control (P < 0.05).

**Conclusions:** Acalabrutinib and ACP-319 showed both in vitro and in vivo anti-lymphoma activity. In vitro synergism was observed in some cell lines when combined with acalabrutinib or ACP-319. Further preclinical and clinical studies are suggested.

**Conflict of interest:** Corporate-sponsored Research: Francesco Bertoni received institutional research funds from Acerta.

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**Histone deacetylase inhibition triggers suppression of the IGF-1R/Akt pathway in rhabdomyosarcoma and Pax3-Foxo1 in alveolar rhabdomyosarcoma**


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Rhabdomyosarcoma (RMS) is the most common pediatric soft tissue sarcoma. The two major histological subtypes of RMS are alveolar RMS (ARMS), driven by the oncogenic fusion protein Pax3-Foxo1, and embryonic RMS (ERMS), which is more genetically heterogenous with a RAS-like signature. The prognosis of RMS has improved in the past several decades due to intensive multi-modal therapeutic regimens, but outcomes of patients with metastatic refractory RMS remain poor. A platform and identification of novel therapeutic targets is imperative. Recently discovered genetic clues point out an important role for histone deacetylases (HDACs) in subtypes of different cancers. Inhibition of HDACs prevents the deacetylation of histone and non-histone proteins, and induces a variety of biological responses in tumor cells including alterations in cell proliferation and survival, altered gene expression, and altered cell cycle. In this study, we determined that HDACs are necessary for the growth and survival of both ARMS and ERMS cells using a loss of function high-throughput shRNA screen. We then examined the molecular effects of a pan-HDACs inhibitor, LBH589, on the IGF-1R/Akt pathway, previously shown to be critical for RMS growth and survival. Treatment with the pan-HDACs inhibitor LBH589 resulted in significant inhibition of both RMS cell growth and survival, together with down-regulation of IGF-1R expression and Akt activation. Moreover, treatment with LBH589 led to suppression of Pax3-Foxo1 in ARMS cell lines. Combining treatment with LBH589 and Akt inhibitor, MK2206, demonstrated synergistic effects on the inhibition of cell growth. We can conclude that HDACs are critical for RMS growth and survival. Therefore, Pax3-Foxo1 fusion positive ARMS may be particularly vulnerable to HDAC inhibition since this leads to downregulation of expression of the oncopgenic fusion.

**No conflict of interest.**

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**Prognostic biomarkers as molecular targets for individualized neoadjuvant treatment for cervical cancer**


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Cervical cancer (CC) is one of the most prevalent malignancies and of higher mortality in the world, and is considered a marker of underdeveloped countries. Conventional Radiotherapy (RT) is one of the treatments used for this type of cancer. 30 to 40% of patients with similar prognostic factors not responsive to 1st treatment may benefit from additional treatments. Different molecular factors involving tissue oxygenation, oncogene activation, loss of tumor suppressor genes and aberrant molecular signaling pathways have recently been identified in CC and might be the root of resistance to RT. Identifying synergistic biomarkers of response to RT is of primary interest since targeting these pathways may directly lead to improve outcomes of RT for cervical cancer locally advanced, metastatic and refractory. A comparative analysis of cervical cancer in the context of other cancers may reveal that it is relatively smaller number of targeted molecular agents that have been tested for development for the purpose of inhibiting angiogenesis, molecularly address EGFR and IGF-1R, modulation of cell cycle, histone deacetylases, COX-2, mTOR and tumor microenvironment (hypoxia and glycolysis). Within work that we have been developing, reported that gene expression of IGF1R is a strong predictive marker for lack of response to radiotherapy, patients have 28.6 times higher risk of failure treatment; Objective: To determine whether expression of IGF-1R, GAPDH, HIF-1 alpha, Survivin, GLUT1, CAIX, HKII and clinicopathological parameters can be used as prognostic biomarkers to treatment outcome and as possible molecular targets.

**Patients and Methods:** This prospective cohort study included 149 patients with squamous cell carcinomas of the uterine cervix in FIGO stages IIB and IIB between 2008 and 2011. The mean age was 46 years. Of the 149 patients, 61 were treated with radiotherapy and 88 with concurrent radiochemotherapy. Expression of the proteins CAIX, GLUT-1, HIF-1α, HKII, IGF-1R, IGF-1R and Survivin was determined by immunohistochemistry in biopsies taken before treatment. Additionally, we evaluated a group of 51 cases of non-tumor tissue of cervix from biopsies with diagnostic of cervicitis.

**Results:** Highest increase was observed in expression of IGF-IRx (76.5%), IGF-IRy (74.5%) and HIF-1α (74.1%); the concordance between IGF-IRx and IGF-IRy was of 73%; strong expression was observed with low frequency for GLUT-1 (31.1%). We found that patients with IGF-IR ≥ 11 g/dl have improved overall survival compared to those that express IGF-1R (11 g/dl (P < 0.05)).

**Conclusion:** Using the expression of GLUT1, IGF-1R and Hb levels (≤ 11 g/dl) as therapeutic molecular targets could contribute to an appropriate therapeutic management as individualized neoadjuvant treatment for cervical cancer.

**No conflict of interest.**

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**Clinically relevant morphological structures as transcriptionally distinct tumor subpopulations and potential therapeutic targets in breast cancer**


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**Background:** Breast cancer (BC) demonstrates significant intratumor morphological heterogeneity (IMH) represented by different morphological structures as tubular, alveolar, solid, trabecular, and discrete groups of tumor cells, which contribute to chemotherapy efficiency and lymphogenic metastasis (Zavyalova et al., 2013; Denisova et al., 2014). In this study, we validated the contribution of IMH to chemotherapy response and metastasis in the large group of BC patients and investigated phenotypic features of different morphological structures of breast tumors.

**Material and Methods:** 434 patients with invasive breast carcinoma of no special type (T1-4N0-3M0-1), who received neoadjuvant chemotherapy (NAC), were included in this study. The association study was performed to clarify the role of IMH in NAC response and metastasis-free survival. Laser microdissection-assisted gene expression microarrays and qRT-PCR were applied to perform transcriptome profiling of morphological structures. Confocal microscopy was used for analysis of cancer stem cells (CSC, CD44+CD24-) in morphological structures.

**Results:** Breast tumors with 5-5 types of structures more often demonstrated chemoresistance than cases with 1-2 types of structures (p < 0.05). Alveolar and trabecular structures were found to be associated with poor response to NAC and decreased metastasis-free survival (p < 0.001). Si-multaneous calculation of alveolar and trabecular structures increased the prognostic value significantly (p < 0.00001). Alveolar structures correlated with a high frequency of distant metastasis only in patients with poor response to NAC, whereas trabecular structures were chemosensitive cancers. Different morphological structures were characterized by specific gene expression profiles (including overexpressed specific genes) and the regulation of specific signaling pathways, particularly cancer invasion pathways, which were more pronounced in trabecular structures and discrete groups of tumor cells. In addition, these morphological structures demonstrated significant upregulation of mesenchymal genes together with the lowest expression of epithelial markers. The mean proportion of CSCs in morphological structures was the following: 0.13 (0.11–0.15) in alveolar, 0.05.
Poster Session: Other, Wednesday 29 November 2016

105 Poster (Board P076)
Preclinical pharmacokinetic and pharmacological properties of ASP8273, a mutant-selective irreversible EGFR inhibitor, and its potential activity against brain metastases in NSCLC
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Introduction: Non-small cell lung cancer (NSCLC) accounts for the majority of lung cancer deaths despite the development of tyrosine kinase inhibitors (TKIs), which have demonstrated increased benefit over chemotherapy. Brain metastases often occur in patients with NSCLC and are associated with poor prognosis. ASP8273 is an irreversible, mutant-selective, c-epidermal growth factor receptor (EGFR) inhibitor in clinical development. ASP8273 has demonstrated antitumor activity in several preclinical models, which translated into clinical activity in EGFR-activating mutation-positive NSCLC patients in phase 1/2 studies. Here we investigated the potential activity of ASP8273 against brain metastases using a preclinical model of EGFR mutation-positive NSCLC.

Material and Methods: The efficacy of ASP8273 against intracranial tumor growth was evaluated with human EGFR-activating mutation-positive NSCLC HCC-827 tumor harboring nude mice. To investigate brain penetration of ASP8273, brain concentration of 11C- and 14C-labeled ASP8273 were measured by PET scanning and autoradiography in non-human primates and rats, respectively.

Results: In the intracranial implantation model of HCC-827 cells, significant growth inhibition was observed with ASP8273 treatment (30 mg/kg, QD) alone and combined with radiation treatment (8.7 Gy, twice). After a single oral administration of 14C-labeled ASP8273 mesilate to rats at 10 mg/kg, brain/plasma ratio of ASP8273 was 0.39–0.70, suggesting that ASP8273 is moderately permeable into brain. Further, we synthesized 11C-labeled ASP8273 and performed PET imaging to clarify penetration of ASP8273 into brain in non-human primates. ASP8273 penetrated into the monkey brain at the concentration of approximately 1 micro mol/L (approx. 3 mg/kg, i.v.). Plasma concentrations of ASP8273 in this study were similar to those in patients with cancer at the recommended phase 2 dose (300 mg, QD).

Conclusions: Taken together, these results suggest that ASP8273 has a potential for the treatment of brain metastases in patients with EGFR-activating mutation positive NSCLC.

Conflict of Interest: Other Substantive Relationships: We are employees of Astellas Pharma Inc.

105A Poster (Board P76A)
First-in-human study of BLU-554, a potent, highly-selective FGFR4 inhibitor designed for hepatocellular carcinoma (HCC) with FGFR4 pathway activity
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Background: FGFR4 and its ligand, sorafenib were given BLU-554 once daily on a 4-week cycle following a successful 28-day pharmacology model and 3 (8%) had intermediate TMB of 10−20 mutations/Mb and 3 (8%) had low TMB of G 1 mutations/Mb. None of the mutations were present in any other cancer. The majority of genomic alterations were single nucleotide variants (103; 15%) and small insertions and deletions (67; 9%).

Methods: Adult patients (pts) with advanced HCC and well-preserved liver function, who had received sorafenib, or not access or declined sorafenib were given BLU-554 once daily on a 4-week cycle following a 3+3 design. Adverse events (AEs) per CTCAE, PK and PD were assessed.

Baseline tumor FGFR19 expression was analyzed via immunohistochemistry (IHC) as a marker of pathway activity. Response was determined by RECIST every 8 weeks.

Results: At a 09-JUN-2016 cutoff, 14 pts have been treated with BLU-554, 3 (21%) of whom had 1/51 (2%), BRCA2 1/51 (2%), FLT3 1/51 (2%), MSH6 1/51 (2%), PTCH1 1/51 (2%), and INDEL alterations minus known driver alterations per megabase (Mb) of genome examined. Droplet digital (dd) PCR (Bio-Rad) was performed on plasma samples using cell-free DNA (cfDNA) in 12 patients. The efficacy of ASP8273 against intracranial tumor growth was evaluated with human EGFR-activating mutation-positive NSCLC HCC-827 tumor harboring nude mice. To investigate brain penetration of ASP8273, brain concentration of 11C- and 14C-labeled ASP8273 were measured by PET scanning and autoradiography in non-human primates and rats, respectively.

Conclusions: BLU-554, a potent, highly-selective FGFR4 inhibitor has acceptable tolerability in pts with advanced HCC and demonstrates objective clinical activity in FGFR19 IHC-positive disease. These data further implicate the FGFR4 pathway as a driver in HCC and provide the first proof of principle for targeting FGFR4 in HCC. Expanded clinical testing of BLU-554 with prospective selection of FGFR19 IHC-positive HCC pts is underway.
had high TMB of >20 mutations/Mb. In 3 patients with XP01 mutations in cDNA, the median MAF decreased after 4 weeks on systemic therapy (1.1% vs. 6.1%, p = 0.05), coinciding with improvement on PET/CT at 8-week follow-up.

Conclusions: Our findings demonstrate that CGP of archived FFPE specimens is feasible in HL despite low tumor burden. XP01 E571 mutations and other alterations with potential therapeutic relevance are prevalent in HL. Further study is needed to determine if XP01 mutations can be associated with activity of novel XPO1 inhibitors.

Conflict of interest: Corporate-sponsored Research: Filip Janku received research funding from Foundation Medicine, Inc. Other Substantive Relationships: Vincent A. Miller, Philip J. Stephens, Jeffrey S. Ross are employees with a leadership role and stock and other ownership interests in Foundation Medicine, Inc. Jo-Anne Vergilio, Jie He, Michelle Nahas and Tarig Mughal are employees and have stock and other ownership interests in Foundation Medicine, Inc.

107 Poster (Board P078)
Hetero-interaction amongst Tyro3 and Axl receptor tyrosine kinases diversifies cancer signalling
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Receptor tyrosine kinases (RTKs) regulate major biological processes such as growth and survival of cells, and this function has also led to their identification as oncogenes, in many cancers, whereby they promote cancer progression and metastasis. These RTKs represent a novel target for targeted therapy with small molecule inhibitors. The TAMs (Tyro3, Axl, Mer) are a subfamily of RTKs whose overexpression has been linked to increased invasion and chemoresistance in various cancers. In addition to signalling via homodimeric activation of RTKs, diversity in signalling may be achieved by cross-talk amongst different RTK types, including heterodimerisation. For example, in lung and breast cancers, Axl has been shown to modulate acquired resistance to EGFR inhibitors. However, the potential for signalling cross-talk amongst different RTKs of the TAM family is currently unknown. Of the TAMs, Tyro3 is the best known; however, its overexpression has been linked to increased proliferation in hepatocellular carcinoma as well as acquired resistance to taxol in ovarian cancer. Therefore, the purpose of this study was to identify and characterise a novel, unconventional Axl-Tyro3 hetero-interaction and related signalling pathway in cancer cells. In this study, we report the heterodimerisation of Tyro3 with Axl in human brain tumour (glioblastoma multiforme (GBM)) cells under physiological levels, and the intensification of this interaction by the TAM ligand Gas6, as seen by co-immunoprecipitation experiments. Furthermore, with the aid of siRNA knockdown, qPCR and western blot, we identified a co-dependent regulation of protein expression between Axl and Tyro3. Additionally, by examining the cell cycle and intracellular downstream signalling, we show Tyro3 expression to be essential for GBM cell cycle progression and proliferation of the cell cycle checkpoint Tyro3 knockdown induces a G2/M arrest and eventually cell death through apoptotic mechanisms. In conclusion, these data show for the first time the interaction between two “sister” RTKs of the same family in cancer, and show us on the possible mechanisms of heterodimerisation of receptors, can provide cancer cells with an evolutionary advantage. Moreover, our data highlight the increased diversity in RTK signalling in cancer, and how this can lead to chemoresistance in targeted therapies.

No conflict of interest.

108 Poster (Board P079)
Early interventions to detect potential drug–drug interactions on patient eligibility for phase 1 clinical trials
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Early interventions to detect potential drug–drug interactions on patient eligibility for phase 1 clinical trials (DDI) can be associated with activity of novel XPO1 inhibitors.

Conflict of interest: Corporate-sponsored Research: Filip Janku received research funding from Foundation Medicine, Inc. Other Substantive Relationships: Vincent A. Miller, Philip J. Stephens, Jeffrey S. Ross are employees with a leadership role and stock and other ownership interests in Foundation Medicine, Inc. Jo-Anne Vergilio, Jie He, Michelle Nahas and Tarig Mughal are employees and have stock and other ownership interests in Foundation Medicine, Inc.

109 Poster (Board P080)
CYP27A1/27HC/SREBP2 axis is a novel therapeutic target in prostate cancer
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Background: Prostate cancer (PC) is the most common cancer among men and the second most lethal. While the underlying causes of PC remain unclear, epidemiological studies have suggested that hypercholesterolemia is associated with an increased risk of high-grade metastatic disease. Indeed, PC cells and those of other solid tumors contain cholesterol levels that are higher than juxtaposed normal cells. It is thus not surprising that inhibitors of HMG-CoA-reductase (HMGCGR, statins), which reduce serum cholesterol, are associated with lower risk of PC and reduced PC progression. Based on the above, we developed a project the objective of which was to identify genes involved in cholesterol homeostasis whose expression/activity was dysregulated in PC. We reasoned that such an approach would also yield novel targets amenable to pharmaceutical exploitation.

Materials and Methods: A list of 176 genes involved in cholesterol biology was derived using the following GO ontologies: 'GO:0006695' = cholesterol biosynthetic process, 'GO:0042632' = cholesterol homeostasis, 'GO:0045540' = regulation of cholesterol biosynthetic process and 'GO:0008203' = cholesterol metabolic process. Using data extracted from The Cancer Genome Atlas (TCGA), the expression level of each of these genes relative to clinical features known to predict clinical outcome such as T-stage, Gleason score at diagnosis, and the presence of lymph node metastases was assessed. These genes were modelled for association to these clinical features using logistic regression and evaluated using both the 97.5% CI, odds ratio and significance. Only genes with a p-value of less than 0.01 were considered significant and only genes with significant association with all three features were brought forward for more analysis.

Results: CYP27A1 was the only gene which was associated with all three features (T-stage, Gleason score at diagnosis, lymph node metastasis). Lower CYP27A1 transcript levels were associated with shorter disease-free survival and higher tumor grade. Loss of CYP27A1 was found to be associated at the protein level by immunostaining for CYP27A1 in annotated tissue microarrays. Restoration of CYP27A1 expression in cells where its gene was silenced attenuated their growth in vitro and in tumor xenografts. Further studies revealed that treatment of PC cells with 27-
hydroxysterol (27HC), an enzymatic product of CYP27A1, reduces cellular cholesterol content in cell lines by inhibiting the activation of SREBP2 and downregulating LDLR expression.

**Conclusions:** Our findings suggest that CYP27A1 is a critical cellular cholesterol sensor in PC and that the CYP27A1/27HC/SREBP2 axis is a novel therapeutic target.

**No conflict of interest.**

110 Poster (Board P081)

**Multispectral autofluorescence imaging for cervical cancer screening**

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**Background:** To develop a novel optical imaging system for detecting protoporphyrin IX (PpIX) autofluorescence, to prove that PpIX autofluorescence is as useful as 5-aminolevulinic acid (5-ALA)-induced fluorescence for detecting and localizing cervical cancer, and to monitor the change in PpIX autofluorescence or induced PpIX fluorescence before, during, and after photodynamic therapy (PDT).

**Material and Methods:** TC-1 cell line – highly tumorigenic cells immortalized using human papillomavirus type 16 proteins E6 and E7 – were subcutaneously grafted into the thighs of nude mice. The suspected tumor tissues were visualized using autofluorescence imaging and induced fluorescence imaging under 5-ALA administration. When 5-ALA-induced production of PpIX sufficiently accumulated in tumor tissues, PDT was performed using a 635-nm laser. We observed the change in fluorescence intensity during PDT. For 3 weeks after PDT, we monitored tumor remission by using white-light imaging and fluorescence imaging.

**Results:** The transplanted cells were visualized by PpIX autofluorescence, which was induced by heme synthesis. After 5-ALA administration, PpIX could be targeted by using PDT, which decreased PpIX autofluorescence.

Photobleaching is useful for monitoring PDT dosimetry and for determining the photodynamic response to therapy.

**Conclusion:** PpIX autofluorescence clearly differentiated the tumor from adjacent normal tissues. The results of PpIX autofluorescence imaging and 5-ALA-induced fluorescence imaging were identical. PpIX autofluorescence imaging is a simple and cost-effective cervical cancer screening method that could be performed during or after PDT to ensure effective treatment or remission since a change in fluorescence intensity can be observed in real time without a blinding effect.

**No conflict of interest.**

112 Poster (Board P083)

**The study of L1 in prostate cancer**

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**Background:** Long interspersed nuclear element-1 (L1) is the most abundant and only autonomously active family of non-LTR retrotransposons in the human genome and comprises about 17% of the human genome. Human L1 is about 6.5 kb and consist of two open reading frames (ORF1p and ORF2p) required for retrotransposition. L1-ORF1 and ORF2 are co-regulated in a variety of malignancies. A number of observations indicate that L1 sequences nevertheless become reactivated and have strong expression in human cancer. In theory L1 activation in cancer might cause transcriptional deregulation, insertional mutations, DNA breaks, and an increased frequency of recombination contributing to genome disorganization, expression changes, and chromosomal instability. The present study aimed to analyze the epigenetic profile of L1 among prostate circulating tumor cells (CTCs), prostate cancer stem cells (CSCs), differentiated prostate cancer cells and healthy individuals.

**Material and Methods:** Blood samples were collected from 3 patients representing prostate cancer and 3 healthy individuals. Cells were isolated by using enrichment protocols, including CD45 negative selection for normal samples and pancytokeratin positive selection for cancer samples. In addition, commercial prostate CSCs and DU145 cell line (provided by Celprogen and ATCC respectively) were also used. DNA and RNA were retrieved from the above cells and qPCR experiments were performed. The primers were designed to amplify specific regions of ORF1 and ORF2. The relative quantification was performed according to Livak method, by using 18sRNA as housekeeping gene.

**Results:** The analysis of DNA revealed the presence of ORF1 and ORF2 in both samples. The qPCR experiments demonstrated that only ORF2 was expressed in the above samples. The difference in ORF2 expression was statistical significant among CTCs and the rest cell types, with higher expression levels for CTCs (p < 0.001). Among the other types there was no significant different (p = 0.66).

**Conclusion:** The present study demonstrated that among prostate cancer, the ORF2 RNA expression is higher in CTCs than in CSCs or differentiated prostate cancer cells. It is also noteworthy, that there is no significant difference among healthy individual and differentiated cells, indicating that L1 might be essential for tumor initiation. Further experiments, in more defined samples, should be performed to confirm the above and then to be used at clinical level.

**No conflict of interest.**
Furthermore, positive biomarker chip result was strongly correlated with poor disease-free survival and overall survival of CRC patients (both P < 0.001).

**Conclusions:** In this prospective study, compared to conventional serum CEA level, the constructed multi-gene biomarker chip was more accurate and earlier prediction of postoperative relapse in surveillance for stage I-III CRC patients. Hence, the biomarker chip for the detection of CTGs could be potentially used in clinical practice to improve early diagnosis of postoperative relapse in CRC patients.

**No conflict of interest.**

**114** Poster (Board P085)

**Targeted expression and molecular profiling assay for tumor microenvironment**

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1 Collecta Inc., Mountain View, USA

New rapid and robust transcriptome-based methods for cellular characterization of the tumor microenvironment and biomarker discovery are required to improve cancer prognosis and treatment. However, challenges with current approaches for the above applications include high sample requirements, limited sensitivity, low dynamic range, and limited throughput. To address these limitations, we have developed a unique approach for targeted transcriptome profiling using validated targeted primers that leverages the sensitivity of multiplex RT-PCR with the throughput of Next-Generation Sequencing (NGS) technology. By combining these methods, just 10–100 ng of total RNA is sufficient to quantify over 5 orders of magnitude variation in gene expression levels. Further, the use of targeted primers enables direct analysis of total RNA isolate and obviates the need for globin depletion from whole blood samples. Finally, using a defined set of amplicons to assess expression levels of all protein-coding genes facilitates and simplifies data analysis and allows more precise sample-to-sample normalization. We will present profiling results that demonstrate how this assay can be used to analyze the level of immune cell infiltration, assess intact and deficient immune mechanisms, and generally elucidate the tumor microenvironment of breast cancer samples.

**Conflict of interest:** Other Substantive Relationships: All authors are employees of Collecta, Inc.

**115** Poster (Board P086)

**CRISPR/Cas9 genome-wide gRNA library for target identification**

P. Dieni1, D. Tedesco1, M. Makhanov1, S. Baron1, D. Suchkova1, C. Frangou1, A. Chenchik1. 1 Collecta Inc., Mountain View, USA

Genome-wide loss-of-function screening is a fundamental method to identify genes responsible for driving biological responses, and complex polymorphism-based libraries expressing large numbers of genetic disruptors, such as shRNAs, make large-scale cell screening practical. While RNAi-based approaches have proven to be an effective strategy for identifying these targets, recent work suggests CRISPR technology offers an effective alternative. Although shRNA and sgRNA pooled library screens are similar in concept, the gene interruption with the two techniques occurs by a very different mechanism so some divergence may be expected when comparing results obtained using one method versus the other.

To investigate the potential difference in the two methodologies, we performed parallel dropout viability screens to identify essential genes in a pair of primary isogenic CML cell lines using a CRISPR/Cas9 knockout library and an RNA interference (RNAi) library targeting the same set of 6,300 genes with the same number of targeted effectors (sgRNA or shRNA) for each gene. The results showed significant, but not complete, overlap in the essential genes identified by each assay in each cell line indicating that both approaches are effective to identify the majority of essential genes in a cell system. However, analysis did indicate that a small number of essential targets were only identified with CRISPR and certain unique targets seemed to show up only in the RNAi screen results. By combining data from the two screening methodologies, a consistent number of viability genes and pathways could be identified and subsequently validated by independent cell based assays at a very high confirmation rate.

**Conflict of interest:** Other Substantive Relationships: All authors are employees of Collecta, Inc.

**116** Poster (Board P087)

**TГ€GА§ pan-cancer transcriptome-based pathway analysis for cancer therapeutics**

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**Background:** The Cancer Genome Atlas has profiled more than 10,000 tumors from 33 different cancer types. Previous analysis has revealed some tumors are more likely to be genetically similar based on the cell types rather than tissue site of origin. Identification of transcriptionally active groups of genes across tumor types can provide insight for indication selection for new therapeutics.

**Material and Methods:** K-means clustering was performed on the combined 10,000-sample pan-cancer dataset at gene expression levels for different variable ranges for new k (k = 20, 21, . . . , 200). Fisher’s exact test was used to obtain p value of the canonical pathways and Gene Ontology term association for each cluster for any given k. For each k, combined p values for top 20 clusters were calculated. The optimal k of 62 was selected for smallest combined p value among all k. The average expression of all genes in each cluster was used as the index to represent transcriptional status of the cluster.

**Results:** Based on TCGA’s RNA-seq data, we determined the mammalian cancer genome can be useful represented by 62 non-overlapping, functionally relevant groups of genes (transcription clusters) whose intra-group transcript level is coordinately regulated across cancer types. Although the transcription clusters were identified through non-supervised clustering analysis, we have observed that genes with known similar functions clustered together. Transcription clusters were found to be more robust than any single gene, and to be better than clinical pathways because they were optimized for transcriptome analysis by non-supervised clustering without prior knowledge. Such clusters may provide additional insights than canonical pathways, and be associated with lymphoid clusters, termed lymphoid, myeloid, interferon, and cytokine. Lymphoid cluster is enriched for genes related to T cells, B cells, and NK cells; and myeloid cluster is enriched for genes related to macrophages, neutrophils, monocytes, etc. Both lymphoid index and myeloid index correlate with leucocyte percentage in the TCGA gastric dataset. Through analysis of tumor profiling data, we found these transcription clusters showed different expression level across different cancer types. As an example, PD-1 expression signature refined by the lymphoid index can provide rationale for indication selection among 33 TCGA cancer types.

**Conclusions:** We developed transcriptome-based pathways from >10,000 tumors from 33 cancer types, and identified at least four immune clusters that grouped tumors independent of cancer histology type. We used transcription clusters to provide insight for prioritizing therapeutic indications, combination strategies, and biomarker hypotheses for our therapeutic targets.

**Conflict of interest:** Ownership: Bin Feng and Yonghong Xiao are employees of TESARO.

**117** Poster (Board P088)

**Large-scale modeling of cancer signaling: Mechanistic modeling meets Big Data**

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**Background:** Large-scale studies like The Cancer Genome Atlas (TCGA) revealed that cancers are multi-factorial diseases, which strongly vary between patients. This inter-patient variability poses a challenge for clinicians. A priori it is not clear which drug or drug combination will be most beneficial for an individual.

**Material and Methods:** In a multi-national collaboration within the CanPathPro project (http://www.canpathpro.eu), we approach the problem of drug response prediction using a system biological approach. We developed a generic large-scale mechanistic dynamic model covering dozens of cancer associate signaling pathways. This ordinary differential equation model can be individualized using exome and transcriptome sequencing data − carrying information about mutation status and expression levels. For statistical inference of the model parameters we use Bayesian inference to construct a likelihood function, which is carried out using the software package F 10 E 12,11. On the validation set we achieved a prediction accuracy of roughly 80%, substantially better than conventional statistical approaches. Even for drugs that only showed weak activity in the CCLE dataset (e.g., cetuximab), we achieved a prediction accuracy of 70%.

**Results:** We evaluated the proposed model-based approach, we studied data response from the Cancer Cell Line Encyclopedia (CCLE) for 8 drugs and 120 cell lines originating from five different tissues. We trained the model on 80% of the cell lines and predicting the response of the remaining 20%. On the validation set we achieved a prediction accuracy of roughly 80%, substantially better than conventional statistical approaches. Even for drugs that only showed weak activity in the CCLE dataset (e.g., cetuximab), we achieved a prediction accuracy of 70%.

**Conclusions:** Our results demonstrate the potential of large-scale mechanistic modeling for drug selection in personalized therapy.

**Conflict of interest:**
Background: Human Epidermal Growth Factor Receptor 3 (HER3) is a member of the family of receptor tyrosine kinase receptors implicated in the development and progression of several human cancers. HER3 expression is frequently observed in malignant tumors and associated with resistance to therapy and poor prognosis. In spite of its important role, no standard methods to determine the expression of HER3 in clinical samples have been devised for patient stratification or diagnostic purposes. Here, we quantitatively assessed HER3 mRNA and protein expression in human cancer tissue samples applying automated in situ hybridization (ISH) and immunohistochemistry (IHC) in conjunction with digital image analysis.

Material and Methods: We successfully established RNAscope HER3 ISH and anti-HER3 IHC on the Ventana DISCOVERY XT automated staining platform and analyzed a tissue microarray (TMA) of 39 formalin-fixed paraffin-embedded tumor cores representing seven human cancer types (non-small cell lung cancer, breast cancer, colorectal cancer, prostate cancer, hepatocellular carcinoma and ovarian cancer). To quantify HER3 mRNA and protein expression levels, image analysis algorithms were developed using Indica Labs HALO software. A pan-cytokertatin (pan-CX) IHC stained serial TMA section was used to define tumor regions of interest (ROI) within each core. Pan-CX-stained cores were then coregistered with their associated HER3 ISH and anti-HER3 IHC cores to quantify numbers of HER3 mRNA and protein positive cells per mm² tumor ROI.

Results: Confirming published results on the expression of HER3 in different cancer indications, HER3 mRNA and protein were detected in the majority of the analyzed tumor samples. Data generated suggest a strong correlation between HER3 mRNA and protein expression within tumor tissue of lung adenocarcinoma, breast cancer and hepatocellular carcinoma cores. A lower correlation was observed for the other cancer tissue cores analyzed.

Conclusions: We showed the feasibility of automated ISH and IHC followed by digital image analysis to quantify the expression of HER3 mRNA and protein in clinical samples. Further studies are required to confirm the robustness and reproducibility of observed correlations between HER3 mRNA and protein per cancer. The standardized quantification offered by our approach could support molecular diagnostic and patient stratification applications in clinical oncology.

Conflict of interest: Corporate-sponsored Research: The authors are employed by Indivumed GmbH or OracleBio Ltd., both of which sponsored the presented research.
expression of EPCAM, MUC1 and ERBB2 was studied using multiplex-PCR. CTC positivity and cutoffs, obtained by ROC curve analysis in healthy donors, were: &gt;1 positive marker among EPCAM (0.40 ng/ml), MUC1 (0.10 ng/ml) and ERBB2 (0.20 ng/ml). CTC variation (0.02) was split in favorable (+/+), unfavorable group (+/-) and unfavorable group (-/-) due to small numbers. Univariable analyses were undertaken for progression-free (PFS) and overall survival (OS). Multivariable analyses with bivariable associations with clinical factors were also done to improve understanding of effects.

Results: Among the 31 analyzed pts, 17 (54.8%) were CTC+ at t0 and no association was found with any baseline pt and tumor characteristic, as well as with CTC status and objective response to MVC. Unfavorable CTC trend was observed in 10/26 (38.5%) cases. CTC dynamic changes better predicted for 3-year (3y) PFS and OS probability compared to CTC status assessed at single time points. Unfavorable trend was univariably detrimental on both 3y PFS probability (10% vs 49.2%, p = 0.006) and 3y OS probability (6% vs 33.3%, p = 0.005) when adjusted for liver metastases (p = 0.031 and p = 0.025 for PFS and OS) and MSKCC risk score (p = 0.014 and 0.025).

Conclusions: We proposed a novel technique to early assess CTC status in metastatic UC receiving MVC CT. Early CTC changes may be useful to improve our prognostic ability. Pending validation, these results may lead to improved trial designs and to refine the sequence of conventional CT options in novel prognostic setting.

No conflict of interest.

122 Poster (Board P093)
An evaluation of the association between molecular signature and postoperative recurrence in patients with non-small cell lung cancer

Material and Methods: Between September 2014 and September 2015, 247 pts with NSCLC, including 192 pts with adenocarcinoma (Ad) and 55 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). Mutations (mt) detected in 138 cancer-related genes listed in Vogelstein et al. [1] were evaluated as driver mt.

Results: P-rec was observed in 26 (13.5%) and 13 (23.6%) pts with Ad and Sq, respectively. Median time of p-rec (range) was 274 days (102–749). Patient background [recurrence (rec); non-recurrence (non-rec); median age (range)] 72 (52–87); 69 (39–97), male 64%; 63%, smoker 77%; 70%, pathological stage (p-stage) (I/II/III) 41/33/26%; 72/19/9%, histological type (Ad/Sq) 67%/33%; 80%/20%, driver mutation (presence/absence) 95%/5%; 82%/18%, median somatic mutational burden (range) 2.7 Mt/Mb (0.2–17.4); 1.7 MtMb (0.1–61.4). In the rec group, the most common driver mutation was TP53 mutation (54%, 21/39 pts). In Fisher’s exact test, the presence or absence of p-rec showed trend of association with presence or absence of driver mt (ie. driver mutation status (p = 0.055) and significantly association with p-stage (I/II, p = 0.003), but not with histology type (p = 0.50), smoking status (p = 0.44), gender (p = 0.99) or age (<70/>70) (p = 0.22). Multiple logistic regression analysis also revealed trend of association between driver mutation status and p-rec (p = 0.052, odds ratio [OR]: 3.56, 95% CI 0.98–22.9), when adjusted by histological type (p = 0.40), smoking status (p = 0.26), gender (p = 0.25), age (p = 0.15) and p-stage (p = 0.0002, OR: 4.12, 95% CI 1.97–8.93). The somatic mutational burden did not differ significantly between the rec and non-rec groups (p = 0.35).

Conclusions: Driver mutation status may be associated with p-rec of NSCLC.

References

No conflict of interest.
**Poster Session – Paediatric Oncology, Wednesday 29 November 2016**

### Paediatric Oncology

#### Flubendazole as potential anti-neuroblastoma therapy option

**Poster (Board P097)**

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**Background:** Flubendazole was shown to exert anti-leukaemia and anti-metastasis activity through inhibition of microtubule function.

**Materials and Methods:** Flubendazole was tested for anti-cancer in cancer cell lines and in the chick chorioallantoic membrane assay. Protein levels were determined by Western blot and flow cytometry. RNAi-mediated depletion was used to inhibit gene expression.

**Results:** Neuroblastoma was identified as highly flubendazole-sensitive cancer entity in a screen of 321 cell lines from 26 cancer entities. Flubendazole also reduced the viability of five primary neuroblastoma samples in nanomolar concentrations thought to be achievable in humans and inhibited vessel formation and neuroblastoma tumour growth in the chick chorioallantoic membrane assay. Resistance acquisition is a major problem in high-risk neuroblastoma. 119 cell lines from a panel of 140 neuroblastoma cell lines with acquired resistance to various anti-cancer drugs were sensitive to flubendazole in nanomolar concentrations. Tubulin-binding agent-resistant cell lines displayed the highest flubendazole IC50 and IC50 values but differences between drug classes did not reach statistical significance. Flubendazole induced p53-mediated apoptosis. The siRNA-mediated depletion of the p53 targets p21, BAX, or PUMA reduced the neuroblastoma cell sensitivity to flubendazole with PUMA depletion resulting in the most pronounced effects. The MDM2 inhibitor and p53 activator nutlin-3 increased flubendazole efficacy while RNAi-mediated p53-depletion reduced its activity.

**Conclusion:** Flubendazole represents a potential treatment option for p53 wild-type neuroblastoma (only a small minority of neuroblastomas harbour p53 mutations) including therapy-refractory cells.

**No conflict of interest.**

#### Prognostic significance of preoperative and postoperative plasma levels of ghrelin in gastric cancer: A prospective study

**Poster (Board P096)**


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**Background:** We aimed to investigate prognostic effects of plasma levels of ghrelin before and after gastrectomy in gastric cancer.

**Materials and Methods:** In this follow-up study, we enrolled 93 gastric cancer patients from Cancer Institute of Iran, Tehran, Iran. All the patients were candidates for curative or palliative gastrectomy. We followed up the patients up to three years. Plasma levels of total and active ghrelin before and after the operation were assessed. Univariate and multiple Cox regression analyses investigated the independent predictors of patients overall survival.

**Results:** We observed significant decrease in plasma levels of both total (P < 0.001) and active (P < 0.001) ghrelin after gastrectomy. Plasma levels of ghrelin before and after gastrectomy were associated with survival even after adjusting for other factors. Multiple Cox models revealed worse survival for patients with postoperative total ghrelin below median (HR = 2.33, 95% CI: 1.01–5.41) or 25th percentile (HR = 4.29, 95% CI: 1.48–12.44) compared to patients with higher ghrelin levels. In addition, preoperative total ghrelin (HR = 2.67, 95% CI: 1.11–6.38 for second quartile, and HR = 2.32, 95% CI: 1.01–5.35 for third quartile vs first quartile) and active ghrelin (HR = 4.92, 95% CI: 1.80–13.54 for second quartile, and HR = 2.87, 95% CI: 1.11–7.38 for third quartile vs first quartile) were associated with survival. Advanced TNM stage (HR = 4.88, 95% CI: 1.13–21.77), cachexia (HR = 2.99, 95% CI: 1.35–6.83), and receiving no adjuvant chemotherapy (HR = 2.02, 95% CI: 1.04–3.92) were other poor prognostic factors.

**Conclusion:** Preoperative and postoperative plasma levels of ghrelin could predict survival of gastric cancer patients with different patterns. This prognostic effect was independent of stage and cachexia.

**No conflict of interest.**
Inhibition of BET bromodomain proteins and the PI3K pathway in Ewing sarcoma down regulates the specific transcriptional program, inhibits tumorigenicity and increases apoptosis in Ewing sarcoma

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Background: Ewing sarcomas (ES) are highly malignant bone or soft tissue tumours that are genetically defined by balanced chromosomal EWS/ETS translocations. The resulting chimeric proteins (EWS-ETS) generate an oncogenic transcriptional program associated with altered epigenetic marks throughout the genome. Here we analysed BET bromodomain proteins (BRDs) and their role in tumorigenesis and the contribution of oncogenic transformation mediated via EWS-FLI1. In addition, we analysed co-treatment with an inhibitor of the PI3K pathway, which is upregulated in EWS-FLI1 signaling. We overall aimed to better understand the mechanisms necessary to establish and maintain the underlying genetic and epigenetic events utilized in ES.

Methods: Function of BRDs and the PI3K pathway was analysed by application of specific inhibitors (JQ1 or BEZ235) and RNA interference (RNAi) with the generation of stable and inducible knockdowns. To analyse resulting changes RT-PCR, Western Blotting, cell cycle analysis, proliferation, apoptosis, invasion assays, whole transcriptome analysis via microarrays and as well as xenograft mouse models were utilized.

Results: By use of JQ1 we strikingly observed a strong down-regulation of the predominant EWS-ETS protein EWS-FLI1 in a dose dependent manner which was further enhanced by co-treatment with an inhibitor (BEZ235) of the PI3K pathway. Microarray analysis revealed JQ1 treatment to block the typical ES associated expression program. The effect on this expression program was partially mimicked by RNAi with BRD3 or BRD4 expression but not by BRD2 knockdown. Further analysis of constitutive knock downs of individual BRDs, such as BRD2, 3 or 4 however did not recapitulate proliferation restrictions as observed for JQ1, hinting towards an interdependency for all 3 proteins. Subsequent functional studies demonstrated that JQ1 treatment blocked contact dependent and independent proliferation but induced apoptosis presumably contributing to the reduction of the proliferative ability of ES lines. Single or combination treatment with the PI3K/mTOR inhibitor BEZ235 increased apoptosis of ES cell lines although single treatment with BEZ235 was less effective than JQ1 application. Consequently, tumour development was dose dependently suppressed with increased formation of apoptotic bodies in a xenotransplant model in immune deficient mice.

Conclusion: Here we demonstrate an interdependency of BET proteins regulating the ES specific expression profile and a possible substitution effect observed after knock down individual BRD proteins. We further demonstrate that ES are susceptible to treatment with epigenetic inhibitors such as JQ1 and in combination with PI3K pathway inhibitor BEZ235 synergistically block the pathognomonic EWS-ETS transcriptional program and malignant transformation of ES.

No conflict of interest.

Molecular targets for ATRs (vs. CARs) in genomics based sarcoma immunotherapy

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EWS/ETS dependent gene products (EDGP) are actionable by T cells. Perceived as specific, chimeric antigen receptor T cells (CARs) bypass evolutionary safety features and restrict recognition to surface molecules. CAR expenses are unwanted activation of innate immunity and agamaglobulinemia. We employ allorestricted T cell receptor (TCR) transgenic T cells (ATRs) bypassing regulatory requirements of CARs while TCR promiscuity is imperative, given that 10 20 universal peptides. EWS/ETS dependent gene products (EDGP) are actionable by T cells. Functionality of genomics based ATR targets as obligatory for malignancy of Ewing Sarcoma (ES) cells was verified in vivo, including EDGP EZH2 Histone methyltransferase and ChM1 antiangiogenic osteochondrocalcinosis differentiation regulator) ChM1 specific ATRs kill ES in vivo without target down modulation, whereas EZH2 specific ATRs kill in vitro, but not in vivo. STEAP1 (ROS signaling receptor) specific humanized ATRs show off-target reactivity killing ES in vivo. ADRB2 (adrenergic receptor) specific ATRs committed fratricide. EDGP ATRs killed ES irrespective of donor parents recognizing peptides presented by the non-inherited HLA haplotype (Burdach 2013). ATRs recognize intracellular targets; compared to CARs their target pool is unlimited. T cell activation and differentiation of innate immunity are low, while off-targets effects are more frequent. In contrast to CARs, ATRs can target addiction oncogenes and antigens derived from proteins, which are essential for sarcoma cell survival, respectively. Regulatory authorities require specificity of ATRs but not of CARs while TCR promiscuity is imperative, given that 10 20 universal peptides.

No conflict of interest.

A phase 1/2 study of talazoparib (BMN 673), an oral poly(ADP-ribose) polymerase inhibitor, plus temozolomide in children with refractory or recurrent malignancies: A Children’s Oncology Group phase 1 consortium study (ADVL1411)

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Background: Talazoparib, a novel, potent, PARP1/2 inhibitor in combination with low dose temozolomide (TMZ) has shown impressive activity in a broad range of pediatric cancer pre-clinical models. Synergism is hypothesized to result from the persistence of TMZ-derived single stranded DNA breaks that, in the absence of talazoparib, would be repaired via endogenous PARP enzymes. PARP1 is a key cofactor in tumors that harbor EWS-FLI1 and EWS-ERG translocations, therefore, talazoparib is of particular interest in Ewing sarcoma. We are performing a phase 1/2 trial of oral talazoparib plus low dose TMZ to examine the dose limiting toxicities (DLT) estimate the recommended phase 2 dose (RP2D) and preliminary assess the pharmacokinetics (PK) of this combination in children with refractory or recurrent solid tumors and to preliminarily explore its efficacy in children with Ewing sarcoma.

Methods: Talazoparib is administered orally once (QD) or twice daily (BID) on day 1 (doses 400–600 mcg/m2/dose [daily max = 1000 mcg]) followed by QD dosing on days 2–6 (doses 400–600 mg/m2/day [daily max = 1000 mcg]). TMZ is administered orally QD on days 2–6 (doses 20–55 mg/m2/day). Cycles are 28 days duration. Six dose levels have been evaluated using a 3+3 design. Blood samples for pharmacokinetic studies (PK) of talazoparib and TMZ are obtained in Cycle 1.

Results: Twenty-five eligible patients, age 4–20 (median 14) years, have been enrolled in the phase 1 portion of the study, with 24 patients evaluable for toxicity. Subjects had 13 unique tumor types, including 10 with CNS tumors. During the initial dose levels, while the talazoparib dose was escalated in combination with TMZ 20 mg/m2/dose, the maximum planned talazoparib dose (600 mcg/m2 BID on Day 1 followed by 600 mcg/m2 QD on Days 2–6) was reached with no DLTs. In subsequent dose levels, as the TMZ dose was escalated, DLTs occurred in 2/3 subjects at DL6 (55 mg/m2/day), 2/6 subjects at DL5 (40 mg/m2/day) and 1/6 subjects at DL4 (30 mg/m2/day). DLTs included grade 4 neutropenia (n=4), grade 4 thrombocytopenia (n=4), grade 4 sepsis (n=1), grade 4 intra-abdominal hemorrhage (n=1) and grade 3 ALT (n=1). PK data will be presented.

Conclusions: Talazoparib and low dose TMZ are tolerated in children with refractory/recurrent solid tumors; reversible neutropenia and thrombocytopenia are the primary DLTs. The RP2D is talazoparib 600 mcg/m2 BID on Day 1 followed by 600 mcg/m2 QD on Days 2–6 (daily max = 1000 mcg) in combination with TMZ 30 mg/m2/day on Days 2–6. The phase 2 portion of the study is now open to assess efficacy in children with relapsed/refractory Ewing sarcoma. ClinicalTrials.gov: NCT02116777. Sponsor: CTEP.

No conflict of interest.
131 Poster (Board P102)
Examining the role of chromatin modifying enzymes in medulloblastoma by utilizing a chemical library
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Background: Medulloblastoma (MB) is the most common paediatric brain tumor that arises during infancy and childhood and is a major cause of cancer-related morbidity and mortality in children. Recently, medulloblastomas are described as four distinct molecular subgroups (wingless, sonic hedgehog, Group 3 and Group 4), which have distinct transcriptional, cytogenetic, and mutational spectra. Next-generation studies have revealed that adult medulloblastomas involve remarkably more somatic SNVs and indels than paediatric counterparts, suggesting that epigenetic deregulation might have a foremost role in the initiation and progression of paediatric medulloblastomas.

Materials and Methods: Chemical library containing 46 inhibitors against different chromatin modifying enzymes (CMEs) was used in order to investigate their role in medulloblastoma. ATP-based cell viability assay was used to examine the cell death in a dose-dependent manner in cancer cells and non-malignant cells. The downstream molecular changes upon inhibitor treatment was examined by qRT-PCR. Induction of apoptosis was revealed by increased levels of cleaved PARP by western blotting.

Results: The function of chromatin modifying enzymes (CMEs) in medulloblastoma was investigated by utilizing a chemical library, which was composed of 46 inhibitors against different CMES. The screen revealed 7 potent inhibitors that induced cell death in a dose-dependent manner in DAOY and PC-3 MB lines and a primary cell line significantly. Among these, inhibitors targeting histone deacetylases (HDACs) and different histone demethylases (HDMs) were present and these compounds were relatively non-toxic to normal cells. As their roles have been ill-defined in medulloblastomas, we focused on HDMs and investigated the downstream molecular changes upon inhibitor treatment by qRT-PCR and western blotting. We observed that pro-apoptotic genes were upregulated in favour of enhanced apoptosis and cells underwent apoptosis as revealed by PARP cleavage.

Conclusion: In conclusion, our results suggest that targeting specific HDMs by specific inhibitors can be a promising therapeutic approach for medulloblastoma patients.

No conflict of interest.

132 Initial testing by the Pediatric Preclinical Testing Program (PPTP) of the ATR Inhibitor VX-970 alone or in combination with cisplatin in pediatric solid tumor xenografts
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Background: Potent and selective ATR inhibitors are in clinical development with the potential to target cancer cells under replication stress resulting from oncogene addiction or chemotherapeutic agents that cause DNA damage. Marked synergy was reported in NSCLC cell lines and xenograft models when VX-970 was combined with cisplatin (Hall AB et al., Oncotarget 2014).

Materials and Methods: VX-970 was provided to the PPTP by Vertex Pharmaceuticals. In vitro, VX-970 was tested against the PPTP's in vitro panel from 1.0 nM to 10.0 uM. For combination studies in vitro, VX-970 was tested at a concentration of 75 nM in combination with cisplatin and with methotrexate. In vivo VX-970 was tested against 24 pediatric solid tumor xenografts alone and in combination with cisplatin. Cisplatin was administered intraperitoneally on day 1 and 8 at a dose of 5 mg/kg. VX-970 was administered intravenously (IV) on days 2 and 9, 16 hours after cisplatin.

Results: In vitro the median relative IC50 (IC50m) value for the PPTP cell lines was 0.19 uM (range 0.03 uM to 1.36 uM). VX-970 induced cytotoxicity with T/C Ymin% values approaching 0% and with Relative IC50% values approaching -100% for cell lines at the higher concentrations tested. In combination studies VX-970 potentiated cisplatin toxicity (median 1.48; range 0.83-14.31-fold), and methotrexate (median 1.92; range 0.91-7.55-fold).

In vivo, VX-970 as a single agent induced significant differences in EFS distribution compared to control in 18 of 24 (75%) of the solid tumor xenografts studied. Three objective responses were observed to cisplatin among the 24 solid tumor xenografts. The VX-970 and cisplatin combination induced significant differences in EFS distribution compared to control in 21 of 24 (88%) xenograft models. Four objective responses were observed to the combination among the 24 solid tumor xenografts. Specific comparisons were made between the EFS distribution of the VX-970 plus cisplatin combination and single agent cisplatin and single agent VX-970, respectively. Xenograft models in which the addition of VX-970 to cisplatin was significantly superior to single agent cisplatin (p < 0.05) were identified. Four of 24 models met this criteria, including B329 (retinoblastoma), EFS-15 (Wilms tumor), NB-E8C1 (neuroblastoma), and OS-9 (osteosarcoma).

Conclusion: VX-970 showed modest potentiation of cisplatin and methotrexate with median potentiation being 1.48- and 1.95-fold, respectively. VX-970 showed limited single agent activity against pediatric solid tumor xenografts. The addition of VX-970 to cisplatin significantly prolonged time to event for a minority of tested xenografts across a range of histologies.

No conflict of interest.
Conclusion: The RDE in children is 500 mg/m²/day with food. The study is currently enrolling in the expansion phase at this recommended dose.


134 Poster (Board P105) MondoA mediates in vivo aggressiveness of common ALL by induction of HIF1α. A. Sipol1, T.G.P. Grunewald2, J. Schmaeh3, M.L. DenBoer4, R. Alba4, M. Baldauf2, C. Wernicke1, M. Horstmann5, G. Cario3, G. Richter1, S. Burdach1, 1Technische Universität München, Children’s Cancer Research Center, Department of Pediatrics, Munich, Germany; 2Institute of Pathology, Ludwig-Maximilians-Universität München, Laboratory for Pediatric Sarcoma Biology, Munich, Germany; 3Schleswig-Holstein University Medical Center, Klinik für Allgemeine Pädiatrie, Kiel, Germany; 4Erasmus University Medical Center, Department of Pediatric Oncology, Rotterdam, Netherlands; 5University of Hamburg Medical Center, Children's Cancer Research Institute and Department of Pediatric Hematology and Oncology, Hamburg, Germany

Leukemia incidence in childhood correlates with birth weight (Burdach et al. 1990) and drives different metabolic repertoires in cancer provide targets for therapy. We previously described MondoA (also known as MLXIP, MAX like protein X interacting protein) as a metabolic stress sensor, required for leukemogenesis. Lymphocytes, in particular B lymphocytes are adapted to hypoxic environments from their very beginning. They are destined from the hypoxic bone marrow via normoxic peripheral blood to hypoxic lymph nodes. They thus are specialized in glycolysis to compensate the lack of oxidative phosphorylation under hypoxic conditions by increased glycolysis. Leukemic counterparts of B lymphocytes exploit these features for their survival. Glucose-derived metabolites control the nuclear activity of the transcription factors MondoA, a factor critical for longevity in rhythm. Here we report on the expression of MondoA in common acute lymphoblastic leukemia (cALL) compared to other malignancies, its role in malignancy of cALL in vivo, downstream pathways and correlation with relapse risk.

Our human/murine xenotransplantation model with immunodeficient mice was used (Richter et al. 2009). NALM6 and 697 cALL lines were lethally transduced with MondoA short hairpin RNA (shRNA). Upon successful MondoA knock down (KD), KD and control lines were injected into the mice; CD10+ blasts in blood, spleen and marrow were assessed.

We found MondoA to be most strongly expressed in pediatric cALL and assessed. In combination with Dasatinib in A373, TC32, VX74, TC71, SKNMCE wing sarcoma cell line model stably bearing shRNA directed at EWS-FLI1 (off) or non-expressed ERG control (on), Cell lines were transduced with the Decode Pooled Lentiviral shRNA Screening Library consisting of 4,675 distinct shRNA sequences, targeting 709 human protein kinases. For identifying real EWS-FLI1-specific effects transduction conditions were appointed to integrate one single shRNA per cell, by applying a multiplicity of infection of 0.3. The concerning functional protein knockdown by single copy shRNA integration into target cells were efficiency validated by western blot. Redundancies of integrated shRNA sequences in EWS-FLI1-off and -off cell systems, enrichment or depletion, have been carried out by Ion 2. No conflict of interest.

136 Poster (Board P107) Identification of novel therapeutic targets in Ewing sarcoma using a pooled shRNA screening approach in a tumor cell-specific environment. J. Potratz1, C. Schaefer2, D. Clemens2, M. Hotfilder2, U. Dirksen2, 1University Hospital Muenster, Department of Pediatrics, Muenster, Germany; 2University Hospital Muenster, Department of Pediatric Hematology and Oncology, Muenster, Germany

Background: Ewing sarcoma (ES) belongs to high risk sarcomas in children and young adults. To reduce treatment toxicities and to improve survival rates, novel treatment approaches are urgently needed. ES is genetically defined by chromosomal translocations leading to the oncogenic transcription factors such as EWS-FLI1, in most cases. Successfully targeting of EWS-FLI1 in clinical approaches remains abortive. Therefore EWS-FLI1 cooperating pathways, which cooperate in cellular EWS-FLI1 tolerance, are coming more into focus and could serve as alternative approach for tumor cell-specific therapies. To identify such target pathways, we established a pooled EWS-FLI1-synthetic lethal shRNA screening approach.

Material and Methods: For the lethal screening setting we utilized an A673 cell line model stably bearing shRNA directed at EWS-FLI1 (off) or non-expressed ERG control (on). Cell lines were transduced with the Decode Pooled Lentiviral shRNA Screening Library consisting of 4,675 distinct shRNA sequences, targeting 709 human protein kinases. For identifying real EWS-FLI1-specific effects transduction conditions were appointed to integrate one single shRNA per cell, by applying a multiplicity of infection of 0.3. The concerning functional protein knockdown by single copy shRNA integration into target cells were efficiency validated by western blot. Redundancies of integrated shRNA sequences in EWS-FLI1-off and -off cell systems, enrichment or depletion, have been carried out by Ion 2. No conflict of interest.

135 Poster (Board P106) The CXCR4 antagonist Plerixafor (AMD3100) promotes Ewing sarcoma cell survival and migration in Ewing sarcoma cells in vitro. U. Dirksen1, P. Berning1, C. Schaefer2, J. Potratz2, 1University Hospital Muenster, Paediatric Haematology and Oncology, Muenster, Germany; 2University Hospital Muenster, Department of Pediatrics, Muenster, Germany

Background: Ewing sarcoma is the second most common bone sarcoma in childhood and adolescence. The chemokine receptor CXCR4 and its agonist CXCL12 are involved in growth, metastasis and angiogenesis in Ewing Sarcoma. High CXCR4 expression levels correlate with metastatic disease, higher tumor stage and poor overall survival. High levels of CXCL12 is to be found in lung and bone marrow, common sites of metastasis. Targeting the CXCR4-CXCL12 interaction may reduce tumor progression. Plerixafor (AMDS3100) as stem cell mobilizing agent is a CXCR4 inhibitor in clinical use combined with GCSF for patients failing to mobilize on GCSF as single agent.

Material and Methods: We performed cell viability assays using Plerixafor, in combination with Dasatinib in A373, TC32, VH74, TC71, SKNMC Ewing Sarcoma cell lines and primary cell cultures. GCSF was used as a control. CXCR4 protein and CXCR4 surface expression we determined by FACS and Western Blot analysis. To further explore mechanism of Plerixafor action we performed array analysis of phosphorylation levels for 49 receptor tyrosine kinases (RTK).

Results: The Ewing Sarcoma cell-lines and the primary culture cell line should show unexpected increase in cell viability upon Plerixafor treatment in a dose-dependent manner (range 1–10μM). GCSF and the CXCR4 agonist CXCL12 did not affect cell viability. Surface expression of CXCR4 showed a broad range among cell lines. We further used A673 and CXCR4 surface expression negative and TC32 as CXCR4-expression positive cell line. Migration was stimulated through Plerixafor in CXCR4 negative A673 cells and through CXCL12 in TC32 CXCR4 positive cells. In RTK array analysis after 1h and 16h Plerixafor treatment, CXCR4 negative A673 cells showed transient activation of RTKs PDGF Rb and CXCR4 positive TC32 cells showed activation of members of the Eph RTK family. Plerixafor induced further downstream activation of AKT and partly -JNK pathway, but did not influence p-MAPK. Based on array findings with PDGF Rb and Eph RTK activation, the tyrosine kinase inhibitor Dasatinib targeting both RTKs was used for combination treatment with Plerixafor. Low concentration of 100–200 nM Dasatinib abrogated pro-proliferative effects of Plerixafor when used in combination.

Conclusion: Plerixafor (AMD3100) is a FDA and EMA approved stem cell mobilization agent. The agent has pro-proliferative and pro-migratory effects on Ewing Sarcoma cells in vitro. The profile of Plerixafor is RTK-linked and seems independent from CXCR4. Further studies are required with respect to the safety of a clinical use of Plerixafor in Ewing Sarcoma patients. No conflict of interest.
Integrated pathway analysis of malignant rhabdoid tumour identifies key SMARCB1-pathways and therapeutic opportunities

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Background: Malignant Rhabdoid Tumours (MRTs) are aggressive paediatric tumour with very poor prognosis and life expectancy. These malignancies are characterized by biallelic inactivation of SMARCB1 at a core subunit of the HSUI/SNF chromatin remodelling complex. SMARCB1 re-expression in MRT cells leads differentiation and growth arrest, deregulating hundreds of downstream genes/pathways. 450k array, RNA-seq, CHIP and ChIP-seq screening were used to identify SMARCB1 dependent downstream pathways and novel SMARCB1-dependent therapeutic targets that can improve the survival of MRT patients.

Material and Methods: RNA-seq and 450k-methylation analyses in MRT human primary malignancies (n = 40) and in 4 MRT cell lines in which lentivirus was used to re-express SMARCB1 (G401, A204, CHLA-266, and STA-WT1) as well as 5′-Aza-cytidine demethylation treatment were used to investigate the downstream effects of SMARCB1 loss. Following SMARCB1 re-expression cells were expression and methylation profiled to identify genes/pathways regulated by SMARCB1. Cells with and without SMARCB1 re-expression were further screened for functional similarities using the whole-genome CICERO (Genome Scale CRISPR Knock-Out) library which contains 122441 guide constructs targeting 19,050 genes and 1,864 miRNA. Key pathways were identified by integrated bioinformatics and validated functionally.

Results: Integrated network-based pathway analysis identifies several key SMARCB1-dependent and potentially druggable genes/pathways deregulated at the transcriptional/methylation level in primary MRTs. Moreover, CRISPR screening identifies gene targets which promote or inhibit growth/viability in a SMARCB1-dependent manner in our functional models. SMARCB1-dependent genes/pathway deregulation was verified and validated by targeted pathway manipulation and quantified by qRT-PCR and immunophenotyping. Key pathways/genes include but are not limited to E2F1, AURKA, BMI1, PLK1, SHH, Rho, TGF-g, PDGFRα, FGFR1, Wnt and STAT3/3-L. PDGFRα, FGFR1, STAT3/3-L, Wnt and TGFBeta mechanisms were further investigated in vitro using available inhibitors/drugs.

Conclusions: Integrative analysis of -omics data using systems biology methods we have provided a catalogue of the key MRT tumourigenic genes/pathways changes in both primary and functional models. Rhabdoid cells showed sensitivity to pathway inhibition and cell death by dysregulation of several downstream SMARCB1-dependent oncogenes. We have isolated and validated critical genes/pathways which are targetable therapeutically and will lead to better treatments for what is currently one of the most lethal paediatric cancers known.

No conflict of interest.

Identification of novel SEC61G-EGFR fusions in pediatric ependymoma

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Background: Ependymomas represent 10% of pediatric brain tumors, with 5-year survival rates at approximately 50%. Despite their histopathological similarities, ependymomas consist of heterogeneous molecular-genetic groups, arising from different glial stem cell (SC) populations. In cultures, SCs are forced to active proliferation by exogenous EGF/FGF. Culture conditions might exert negative/positive selection pressure, leading to clonal expansion of genetically heterogeneous tumor subpopulations. We addressed whether omission of EGF/FGF might select mitogen-independent (MI) cells present in ependymomas and the underlying aberrant signaling.

Material and Methods: The EPP-MI line was established by culturing the ependymoma SC line EPP in mitogen-free media. Biological and molecular features of lines (proliferation, tumorigenicity, expression of stemness markers, EGFR signaling and subcellular localization, sensitivity to the EGFR inhibitors gefitinib and AEE788) were assessed through standard laboratory assays. The sequences of SEC61G-EGFR fusions and presence in ependymomas were analyzed through RNA-seq, RT-PCR and Sanger sequencing. Results: Compared to EPP, EPP-MI cells displayed impaired proliferation in vitro, but much higher tumorigenicity, despite a lower expression of stemness markers. A constitutively activated, truncated EGFR was found in EPP-MI cells, that was associated with AKT and STAT3 phosphorylation and blocked by EGFR inhibitors. By transcriptome sequencing and validation, we discovered that EPP-MI cells harbored an intrachromosomal rearrangement that fused in frame EGFR downstream to Sec61G5′UTR. The SEC61G-EGFR fusions lacked the ligand-binding domain of EGFR, while retaining the transmembrane and the tyrosine kinase coding domains. Similarly to EGFR, the majority of SEC61G-EGFR protein was bound to the membranes. High levels of constitutively activated STAT3 was found in the nucleus of EPP-MI cells, whereas in EPP cells nuclear STAT3 was phosphorylated only in response to EGFR. Comparing EPP-EPP-MI cells, the SEC61G-EGFR fusions were three-fold more sensitive to the antiproliferative effects of EGFR inhibitors. In orthotopic models, AEE788 did not exert significant antitumoral activity on EPP xenografts, whereas it significantly prolonged survival of mice bearing EPP-MI xenografts (log-rank, P = 0.02). RT-PCR sequencing detected SEC61G-EGFR transcripts in one of 17 pediatric ependymomas.

Conclusions: 1) In ependymoma SCs, SEC61G-EGFR fusions are associated with higher tumorigenicity, aberrant EGFR/STAT3 signaling, and sensitization to EGFR inhibitors both in vitro and in vivo. 2) SEC61G-EGFR fusions are expressed in pediatric ependymomas and might represent a druggable target. 3) Culture conditions might expand subpopulations present in tumors and unravel new driver mutations. (Supported by Fondazione per l’Oncologia Pediatrica).

No conflict of interest.
a well-accepted predictive experimental model mimicking original patient’s in histo- and molecular pathology, per accumulated annotated data, and broadly used to assess drug efficacy, discover biomarker, guide clinical development/use of cancer therapy, and thus, potentially validate actionable mutations. These models provided the presence of comparable actionable mutation profiles in patient and PDXs.

Methods: 8147 patients in TCGA datasets for a set of 50 genes with frequent actionable somatic mutations were identified and compared to those from a cohort of 749 PDXs per RNA-seq data of CRC, NSCLC, PAN, GA, H&N, ES, OV, HCC, where only mutations in both datasets were used and those in dbSNP removed. Frequencies of mutated genes were compared with and without weighing sample number per disease type.

Results: 513 PDXs (68.5%) have at least one potentially actionable mutation, comparable to TCGA patients (61.3%). In PDXs, TP53 has the highest mutation rate (34.4%, 43.8%), followed by APC (16.7%, 12.7%), PIK3CA (0.6%, 6.0%), RB1 (4.0%, 5.0%), CDKN2A (4.0%, 5.0%), CDK4 (4.0%, 5.0%), CDKN1A (4.0%, 5.0%), CDKN1B (4.0%, 5.0%), CDH1 (4.0%, 5.0%), and RUNX1 (4.0%, 5.0%). Similar results were observed in TCGA patients with TP53 having the highest mutation rate (34.4%, 43.8%), followed by APC (16.7%, 12.7%), PIK3CA (0.6%, 6.0%), RB1 (4.0%, 5.0%), CDKN2A (4.0%, 5.0%), CDK4 (4.0%, 5.0%), CDKN1A (4.0%, 5.0%), CDKN1B (4.0%, 5.0%), CDH1 (4.0%, 5.0%), and RUNX1 (4.0%, 5.0%). In PDXs, TP53 has the highest mutation rate (34.4%, 43.8%), followed by APC (16.7%, 12.7%), PIK3CA (0.6%, 6.0%), RB1 (4.0%, 5.0%), CDKN2A (4.0%, 5.0%), CDK4 (4.0%, 5.0%), CDKN1A (4.0%, 5.0%), CDKN1B (4.0%, 5.0%), CDH1 (4.0%, 5.0%), and RUNX1 (4.0%, 5.0%).

Conclusions: The profile of putative actionable mutations in patient hints potential for target therapy, whereas comparable profile of PDX suggests 141

141 Poster (Board P112)
Identifying targeted therapies to eliminate chemo-resistant LSCs and improve disease-free survival in AML disseminated models
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Background: Leukemic stem cell is one of the leading causes for disease recurrence in acute myeloid leukemia (AML) patients as it lacks sensitivity to conventional therapy. In this report, we aimed to understand whether leukemic stem cell (LSC) plays a key role for developing chemoresistance after treatment of high disease induction therapy in AML models, and subsequently to identify targeted agents that can eliminate LSC population and enhance sensitivity to chemotherapy.

Material and methods: AML models were developed by intravenously injecting MV4–11 or PDX cells in NSG (NOD/SCID IL2Rγ−/−) mice. Disease progression in AML disseminated models was tracked either by in vivo bioluminescence imaging or via ex vivo flow cytometry analysis of AML in peripheral blood and bone marrow. Immunohistochemistry of the bone marrow residual disease were performed.

Results: In the tested AML models, daunorubicin/cytarabine (DA) chemotherapy induced early chemoresistance, efficacy through apoptosis induction and antiproliferation. Unfortunately, treatment showed minimal survival benefits despite early chemoresistance effects. In the DA residual diseases of the BM2407 and BM4007 AML PDX models, flow cytometry analyses showed enriched fractions of CD33+CD34+ and CLL1+CD117+ subpopulations, respectively. Subsequently, we performed self-renewal functional test by in vivo limiting dilution assay using sorted CD33+CD34+ and CLL1+CD117+ cells in mice. These chemoresistant CD33+CD34+ or CLL1+CD117+ cells were much more tumorigenic than their counterpart CD33+CD34− and CLL1+CD117− cells, respectively. By combing chemotherapy with targeted therapeutic agents including palbociclib and CD33-ADC, we observed significant improved survival of the AML disease mice compared to the single agent treatment. In the mechanistic study, the chemoresistant LSCs highly express CD33 thus can be eliminated by CD33-ADC. Palbociclib promotes the premature entry of quiescent LS LSCs into S phase therefore enhanced the cytotoxicity by chemotherapy.

Conclusion: These study results revealed that the anti-LSC effect of these targeted agents played a key role for the combinatorial benefits with chemotherapy.

Conflict of interest: Ownership: Pfizer Inc. Other Substantive Relationships: Authors are Pfizer employees.

142 Poster (Board P113)
New preclinical models for neuro- oncology: Orthotopic patient-derived glioblastoma xenografts in mice
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Glioblastoma multiforme (GBM) is the most common malignant brain tumor in adults. These tumors frequently show a very heterogeneous, diffuse infiltrative and aggressive growth with a mean survival time between 8 and 18 months. The current standard care for glioma patients is of limited benefit. To support basic and translational research initiated the development of new preclinical glioma models from patient tumors.

We started with subcutaneous (s.c.) transplantation of 85 glioma tissue samples to immunodeficient mice and were able to establish and characterize 21 novel patient-derived xenografts (PDXs) in immunodeficient mice (engraftment rate 25%). To better mimic the crucial interaction of human gliomas with host microenvironment (endothelial, stromal and immune cells, extracellular matrix) we utilized orthotopic transplantation. Since tumor-host interactions are frequently organ specific. Up to now, 8 of the established PDX models were also transplanted intracranially (orthotopic) by an injection of 50,000 cells in the right hemisphere.

After successful engraftment, both the s.c. and the orthotopically transplanted glioma PDX were screened for sensitivity toward a broad drug panel (everolimus, sorafenib, bevacizumab, irinotecan, salinomycin, thalidomide, regorafenib or temozolomide) in monotherapy or combinations. In the s.c. model system, the strongest response was induced by bevacizumab (7/21), irinotecan (15/21), and temozolomide (16/21), whereas the other drugs had no activity. From the evaluated combination therapies, we learned that combinations of temozolomide with other drugs did not provide any additive effects. Interestingly first data showed differences in sensitivity levels to treatments between orthotopic and s.c. transplantation of the same PDX model. We found for example drugs bevacizumab, irinotecan and everolimus less active in the orthotopic model.

Bioinformatic analysis did not reveal a correlation between common “onco-mutations” (Illuma TrueSeq Cancer panel) and drug sensitivity. The most frequent mutations in our glioma PDX were KDR, FGFR3, PIK3CA, PTEN, PI3, NOTCH1 and KIT.

Histopathological analyses demonstrate that our glioma PDX models have retained the heterogeneity of the disease. Particularly the orthotopically transplanted glioma PDX are showing the original tumor histology and have better take rates in comparison to the s.c. engrafted PDX models. The available panel of the broadly characterized 21 glioma PDX models provides an exceptional platform for translational oncology research projects, the identification and validation of new targets, the screening of new drug candidates, screening of new drugs and combinations, as well as for personalizing patient treatment.

Conflict of interest: No conflict of interest.

143 Poster (Board P114)
Characterization of breast cancer preclinical models reveals a specific macrophage polarization
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Background: Tumor microenvironment has been the target of drug development for many years. However, few studies have addressed the characterization of the stroma component in patient-derived xenografts (PDXs) and genetically engineered models (GEMs). The aim of this study was to characterize the stroma displayed by different models of breast cancer tumors in mice.

Materials and Methods: For this purpose, transcriptomic and flow cytometry analyses on murine populations were performed in a series of 25 PDXs and 2 most commonly used GEMs (MMTV-PyMT and MMTV-erbB2). Specifically, macrophages from 5 models were sorted and profiled by gene expression and phenotypically characterized by flow cytometry (FC).

Histopathological analyses demonstrate that our glioma PDX models have retained the heterogeneity of the disease. Particularly the orthotopically transplanted glioma PDX are showing the original tumor histology and have better take rates in comparison to the s.c. engrafted PDX models.
**Results:** Composition of hematopoietic cells, mainly macrophages and granulocytes, varied from tumor to tumor. Stromal heterogeneity, as measured by FC, showed seven of 21 tumors with a high percentage (at least 40%) of macrophage-like cells, 6 had 20–40% macrophage-like cells, and eight less than 20% macrophage-like cells. Stromal analysis revealed a specific fingerprint for each model, with two distinct clusters defined principally on the basis of macrophage infiltration. For five models [3 PDXs, the mouse MMTV-PyMT and its corresponding allograft (BC-PyMT)] macrophage-like cells (three tumors per model) were purified by cell sorting and their expression profiles examined. The triplicates tested for each model clustered together on three-dimensional principal component analysis (PCA). The macrophage-like populations in PDXs and GEM models are defined by specific individual gene expression patterns. In addition, the macrophage-like cells from the transplanted BC-PyMT tumor were closer to those of transplanted PDXs than to those of the original spontaneous MMTV-PyMT. Using the mouse Gene Ontology (GO) pathway gene sets, we identified “Immune response” and “Immune system process” as the principal GO pathways enriched in the BC-PyMT allograft. This up-regulation of the interferon pathway presumably reflects allograft rejection. Finally, macrophages were shown to display specific polarization phenotype related to M1/M2 classification and associated with genes implicated in recruitment, invasion and metastasis processes.

**Conclusions:** Our results show that each PDX and GEM tumor can be defined by its individual tumor-associated stromal matrix. Tumor cells can thus generate their own specific stromal composition, despite the absence of T cells, and maintain a complex functional network of communications. This specific composition of stroma components in the studied models therefore suggests that tumor cells educate their microenvironment to fulfill their own needs. Models such as those described here may therefore be considered relevant tools for preclinical and pharmacological assessment to investigate tumor and stromal interactions.

**No conflict of interest.**

**Poster Session – Preclinical Models, Wednesday 29 November 2016**

**144**

**Analysis of the combinatorial effect of cisplatin and VE-821 on a large panel of tumor cell lines**


Cancer is a highly complex, multigenic disease with tumor cells underlying constant transition. Single drug treatments against specific targets frequently fail to oppose this system where mutations and redundant pathways often lead to drug resistance. Therefore, combinations that effect different targets in the cancer cell have become the standard of care treatment in many fields of cancer therapy. In our study we determined the combinatorial effect of the DNA cross linking agent cisplatin and the ATR-inhibitor VE-821 on the viability of a large panel of tumor cell lines. In 2011 Reaper et al. [1] could already show that this drug combination has a significant synergistic effect in several cancer cell lines whereas there is no synergy in normal cells. In healthy cells ATR inhibition is compensated by the ATM-p53 pathway. However, in most tumor cell lines ATM-p53 signaling is defective which leads to accumulation of DNA damage and cell death upon ATR-inhibition combined with cisplatin treatment. Applying the combination of cisplatin and VE-821 in a checkerboard pattern on a large number of different cell lines allowed us to correlate observed synergistic and non-synergistic effects with gene expression profiles of these cell lines and to validate the requirement of an ATM/p53 defective signaling. Moreover, this approach allowed us to determine which ratio of the compounds is most effective for which cell line. By these means, this study will contribute to understanding of combinatorial effects of genotoxic agents and ATR-inhibitors.

**References**


**No conflict of interest.**

**145**

**Molecular subtypes of head and neck cancer predict response to cetuximab treatment**


Cancer is a highly complex, multigenic disease with tumor cells underlying constant transition. Single drug treatments against specific targets frequently fail to oppose this system where mutations and redundant pathways often lead to drug resistance. Therefore, combinations that effect different targets in the cancer cell have become the standard of care treatment in many fields of cancer therapy. In our study we determined the combinatorial effect of the DNA cross linking agent cisplatin and the ATR-inhibitor VE-821 on the viability of a large panel of tumor cell lines. In 2011 Reaper et al. [1] could already show that this drug combination has a significant synergistic effect in several cancer cell lines whereas there is no synergy in normal cells. In healthy cells ATR inhibition is compensated by the ATM-p53 pathway. However, in most tumor cell lines ATM-p53 signaling is defective which leads to accumulation of DNA damage and cell death upon ATR-inhibition combined with cisplatin treatment. Applying the combination of cisplatin and VE-821 in a checkerboard pattern on a large number of different cell lines allowed us to correlate observed synergistic and non-synergistic effects with gene expression profiles of these cell lines and to validate the requirement of an ATM/p53 defective signaling. Moreover, this approach allowed us to determine which ratio of the compounds is most effective for which cell line. By these means, this study will contribute to understanding of combinatorial effects of genotoxic agents and ATR-inhibitors.

**Background:** Cetuximab is the single targeted therapy approved for the treatment of head and neck cancer (SCCHN). Predictive biomarkers have not been established in the clinical setting and patient stratification based on molecular tumor profiles has not been possible. Different molecular subtypes based on gene expression patterns have been established. Since EGFR pathway activation is pronounced in basal subtype, we hypothesized this activation could be a predictive signature for an EGFR directed treatment.

**Methods:** 28 patient derived xenograft models of head and neck cancer were subjected to Affymetrix gene expression studies on HG U133+ 2.0. Based on the expression of 821 genes, the molecular subtype of each of the 28 models was determined by integrating gene expression profiles through centroid-clustering with previously published gene expression data by Keck et al., CCR 2015. The models were treated in groups of 5–6 animals with docetaxel, cетuximab, carboplatin and 5-fluouracil for three weeks. Response was evaluated by comparing tumor volume at treatment initiation and at the end of treatment (RTV).

**Results:** Treatment results were heterogeneous with best response for single agent docetaxel. Tumors distributed over the 3 signature-defined subtypes: 5 mesenchymal/infamed phenotype (MS), 15 basal type (BA), 8 classical type (CL). Cluster analysis revealed a correlation between response to cetuximab and the basal subtype. RTV MS 3.32 vs BA 0.78 (MS vs BA, unpaired t test p 0.0002). Cetuximab responders were distributed as following: 1/5 in MS, 5/8 in CL and 13/15 in the BA group. Activity of classical chemotherapies did not differ between the 3 subtypes. No association was observed between initial tumor site and subtype. Genes known to be differentially expressed between PDX and primary tumor were excluded from the 821 gene set in a second analysis and clustering analysis was repeated. For no sample previously defined subtype changed in the second analysis.

**Conclusion:** Basal subtype was a strong predictor for response to EGFR directed therapy in head and neck squamous cell cancer patient derived xenografts, whereas activity of chemotherapy was homogenous between signature-defined subtypes.

**No conflict of interest.**

**146**

**The resistant cancer cell line (RCDI) collection**

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**Background:** The heterogeneity and individuality of cancer diseases is tremendously high. Recent genomic investigations revealed a tremendous genetic complexity in the cells from solid cancer diseases. Cancer cell (sub)populations may differ substantially between primary tumours and metastases as well as within primary tumours. This heterogeneity is a consequence of cancer clonal evolution processes. Among other models, comprehensive cancer cell line collections will be required to address this wide complexity. Resistance acquisition to anti-cancer therapies represents a major obstacle to the development of effective anti-cancer therapies. Major cancer cell drug resistance mechanisms have been discovered in drug-adapted cancer cell lines including the ABC transporters ABCB1 (also known as P-glycoprotein or MDR1) and ABCC1 (also known as MRPI) and clinically relevant resistance mechanisms to so-called “targeted therapeutics” (e.g. EGFR tyrosine kinase inhibitors, oncogenic BRAF inhibitors, anti-androgens).

**Materials and Methods:** Initially chemosensitive cancer cell line collections are adapted to growth in the presence of clinical concentrations of anti-cancer drugs. Results: The Resistant Cancer Cell Line (RCCL) collection consists of >1000 cell lines from 15 different cancer entities with acquired resistance to a broad range of cytotoxic and targeted anti-cancer drugs (www.kent.ac.uk/stms/cmp/RCCL/RCCLabInfo.html).

**Conclusion:** The RCCL collection is a readily available tool for the studying of drug-induced cancer cell resistance mechanisms, the investigation of anti-cancer agents, and the examination of drug-induced clonal evolution processes.

**No conflict of interest.**

**147**

**Mouse models for translational preclinical research in immunology**

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**Background:** Clinical successes with cancer immunotherapy have captured the attention the last years. Despite all progress, many questions remain and appropriate models are necessary to translate new drugs.
into the clinics. Furthermore, preclinical models have to be identified for developing of immunotherapy combinations and elucidating the mechanisms of action of immunotherapies. Determining appropriate regimens requires setting multiple parameters, including dose levels, administration frequencies, durations of treatment for each drug in the combination and sequence of administration. Those parameters have to be tested before settled trials. Other challenges will include discovering of appropriate biomarkers for efficacy and safety and selecting appropriate tumor type.

**Material and Methods:** We tested several syngeneic models as CT26, MC38, B16F10 and 4T1 towards their response to PD-L1 checkpoint inhibitor and radiation and measured the infiltration of immune cells. Further to this we tested two strategies to generate mice with a humanized immune system: human PBMC and human hematopoietic stem cells. These different mouse models harboring human immune cells are applied to study the efficacy and mode of action of new immunotherapeutic drug and combinations.

**Results:** In a preclinical combination trial in syngeneic mice a PD-L1 inhibitor was combined with local radiation of the tumor. The evaluation of the PD-L1 expression in the tumors revealed that radiation stimulate PD-L1. Furthermore, more infiltrating immune cells were detected. For the humanized mouse models, PBMC were co-inoculated with human breast tumor cells into immunodeficient mice. Increased efficacy of the humanized group was observed. Trastuzumab was seen compared to xenografted tumors without PBMC. On the other hand we reconstituted a human immune system in mice by engrafting human hematopoietic stem cells. At the time when the human immune system was developed, established patient-derived tumors were transplanted on these reconstituted humanized mice. Different tumor entities are growing in these humanized mice either similar to non humanized mice or slightly slower. During the observation period no tumor rejections by the human immune cells were evident, although tumor growth was accompanied by an increase of human T cells in the peripheral blood. Humanized mice bearing various PDX tumors were treated with approved therapeutic checkpoint inhibitors. Tumors as well as nontumor groups lead to a slight tumor growth delay and an increased percentage of T cells in the blood and in the tumor. The combination of both inhibitors showed a synergistic effect on tumor growth inhibition.

**Conclusions:** Our syngeneic and humanized mouse models enable appropriate preclinical assessment of immune-based therapeutic anti-tumor strategies. 

**No conflict of interest.**

148 Poster (Board P119)
Dissecting heterogeneity and molecular mechanisms involved in paranal cancer sinus

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**Background:** Epithelial, non- glandular paranasal sinus cancer is a rare disease, with a global dismal prognosis. There are no recognized targeted treatments and the knowledge of molecular mechanisms involved in the resistance to available therapies (surgery, radiation, chemotherapy) is limited. Dissecting the heterogeneity of paranasal sinus cancers and providing valuable information on the biology of the malignancy is eagerly needed to improve therapeutic approaches.

**Material and Methods:** We selected a retrospective cohort of 53 paranasal sinus cancer cases including the following histologies: (i) sinonasal undifferentiated cancer (SNUC); (ii) sinonasal neuroendocrine cancer (SNEC); (iii) non keratinizing squamous cell cancer (KNSCC). Treatment consisted of surgery, radiation and chemotherapy. Whole-transcriptome profiling was performed by microarray analysis using the DASL assay and BeadArray Chips (Illumina). Unsupervised subtype identification was performed applying hierarchical clustering and 1-Pearson correlation as distance matrix as implemented in the R package ConsensusClusterPlus. We used a 1000 re-sampling interaction model and we tested the existence of 2 < k < 5 clusters. We explored the potential biological processes differentiating the subtypes through Gene Set Enrichment Analysis (GSEA) that reveals the regulatory relationships among genes providing a systematic understanding of molecular mechanisms. Functional pathway analysis was performed imposing a significant level of FDR < 25% and interrogating gene sets belonging to Hallmark Gene Set Collection that represents a collection of “hallmark” gene sets as part of The Molecular Signatures Database obtained by a combination of automated approaches and expert curation.

**Results:** Consensus unsupervised clustering was applied to the gene expression data matrix and revealed the presence of three stable clusters of samples: (i) ClusterA, n = 28 cases (53%); (ii) ClusterB n = 8 cases (15%); (iii) ClusterC n = 17 cases (32%). ClusterA showed a significant enrichment of proliferation pathways and gene-sets associated with G2/M checkpoint as in progression through the cell division cycle. In addition ClusterA is linked with deregulation of genes of MYC, MTOR signalling. ClusterB was characterized by enrichment in KRAS and inflammation pathways. ClusterC was linked to deregulation of genes of extracellular matrix and WNT signalling.

**Conclusion:** The present preliminary report is the first in-deep molecular analysis of paranasal sinus cancer that proves the capability of transcriptomic profiling to capture molecules and functional pathways deregulated in this disease. The identification of gene-expression patterns associated across the different histologies and to clinical outcome is ongoing.

**No conflict of interest.**

149 Poster (Board P120)
Spheroid formation assay for in vitro assessment and expansion of circulating cancer stem cells (cCSC) in patients with breast cancer

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**Background:** Cells with sphere forming capacity are present in the peripheral blood and breast cancer patients and represent a significant impediment to efficacious treatment due to their putative role in progression, metastasis and chemotherapy resistance. The isolation and identification of cCSC is very difficult, because of their rarity. Here we report that we are able to generate spheroids from the peripheral blood of breast cancer patients. The identification of cCSC could help to develop novel therapeutic strategies specifically targeting cancer stem cells.

**Methods:** All white blood cells including circulating epithelial tumor cells from 1 ml blood were cultured under conditions favoring growth of spheroids from 72 patients with breast cancer, including a subgroup of 23 patients with metastatic disease. CETCs were determined using the maintrac® method. Gene expression profiles of single CETCs and spheroids of the same patients were analyzed using qRT-PCR.

**Results:** Sphere formation was observed in 79% of patients. The number of spheroids was dependent on stage of disease. Furthermore, the most important factor influencing the growth of spherosomes was the treatment with chemotherapy. Patients treated with chemotherapy had significantly lower numbers of spherosomes compared to patients without chemotherapy. The spheroids were found to consist of cells with cancer stem cell characteristics such as upregulation of stem cell genes ( Sox2, Oct4, Nanog, EpCAM, CD 133,) self-renewal, and high aldehyde dehydrogenase (ALDH) activity. There was no sphere formation in a control group with 50 healthy donors.

**Conclusion:** This study reports on a method for generating and growing spherosomes from peripheral blood and confirms the hypotheses that cells with cancer stem cells properties are present in the blood of patients with breast cancer. Understanding the biology of spherosomes may contribute to the identification of novel therapeutic opportunities.

**No conflict of interest.**

150 Poster (Board P121)
Evaluation of potential predictive biomarker for cetuximab in a panel of colorectal cancer patient-derived xenografts (PDX)

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**Background:** Cetuximab is approved as EGFR targeted therapy for colorectal cancer (CRC) together with activating mutations in the MAPK pathway (KRAS and BRAF) as predictive biomarker. However there is a group of resistant patients for which an improved predictive biomarker is...
needed. We used our recently established panel of 49 CRC PDX for a correlation analysis of cetuximab response with molecular markers in the EGFR signalling.

Methods: Tumor tissues from 87 CRC patients were transplanted into immunodeficient mice, 49 of them engrafted as PDX. The models were characterized with regard to sensitivity to standard care therapy. Mutational screening was performed with Illumina TruSeq® Amplicon – Cancer Panel and gene expression profiling with the Affymetrix® Human GeneChip®. The expression of EGFR receptor family and ligands was examined at mRNA and protein level using real time PCR and Sandwich-ELISA.

Results: Our 49 CRC PDX were derived from 26 primary tumors and 23 liver or lung metastases, for 5 patients PDX pairs derived from samples originated from different time points or locations could be established. Immunohistochemistry confirmed resemblance of the PDX to the original patient sample. The sequencing of patient tumors, paired normal tissues and the corresponding PDX confirmed maintenance of the genetic profile and the encountered mutations reflected the clinical incidence in CRC. The mutational profiles of the PDX pairs were mainly identical. PDX with activating mutations in PIK3CA (n=8) showed a shorter tumor doubling time, compared to wildtype. From the PDX panel, 63% showed a growth delay >50% upon cetuximab treatment. Activating mutations in KRAS and BRAF were individually predictive for resistance, furthermore, PDX models with a mutation in one or more of the three genes (KRAS, BRAF and PIK3CA) showed significantly higher T/C-values than triple wildtype PDX (T/C = 28% vs. T/C = 58%, p = 0.001). This observation extended to the sensitivity for erlotinib, a TK-inhibitor for EGFR. Regarding EGFR ligands, high expression of epi- and amphiregulin, showed a significant correlation to cetuximab sensitivity, pointing out tumors dependent on the EGFR pathway. TGFβ behaved inversely, and its expression also correlated negatively with the response to erlotinib and cetuximab, suggesting a more general role in survival of CRC cells.

Conclusion: The comprehensive characterization of the models allowed identification of molecular factors that correlate with general survival of CRC cells, and elucidate the different roles of the players of the EGFR pathway in sensitivity towards EGFR targeted therapy. Thus, PDX models are well suited for preclinical translational research studies, as they reflect the heterogeneity and dynamics of CRC and can be used for identification or evaluation of potential biomarkers for personalized medicine.

No conflict of interest.

151 Poster (Board P122)

Fully human, preclinical model for the testing of immunotherapies


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Background: The goal of personalized medicine is to stratify individual patients to the most appropriate treatment. As the past has shown, this approach strongly depends on extensive characterization of individual tumors and their sensitivity to therapeutics. We have previously shown that a modular drug testing platform based on precision cut cancer tissue slices is applicable to analyze individual responses of patients to chemotherapeutics, small molecules and therapeutic antibodies. In the context of immunotherapeutic studies, the localization, abundance and activation of immune cells within individual tumors gained in importance. In this study, we showed that viable tumors used within our drug testing platform, exhibit different populations of infiltrating immune cells. Analysis of immune cells was conducted on disaggregated, primary cells from viable tumors by flow cytometric analysis of CD3, CD4, CD8 and CD45. Furthermore, we identified PD1 positive cells among the CD45+CD3+ lymphocyte population, indicating relevance for anti-PD1 targeted therapy in colorectal cancer.

Material and Methods: Tumors from colorectal cancer, used in this study were freshly collected and tissue preparation was conducted within 4 hours after resection. In order to obtain tissue slices, fresh tissue samples were cut using a Krumdieck tissue slicer according to manufacturer's instructions. Precision cut cancer tissue slices were cultured in 24-well plates in proprietary tissue culture medium. Disaggregation of tumor tissue was performed using the Miltenyi GentleMacs. Flow cytometry was performed on the Cyflow space instrument, using primary labeled antibodies.

Results: We successfully prepared single cell suspensions from whole tumors as well as from precision cut cancer tissue slices. Those cells were subjected to flow cytometric analysis. In PDX of eight colorectal cancer patients we detected in mean 10% CD45+ cells; of those cells fewer than 1% were PD1 positive. Furthermore, the CD45+ lymphocyte population was identified as cytotoxic T-cells. Interestingly, predominantly CD4+ cells expressed PD1 (5%), whereas only 0.3% of CD8+ cells were PD1 positive.

Conclusions: In order to optimize preclinical testing of immune-modulatory compounds, preclinical models, which reflect the individual tumor, as well as the individual immune components of the tumor, are mandatory. We have shown here that individual tumors used in the drug testing platform, exhibit immune cells corresponding to the individual patient, which can be detected and quantified by flow cytometry. Therefore, this drug testing platform represents an unique opportunity to test immune-modulatory compounds in a fully human, patient derived model that is close to in vivo situation.

No conflict of interest.
here we introduce a fast and cost-efficient, patient-derived test system encompassing immune cells and 3D tumor spheroids to assess the specific response towards standard-of-care (SoC) as well as investigational therapeutic approaches, including immunotherapy.

**Materials:** Tumor spheroids are established directly from primary RCC material of consenting patients after surgery using limited digestion and cultivated in defined media in the absence of serum. Autologous immune cells (incl. T-lymphocytes, NK cells) are isolated from whole blood by magnetic bead based separation using anti-CD137 and anti-CD56 antibodies, respectively. Isolated T-lymphocytes are either left untreated, broadly activated using super antigens or primed against tumor antigen-specific peptide pools and expanded in culture. Tumor-specific cytotoxicity of immune cells, SoC and investigational compounds as well as their combinations are subsequently assessed in co-culture with respective 3D tumor spheroids using a fluorometric microplate assay. Live cell pre-labeling of tumor spheroids is applied for simultaneous assessment of spheroid disintegration in response to treatment.

**Results:** Activated autologous T lymphocytes induced a robust and significant cytotoxic response in tumor spheroids generated from renal cell carcinoma tissue over time. Moreover, simultaneous disintegration of dye-labelled spheroids in response to activated but not naïve T lymphocytes was observed using live-cell fluorescence microscopy. In addition, the cytotoxicity response of spheroid models established from different patients towards SoC and investigational compounds was assessed. Results obtained are highly reproducible for each patient tested and vary between individual patients indicating interpatient variability in treatment response.

**Conclusion:** Our novel assay system is a useful tool for screening of investigational compounds as well as SoC drugs with regard to their patient-specific tumor targeting efficiency. Fast and economic isolation of tumor spheroids facilitates the generation of patient specific treatment response data within 2–3 weeks after surgery. Importantly – shown here for RCC – our platform comprises 3D tumor spheroids in co-culture with autologous immune cells and thus enables testing of immunotherapeutic compounds alone and in combination with chemo- and targeted therapy. No conflict of interest.

154 Poster (Board P125)

**NAMPT inhibition is a novel synthetic lethal therapeutic approach exploiting nuclear-mitochondrial crosstalk in ERCC1-deficient populations**

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**Material and Methods:** High-throughput proteomic SILAC (stable isotope labeling by amino acids in cell culture) and metabolomic (LC-MS/MS, GC-MS/MS and LC-QTOF) profiling were performed on a unique in-house generated isogenic NSCLC model of ERCC1 deficiency. NAMPT was selected as a main hit in the screen for synthetic lethal interactors. To determine specificity of NAMPT inhibition in ERCC1-deficient and wildtype NSCLC models, sensitivity to selective NAMPT inhibitors (FK866 and GNE617) was further assessed in vitro and in vivo. Mechanistic dissection of the SL link between ERCC1 deficiency and NAMPT inhibition was investigated using metabolomics profiling, electronic transmission microscopy (ETM), and functional analysis of mitochondrial respiration and glycolysis (Seahorse13, Agilent).

**Results:** We found marked metabolic rewiring of ERCC1-deficient populations, including decreased NAMPT levels, decreased NAD+ levels and abnormalities in the tricarboxylic acid (TCA) cycle. These metabolic alterations caused selective exquisite sensitivity to small molecule NAMPT inhibitors, both in vitro – ERCC1-deficient cells being approximately 1000 times more sensitive than their proficient isogenic counterpart – and in vivo. We further sowed that this exquisite sensitivity to NAMPT inhibition is a primary effect of ERCC1-deficiency. ETM microscopy and functional metabolic studies revealed that ERCC1-deficient cells harbor mitochondrial structural and functional defects, as well as downregulated SIRT1/NAD+ axis. Evaluation on patient tissue samples is ongoing and will be presented at the conference.

**Conclusions:** These findings open novel therapeutic opportunities that exploit a hitherto undescribed nuclear-mitochondrial synthetic lethal link between ERCC1 deficiency and NAMPT inhibition in NSCLC, and highlight the potential of targeting DNA repair defect with a useful tool for developing investigational compounds as well as SoC drugs with regard to their patient-specific tumor targeting efficiency. Fast and economic isolation of tumor spheroids facilitates the generation of patient specific treatment response data within 2–3 weeks after surgery. Importantly – shown here for RCC – our platform comprises 3D tumor spheroids in co-culture with autologous immune cells and thus enables testing of immunotherapeutic compounds alone and in combination with chemo- and targeted therapy. No conflict of interest.

155 Poster (Board P126)

**Presence of immune infiltrates in early phases of prostate cancer: Establishment of a preclinical efficacy model to promote immunotherapy development**

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**Material and Methods:** Intact 10–12 weeks old male Noble rats were a.s. implanted with slow-releasing estradiol and testosterone pellets for 6, 13 and 18 weeks. Daily release for testosterone was 0.8 mg and for estradiol 0.08 mg. Control group animals received placebo hormone pellets without hormones. Serum samples were collected during the study to monitor hormone levels, and prostates were removed and processed for histopathological evaluation at the end of the study. **Results:** Hormonal treatment caused an increase in estradiol to testosterone ratio, and the prostates were enlarged. imbalance in hormone-miule induced inflammation in the prostate, followed by formation of prostatic intraepithelial neoplasia (PIN)-like lesions and finally adenocarcinomas in the periurethral region. Inflammatory cells, mainly T-cells were noticed in the vicinity of PIN-like lesions. During the progression of prostate cancer, inflammatory cells disappeared from the adenocarcinoma sites. The prostate, inflammation consisting of perivascular, stromal and periglandular T-lymphocytes and intratumoral neutrophils remained. **Conclusions:** Results of this study indicate significance of hormonal milieu, especially estrogens and androgens, in the development of inflammation and progression of prostate cancer, with a key role for tumor microenvironment. Prevention of ly in prostate cancer may be considered as a promising therapeutic strategy in the prevention of PIN-like lesions during the early phases of prostate cancer, and their disappearance late in the adenocarcinomas, indicate interaction between innate immune system and cancer. Furthermore, this preclinical prostate cancer model that combines immune system and cancer can be utilized when new immunotherapies, combination treatments and prevention possibilities against prostate cancer progression are developed. No conflict of interest.
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Poster (Board P127)  
Prostate cancer cell induced generation of myeloid derived suppressor cells from monocytes ex vivo is inhibited by targeting STAT3 with galleialactalone  
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Background: The transcription factor Signal Transducer and Activator of Transcription Factor 3 (STAT3) is implicated in acquired drug resistance, metastases and immune suppression in prostate cancer (PCa). Elevated levels of myeloid derived suppressor cells (MDSC) have been found in the peripheral blood and tumor site of PCa patients and correlate with disease progression. MDSC play a major role in the suppression of antitumor immunity. STAT3 signaling is involved in accumulation, generation and function of MDSCs. We aimed to investigate if cells with an MDSC phenotype could be generated from monocytes in the presence of PCa cells and if this induction could be blocked by the direct STAT3 inhibitor galleialactalone.  

Materials and methods: Monocytes were isolated from peripheral blood mononuclear cells (PBMC) from healthy donors. Monocytes were co-cultured with PCa cells in transwells allowing cancer cells and monocytes to share culture medium. Monocytes in co-cultures or with conditioned medium from PCa cells were grown for 72 h with or without the STAT3 inhibitor galleialactalone with subsequent analysis by flow cytometry for CD14 and HLA-DR for MDSC generation. PBMC was collected from patients with metastatic PCa and healthy controls and analysed for the expression of CD14 and HLA-DR with FACS analysis. CD14+ HLA-DRlo cells were sorted using BD FACSaria cell sorter, subjected to cytospin and analysed for expression of pSTAT3.

Results: PCa cell lines induced an MDSC phenotype (CD14+ HLA-DRlo) in monocytes from healthy donors, with the greatest induction from the more aggressive PCa cell line DU145. Galleialactalone prevented the PCa cell induced MDSC phenotype in a dose dependent manner. The inhibitory effect of galleialactalone on PCa induction of MDSCs may be due to STAT3 inhibition in both monocytes and PCa cells. The levels of MDSCs (CD14+ HLA-DRlo) were significantly higher in metastatic PCa patients compared to healthy controls and were shown to express pSTAT3.

Conclusions: This study demonstrates that monocytes can induce MDSCs from monocytes ex vivo and that inhibiting STAT3 will block this process. This indicates that STAT3 inhibition may reverse the immunosuppressive mechanisms caused by MDSC activation and that STAT3 inhibitors represents a potential new treatment for advanced PCa where levels of MDSC are increased.

Conflict of interest: Ownership: Shareholders in Glactone Pharma AB (RH, AB, MJ); Board of Directors: Glactone Pharma AB (AB). Other Substantive Relationships: Project manager for Glactone Pharma AB (MJ).

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Poster (Board P128)  
Changes in transcription of the genes from iron and copper metabolic pathways are associated with progression from T1 to T3 stage of renal cell carcinoma  
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Background: Staging of renal cell carcinoma (RCC), based on TNM classification as well as size and regional spread of primary tumors, provides useful prognostic indicator of patient outcome. However, clinical course of the disease varies even among patients within the same staging strata. Thus calls for search of other prognostic factors that could improve prognostication for individual patients. Molecular events, e.g. presence of mutations or changes in transcriptional and epigenetic regulation may provide a clue for personalized disease prognosis. However, molecular events, associated with regional spread of the tumor during progression from stage T1 to T3 are known only partially. We aimed to identify new patterns of gene expression, which point at the mechanisms of local tumor spread.  

Material and Methods: We used microarrays for genome-wide analysis of gene expression in 24 patients with RCC in stage T1 through T3. Unsupervised clustering divided patients into subgroups. Genes with fold changes above 1.5 were used for clustering. Pathway analysis identified among them genes responsible for cell proliferation and mesenchymal transition but surprisingly also genes related to iron and copper metabolism, such as lysyl oxidase or STEAP3.

Conclusion: Growth of tumor and its local spread in RCC patients is associated with changed transcriptional regulation of iron and copper metabolism.

No conflict of interest.

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Poster (Board P129)  
Chimeric anti-podoplanin mAbs produced by CasMab technology possess high antitumor activity via ADCC and CDC activities  
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Background: Podoplanin (PDPN), a platelet-aggregation-inducing transmembrane glycoprotein, is expressed in a variety of tumors, and binds to C-type lectin-like receptor-2 (CLEC-2). PDPN expression is linked to tumor invasion and metastasis. Several anti-PDPN monoclonal antibodies (mAbs) such as NZ-1 showed antitumor and antimetastatic activities by binding to platelet-aggregation-stimulating (PLAG) domain of PDPN. Recently, we developed novel mouse anti-PDPN mAbs, LpMab-2, LpMab-7, and LpMab-23 using the cancer-specific mAb (CasMab) technology.  

Methods: We generated and characterized mouse-human chimeric anti-PDPN mAbs, chlPDPN-2, chlPDPN-7, and chlPDPN-23. We investigated antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) activities of chimeric anti-PDPN mAbs in vitro. Furthermore, we evaluated the in vivo efficacy of chimeric anti-PDPN mAbs using several xenograft models.

Results: Novel chimeric anti-PDPN mAbs showed ADCC and CDC activities against CHO/PDPN, glioblastoma, mesothelioma, or lung cancer cell lines. Treatment with chimeric anti-PDPN mAbs and human NK cells abolishes tumor growth in xenograft models of hPDPN-expressing cell lines.

Conclusion: Chimeric anti-PDPN mAbs produced by CasMab technology suppressed tumor development via ADCC/CDC activities. PDPN should be useful as a novel antibody-based therapy.

No conflict of interest.

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Poster (Board P130)  
The role of hsa-miR-X in cell motility and invasion in triple-negative breast cancer cell lines  
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The aim of this study is to identify the role of hsa-miR-X in motility and invasion in TNBC. Triple-negative phenotype which is an aggressive type of breast cancer lacks expression of ER, HER2 and PR and is known as basal-like carcinoma. Since the lack of targeted therapies and resistance to chemotherapy it is inevitable to identify new molecules for diagnosis and treatment of triple negative breast cancer (TNBC). MicroRNAs could be good candidate molecules which regulate gene expression in many biological processes. Epithelial–mesenchymal transition (EMT) is a process which induces motility and invasion of the cells. E-cadherin is an important mediator of adhesion in epithelial tissues. Loss of E-cadherin can play a critical role in tumor invasive behavior and E-cadherin transcription is known to be regulated by transcriptional suppressors, such as SNAIL. Dysregulation of these molecules could affect the invasion and metastatic capacity of the cell.

In this study three triple negative breast cancer cell lines, BT-20, MDA-MB-468, MDA-MB-231 were transfected with hsa-miR-X or scrambled control siRNA. To check its role in motility and invasion, wound healing and invasion assays were performed respectively. Cell invasion was monitored over a period of 24 hours by xCELLigence real-time cell analyzer using a double-plate and measuring impedance-based signals. Furthermore, EMT markers were analyzed in transfected cells by qRT-PCR to explain the effect of hsa-miR-X in motility and invasion.

We observed that cell motility and invasion decreased in three triple negative breast cancer cell lines (BT-20, MDA-MB-468, MDA-MB-231 cells) after transfected with mimic for hsa-miR-X. Additionally, qRT-PCR experiments demonstrated that transfection of hsa-miR-X decreased the expression level of SNAIL while increasing the E-cadherin expression level.
Wound healing and invasion assays together with qRT-PCR results could support the role of hsa-miR-X in cell motility and invasion via SNAI1 and E-Cadherin dependent manner. It might be concluded that insights into the miRNA research that regulate triple negative breast cancer motility and invasion could lead to novel therapeutic targets.

No conflict of interest.

160 Poster (Board P131)
Towards the next-generation of cancer cell lines: Derivation of an organoid biobank
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Cell lines derived from patient tumours have contributed to our understanding of cancer biology and therapeutic drug response. However, cancer cell lines grown in 2D in vitro culture have several limitations including failing to represent the heterogeneity of cancer. The derivation from clinical specimens of epithelial cells in 3D culture can transform the preclinical cancer setting by better reflecting the biology of the tumour of origin, and by providing more predictive models of patient response to cancer therapies.

The Human Cancer Model Initiative, an international consortium including the Wellcome Trust Sanger Institute (WTSI), Cancer Research UK (CRUK), the foundation Hubrecht Organoid Technology (HUB) and the US National Cancer Institute (NCI), have come together to derive the next-generation of cancer cell lines for the research community. This consortium will derive and genomically annotate in the region of 1000 new cancer models over the next two years. WTSI and CRUK working in collaboration will generate 250 human models, with a focus upon colon, pancreas and oesophageal cancer. Multiple UK clinical sites are contributing human cancer material on a weekly basis. Tissue samples are taken at biopsy or surgical resection and sent immediately to WTSI for organoid derivation. In addition, we receive patient clinical data providing a complete clinical history of the cancer sample received, including age, disease stage and prior lines of therapy.

Derived organoids are subjected to targeted gene sequencing of a cancer gene panel to confirm normal or tumour origin before undergoing whole genome sequencing, RNAseq and drug sensitivity screening. We will be answering key questions to determine the utility of organoids for cancer research. Preliminary work indicates that organoids reflect the molecular alterations present in the donating tumour and retain a degree of polyclonality. Importantly, they are genomically stable in culture, acquiring only a small number of mutations when in long-term culture. We will present results of our on-going efforts evaluating the utility of organoid models and whether they can effectively capture the molecular diversity and treatment responses of patient populations.

No conflict of interest.

161 Poster (Board P132)
Combination immune checkpoint inhibitors for the treatment of human colon carcinoma in NSG mice engrafted with human PBMC
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Over the past decade there has been increasing improvements in the development of preclinical models for the evaluation of the efficacy of checkpoint inhibition-based cancer immunotherapies. Moreover, the recently developed humanized mouse models offer a unique tool to assess the anti-tumor response of the human immune system to checkpoint inhibitors. We have recently established that severely immune-compromised mice successfully engrafted with huPBMC develop graft versus host disease (GvHD) in 5 to 6 weeks. Here we present the response to the checkpoint inhibitors pembrolizumab (anti-PD-1) and ipilimumab (anti-CTLA-4) in the human RKO colon carcinoma (HLA-A*01) xenograft model following the engraftment of HLA-A matched huPBMC in NSG mice. Combination of pembrolizumab with ipilimumab therapy resulted in a significant inhibition of tumor growth. Analysis of the human immune cell subsets showed the majority fraction of the human CD45 cells were T-cells. There was a significant increase in the T-cell population, specifically CD8 T-cells, of the combination ipilimumab + pembrolizumab treatment group as compared to the control group treated with non-specific IgG.

In summary, this study supports the use of the huPBMC-NSG mouse model to test the tumor response to immune-checkpoint based therapies as it shows significant tumor growth inhibition associated with CD8 T-cell expansion. In addition, this study demonstrates that there is a therapeutic window to evaluate cancer treatments before the onset of xenogeneic GvHD in this model.

No conflict of interest.

162 Poster (Board P133)
Experimental design recommendations for PDX pre-clinical trials: Reanalysis of radiation and temozolomide orthotopic survival data in GBM models
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Background: Patient derived xenograft (PDX) preserve salient genetic features of human tumors. Testing candidate treatments across a population of PDXs may be useful to predict the extent of clinical benefit and to identify predictive biomarkers. However, there is little guidance on statistical designs for PDX pre-clinical trials such as a recently reported 1 x 1 x 1 trial design.

Methods: Mayo Clinic has 72 glioblastoma (GBM) PDX models that are routinely used for pre-clinical trials. Using orthotopic tumor survival data collected for radiation (RT), temozolomide (TMZ) and combined RT/TMZ treatments for 39 models, we empirically evaluated trial designs by varying the number of PDX models per treatment group as well as the number of mice per model per treatment. For each scenario, 100 bootstrap trials were performed by randomly selecting animals from each treatment group to identify the design in which 95% of the time the p-values were <0.1.

Results: Analysis of variance models were used to test for differences in survival across treatment groups; PDX model was adjusted for as a covariate when more than one mouse per model was evaluated.

Results: In comparison to placebo survival (mean = 67 days), TMZ alone was associated with two-fold improvement in survival (mean = 148 days) and RT/TMZ with nearly three-fold improvement (mean = 183 days). In our empirical studies, differences in survival could be detected for TMZ alone versus placebo using 35 PDX models and one mouse per treatment/model, or similarly, 20 PDX models and two mice per treatment/model. Differences could be detected for RT/TMZ versus placebo using 30 PDX models and one mouse per treatment/model, or similarly, 15 PDX models and two mice per treatment/model. Comparing RT alone versus RT/TMZ demonstrated that 35 PDX models and one mouse per treatment/model, or similarly, 30 PDX models and two mice per treatment/model could detect a mean difference of 90 days improvement in survival. Amongst the 16 MGMT methylated PDX models, TMZ alone was associated with nearly a three-fold improvement in survival (mean = 188 days) and RT/TMZ with more than a three-fold improvement (mean = 239 days). Differences in survival amongst MGMT methylated PDX models could be detected for TMZ alone versus placebo using 15 PDX models and two mice per treatment/model. Differences could be detected for RT/TMZ versus placebo using 10 PDX models and two mice per treatment/model. Comparing RT alone versus RT/TMZ in MGMT methylated models demonstrated that 15 PDX models and two mice per treatment/model, could detect a mean difference of 143 days improvement in survival.

Conclusions: These results reiterate that the design of PDX pre-clinical trials is dependent on the expected effect sizes (improvement in survival across treatment groups) and demonstrate how existing data can be used to guide design of PDX trials.

No conflict of interest.
level molecular validation in patients' tumor cells. Primary tumor cells from ALL patients were amplified in severely immune-compromised NSG mice to generate PDX ALL cells. For genetic engineering, PDX ALL cells were lentivirally transduced in two consecutive rounds using a blue or green fluorescent protein (mtagFP or eGFP) followed by a knockdown construct. The shRNA was embedded into the context of a microRNA 30 background which allowed expressing the shRNA directly linked to a transgenic marker. The two transfections were performed such that blue cells expressed the corresponding knockdown construct, while green cells of XIAP so that control cells and XIAP knockdown cells could be distinguished by flow cytometry. This setting allowed performing competitive in vivo proliferation assays in which both cell populations could be simultaneously monitored in the same mouse.

Results: Our knockdown strategy reduced XIAP expression by well more than 90% in ALL PDX cells which remained stable over several months of passaging in mice. We found that PDX ALL cells with knockdown of XIAP suffered a severe growth disadvantage over control cells. Blue control cells significantly overgrew green cells with XIAP knockdown. The inhibitory effect of XIAP knockdown was dose-dependent as PDX ALL cells with high knockdown of the knockdown marker showed a more pronounced growth inhibition than cells with low marker expression. In one sample, cells with high knockdown of XIAP completely vanished after 6 weeks.

Conclusion: We established a novel technique enabling molecular target validation in patients' tumor cells in vivo by combining efficient gene silencing in PDX cells with convenient readout systems. This novel approach enables prioritizing putative therapeutic targets on a convenient, molecular and cellular level. XIAP plays an essential role in ALL and ALL high knockdown of XIAP completely vanished after 6 weeks. treatment cancellation with low marker expression. In one sample, cells with high knockdown of XIAP completely vanished after 6 weeks.

No conflict of interest.

164 Poster (Board P135)

Development and characterization of HER2+ T-DM1-resistant breast cancer PDX models

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Background: Ado-trastuzumab emtansine (T-DM1) is an antibody–drug conjugate (ADC) approved for treatment of high HER2 expressing (3+), trastuzumab resistant breast cancers. While this agent is initially effective, resistance often develops. To aid in developing new therapies for HER2+ T-DM1-resistant breast cancer and better understand resistance mechanisms, we developed two T-DM1-resistant breast PDX models designated ST1616B/TDR and ST1360B/TDR in athymic nude mice and characterized both for receptor expression, genomic alterations and in vivo drug sensitivity.

Materials and Methods: ST1616B/TDR and ST1360B/TDR models were developed by chronic in vivo drug conditioning of ST1616B and ST1360B parent models over several passages; resistance was confirmed with weekly high dose drug injections. Resulting models were characterized by immunohistochemistry, RNA in situ hybridization and NGS and tested for sensitivity to chronic drug exposure. Characteristics including trastuzumab resistance, pertuzumab and T-DM1 and results compared with data from parent and other HER2+ PDX models. In vivo study endpoints included tumor volume and time from treatment initiation with T/C values and tumor regression regression reported at study completion.

Results: Both TDR models retained high HER2 expression and DNA-based characteristics of their respective parent models. ST1616B/TDR and ST1360B/TDR were found resistant to chronic single agent treatment of trastuzumab, pertuzumab or T-DM1. Interestingly co-administration of pertuzumab and either trastuzumab or T-DM1 restored sensitivity of either agent towards the ST1360B/TDR model.

Conclusion: We have developed and characterized two HER2+ T-DM1-resistant breast PDX models one of which can be sensitized with co-administration of pertuzumab. These models are valuable tools in developing new therapies to T-DM1 resistance.

No conflict of interest.

165 Poster (Board P137)

Preclinical models of patient derived xenografts (PDX) for immuno-oncology research

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Objective: The preclinical evaluation of novel immune checkpoint modulators is dependent on tumor models in mice with functional immune cells. In previous experiments, we have demonstrated that hematopoietic stem cells (HSC) can proliferate and differentiate in vivo in a functional humanized immune system. Further we have shown engraftment of PDX on these humanized mice. In the next step, we developed PD-L1 expression as a target for immuno-therapy with different tumor cell lines and PDX and evaluated the functionality of the humanized mice by the treatment with the checkpoint inhibitors ipilimumab and nivolumab.

Methods: For humanization of mice HSC from cord blood were transplanted intravenously into 3 week old irradiated nude scid gamma mice. Tumor cell lines and PDX were screened for PD-L1 expression on RNA level by PCR and protein level by FACS, western blot and immunohistochemistry. PD-L1 positive and negative tumor cell lines and PDX models were subcutaneously co-implanted into these humanized mice and followed for growth. Response to the CTLA-4 checkpoint inhibitor ipilimumab and PD-1 checkpoint inhibitor nivolumab was evaluated. Tumor and cells (bone marrow, spleen, thymus) were harvested at the end of the experiments by FACS and exemplary by immunohistochemistry for T cells and other immune cells and the expression of PD-1, CTLA-4 and PD-L1.

Results: All investigated cell lines (e.g. MDA-MB-231, ES-2, Colo205) and PDX models (e.g. colon, head and neck, melanoma, lung) engrafted successfully on humanized mice. 12 from 14 PDX models showed no difference in tumor growth compared to non-humanized mice. These PDX model seem to be not as effective as the remaining 2 PDX models with a reduced growth on humanized mice (due to innate immune response) showed a stronger response to the checkpoint inhibitors.

Conclusion: HSC can be transplanted in immunodeficient mice and establish a functional human immune system. Tumor cell lines and PDX successfully engrafted in the humanized mice and responded to the checkpoint inhibitors.
models can be successfully engrafted on humanized mice – generating a fully human preclinical test system for immuno-oncology. 

**No conflict of interest.**

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**Radiation-induced lung fibrosis is associated with interstitial M2 macrophages and hybrid alveolar macrophages**

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**Background:** Radiation-induced fibrosis (RIF) is a delayed complication of radiation therapy (RT) which affects both normal and tumor tissues. RIF is characterized by the infiltration of fibroblasts and deposition of extracellular matrix components. The role of macrophages in the fibrogenic process is still not well understood.

**Material and Methods:** We analyzed the infiltration of different macrophage subsets in the fibrotic lung of patients treated with definitive RT for head and neck squamous cell carcinoma (HNSCC). We used a panel of immune markers to identify the infiltrating cells, including CD68, CD163, and CD206 for M1, M2, and hybrid macrophages, respectively.

**Results:** We observed a significant increase in the infiltration of M2-polarized macrophages in the fibrotic lung tissue compared to normal lung tissue. The infiltration of M2 macrophages was associated with an increase in the production of cytokines, such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β), which are known to promote fibrosis.

**Conclusions:** Our findings suggest that M2 macrophage infiltration plays a crucial role in the development of radiation-induced fibrosis. Further research is needed to understand the mechanism by which these cells contribute to fibrogenesis and to identify potential therapeutic targets to mitigate RIF.

**No conflict of interest.**

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**Validation of 68Ga-HBED-CC PSMA-PET/CT and multiparametric MRI for gross tumor volume delineation in patients with prostate cancer based on comparison with histology reference**

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**Background:** Radiation therapy for prostate cancer is an effective treatment option, but accurate tumor volume delineation is crucial for optimizing treatment planning. 68Ga-HBED-CC PSMA-PET/CT and multiparametric MRI are imaging modalities that can provide detailed information about tumor extent. The aim of this study was to validate the use of these imaging modalities for gross tumor volume delineation in patients with prostate cancer, with histology as the reference standard.

**Methodology:** Seventeen patients with histologically proven prostate cancer underwent 68Ga-HBED-CC PSMA-PET/CT and multiparametric MRI. Tumor volume was assessed on both imaging modalities and compared to the volume measured on histological slides. Sensitivity and specificity were calculated for each imaging modality.

**Results:** The mean volume of the gross tumor volume (GTV) calculated from PET/CT was 1.1 cm³ (±0.4 cm³) and from MRI was 1.2 cm³ (±0.5 cm³). The sensitivity and specificity for both modalities were 92% and 88%, respectively. A significant correlation was observed between the volumes measured on PET/CT and MRI (p < 0.05).

**Conclusion:** 68Ga-HBED-CC PSMA-PET/CT and multiparametric MRI are accurate imaging modalities for gross tumor volume delineation in patients with prostate cancer. These modalities can provide valuable information for planning radiation therapy, improving treatment outcomes.

**No conflict of interest.**
Antitumor effect for combined therapy of SOCS-1 gene therapy and radiotherapy in esophageal squamous cell carcinoma

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Background: The prognosis for patients with esophageal squamous cell carcinoma (ESCC) remains unsatisfactory in spite of improvements of multimodality treatment, including surgery, radiotherapy and chemotherapy. Previous reports showed that constitutive activation of JAK/STAT signaling as a negative regulatory of various cytokine signaling including JAK/STAT pathway. This study was aimed to evaluate the antitumor effect of SOCS-1 gene therapy using adenoviral vector (AdSOCS-1) as monotherapy and combination therapy with radiation.

Material and Methods: As in vitro, we evaluated cell growth inhibition effect of AdSOCS-1 as monotherapy (1) or combination therapy with radiation (2 and 3). Also, we evaluated growth inhibition effect of ESCC by using JAK inhibitor I and/or FAK-siRNA. As in vivo models (3), ESCC cell line (TE-14) xenograft models consisted of C57BL/6 mice implanted with human ESCC tumor including ESCC, and the activation of STAT3 also was associated with radiation resistance. Suppressor of cytokine signaling-1 (SOCS-1) has been cloned as a negative regulator of various cytokine signaling including JAK/STAT pathway. In this study, we also evaluated antitumor effect of SOCS-1 gene therapy using adenoviral vector (AdSOCS-1) as monotherapy and combination therapy with radiation.

Results: (1) AdSOCS-1 markedly suppressed proliferation of all ESCC cell lines in vitro regardless of activation level of STAT3 and induced apoptosis via inhibiting not only JAK/STAT signaling but also FAK/JNK signaling. Although TE14 which has low activation level of STAT3 was not significantly suppressed only by JAK inhibitor I, the inhibition of JAK/STAT and FAK/JNK signaling by using JAK inhibitor I and/or FAK-siRNA showed the antiproliferation effect regardless of activation level of STAT3 as same as AdSOCS-1. (2) Irradiation for ESCC cell lines induced the activation of STAT3, and also induced anti-apoptosis protein such as Mcl-1 and survivin. The combination of radiation and AdSOCS-1 showed an additive anti-proliferative effect, and anti-apoptosis protein such as Mcl-1 and survivin was suppressed through the inhibited pSTAT3. As in vivo (3), the tumor volume and tumor weight with AdSOCS-1 was significantly lower than AdLacZ. Especially, PDX mice with AdSOCS-1 showed better antitumor effect. We confirmed the expression of SOCS-1 and the inhibition of pSTAT3 and FAK/JNK signaling as same as in vitro by western blotting of AdSOCS-1 injected tumor. Also, Ki-67 index of tumor with AdSOCS-1 significantly decreased compared to AdLacZ, and TUNEL staining showed that SOCS-1 gene therapy induced apoptosis in vitro. Conclusion: Our results indicated that overexpression of SOCS-1 in monotherapy and combination therapy with radiation inhibited the progression of ESCC in vitro. Also, SOCS-1 gene therapy had good antitumor effect in ESCC xenograft mice, especially in PDX mice.

Conflict of interest: None.
enhanced radiation sensitivity of HPV-positive tumors is clearly evident from clinical data. On the cellular level HPV-positive HNSCC cell lines also show an enhanced sensitivity associated with a defect in DNA double-strand break repair and a profound and sustained arrest in G2. The normal function of the radiation-induced G2-arrest is to provide time for DNA repair before the critical passing of mitosis. Interfering with G2-arrest can therefore be expected to result in radiosensitization in these cells but should have little impact on non-proliferating and p53/G1-arrest proficient normal tissue cells. Therapeutic approaches to target the radiation-induced G2-arrest are the inhibition of Chk1 and Wee1 kinases.

**Material and Methods:** Assessment of Chk1 and Wee1-Inhibition by Western blot; assessment of cell cycle distribution by propidium iodide staining and flow cytometry; assessment of cell survival by colony formation assay. HPV+ HNSCC cell lines: UD-SCC-2, UM-SCC-47 and UP1-SCC-154. Chk1-inhibitors: LY260368 and SCH900776; Wee1-inhibitor: AZD-1775.

**Results:** While both specific Chk1-inhibitors efficiently interfered with radiation-induced G2-arrest, Wee1-inhibition resulted in an accumulation of the HPV-positive cells in the S-phase, indicative of replication stalling. Surprisingly already low concentrations of the Wee1-inhibitor AZD-1775 resulted in an activation of Chk1. This feedback mechanism is likely to at least partially counteract the effects of Wee1-inhibition and could be investigated through the combined inhibition of both kinases. Combined inhibition was already effective using explicitly low concentrations of both inhibitors and resulted in radiosensitization of the HPV-positive cell lines but not normal human fibroblasts as a surrogate for normal p53-proficient tissue.

**Conclusion:** Usage of both, specific Chk1 inhibitors as well as a combined inhibition of Chk1/Wee1 inhibition is able to radiosensitize HPV-positive HNSCC but not normal tissue cells and may be suitable in deintensified therapeutic regimes.

No conflict of interest.

**Enhancement of radiosensitivity by the novel anticancer quinolone derivative vosaroxin in preclinical glioblastoma models**

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**Purpose:** Glioblastoma multiforme (GBM) is the most aggressive brain tumor. The activity of vosaroxin, a first-in-class anticancer quinoline derivative that intercalates DNA and inhibits topoisomerase II, was investigated in GBM preclinical models as a single agent and combined with radiotherapy (RT).

**Materials and Methods:** Cellular, molecular and anti-proliferative effects of vosaroxin alone or combined with RT were evaluated in 12 GBM cell lines. Tumor growth delay was determined in U87MG, U251 and T98G xenograft mouse models. Disease free survival (DFS) and Overall Survival (OS) were assessed in orthotopic intra-brain models using luciferase-transfected U251 or U87 cells by bioluminescence and magnetic resonance imaging.

**Results:** Vosaroxin had antitumor activity in clonogenic survival assays with IC50 of 10–100 nM and caused radiosensitization. Combined treatments exhibited significantly higher g-H2AX levels compared to controls. Vosaroxin reduced tumor growth and showed enhanced activity with RT; vosaroxin/RT combined was more effective than temozolomide/RT. Vosaroxin/RT triggered rapid and massive cell death with characteristics of necrosis. Only a minor proportion of treated cells underwent caspase-dependent apoptosis in agreement with in vitro results. Vosaroxin inhibited RT-induced autophagy increasing necrosis. This was associated with increased recruitment of granulocytes, monocytes and undifferentiated bone marrow-derived lymphoid cells. Pharmacokinetic analyses revealed adequate blood-brain penetration of vosaroxin. Vosaroxin/RT increased DFS and OS significantly compared to RT, vosaroxin alone, temozolomide and temozolomide/RT in the U251-luciferase orthotopic model.

**Conclusions:** Vosaroxin demonstrated significant activity in vitro and in vivo in GBM models, and showed additive/synergistic activity when combined with RT in O6-methylguanine methyltransferase (MGMT) negative and positive cell lines.

Conflict of interest: Ownership: Judith Fox is an employee of Sunesis Pharmaceuticals Inc, San Francisco, USA. The other authors disclosed no potential conflicts of interest.

**Clinical Trial Methodology**

175 Poster (Board P001)

**Detecting secondary KIT mutations in the peripheral blood of patients with imatinib-resistant gastrointestinal stromal tumor**

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**Background:** Imatinib resistance in gastrointestinal stromal tumor (GIST) is mostly caused by secondary mutations in KIT. The efficacy of multikinase inhibitors, sunitinib and regorafenib, for treating imatinib-resistant GIST is correlated with the type of secondary mutation. In order to guide the selection of targeted agents, noninvasive analysis to detect mutations is desirable. Circulating tumor DNA (ctDNA) is a part of cell-free DNA (cfDNA) that consists of small fragment of nucleic acids derived from the tumor which is present in the cell-free fraction of blood. Since ctDNA carries tumor-specific mutations, it is an emerging candidate biomarker of resistance to therapy and tumor progression.

**Materials and Methods:** This study included four patients who underwent resection of imatinib-resistant GIST between November 2011 and January 2017. Mutation-specific tumour related sequencing and mutation-specific differential sensitivity analysis of ctDNA was performed using real-time PCR. The sensitivity of ctDNA was measured using next-generation sequencing of 318 semiconductor chips with more than 100,000x coverage. The fraction of ctDNA decreased after treatment in two of three patients. Identical secondary mutations could be detected in ctDNA from the peripheral blood of these patients. This study was approved by the Ethics Committees of Osaka University.

**Results:** Three patients had primary lesions in the stomach and one in the duodenum. All four patients performed curative resection of primary lesions, and received imatinib as adjuvant therapy or treatment for recurrent lesions before imatinib-resistant lesions emerged. Macroscopic curative resection was performed for all imatinib-resistant lesions. Imatinib-resistant lesions showed single nucleotide substitutions in KIT exon 13 in three patients and exon 18 in one patient. Vosaroxin/RT increased disease-free survival and overall survival in two of three patients. Individual secondary mutations could be detected in ctDNA, and the fraction decreased slightly after treatment in two of three patients. Identical secondary mutations could be detected in ctDNA, and the concentrations decreased slightly after treatment in two of three patients. One patient had growth of an imatinib-resistant tumor containing a KIT exon 13 mutation, and the fraction of ctDNA decreased after initiation of sunitinib.

**Conclusions:** Secondary KIT mutations could be detected in ctDNA from peripheral blood samples. Contrary to ctDNA concentration, the fraction of ctDNA decreased after tumor progression. Detection of secondary KIT mutations in ctDNA would be useful for the selection of targeted agents and prediction of antitumor effects.

No conflict of interest.

176 Poster (Board P002)

**Roche-Genentech oncology trials – our experience with data sharing via CSDR**

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**Background:** As part of its commitment to data sharing, Roche-Genentech, with 12 other sponsor companies, shares patient-level data via the multi-sponsor request site ClinicalStudyDataRequest.com (‘CSDR’). As part of this initiative, Roche aims to maximise access to clinical trial data and enable further scientific research, whilst respecting patient privacy and the role of regulators in determining access to new medicines.

**Methods:** We outline the request process, volume of requests received & broad aims, the Roche trials available to request (including oncology products) and details of research involving Roche oncology data.

**Results:** ‘CSDR’ enables third-party researchers to request data from sponsor clinical trials, as well as enquiring about trials not listed as available. Central to the process is an Independent Review Panel (IRP), managed by Wellcome Trust, an independent global charitable health foundation. Researchers create a research proposal; this includes lay summary, statistical analysis and publication plans. Once approved by the IRP and data sharing agreement signed, anonymised data/documents are

Conflict of interest: Ownership: Judith Fox is an employee of Sunesis Pharmaceuticals Inc, South San Francisco. The other authors disclosed no potential conflicts of interest.
Trabectedin is a marine-derived antineoplastic drug registered for the treatment of STS after failure of anthracyclines and ifosfamide, or for patients who are unsuited to receive these drugs. Trabectedin has a unique mechanism of action that combines a direct growth-inhibitory effect on tumor cells with its ability to affect tumor-associated macrophage, which induces changes in the tumor microenvironment contributing to its antitumor activity. The pharmacokinetic (PK) of trabectedin is characterized by liver extensive metabolism and excretion through the biliary route. The urinary elimination of the drug and metabolites is negligible, suggesting that renal function does not play a significant role in drug clearance (Cl). Since limited data are available in patients with reduced creatinine clearance (CrCl), the objective of this study was to evaluate the PK of trabectedin in patients with abnormal renal function, including patients aged >75 years and/or with a physiological decrease of CrCl.

Materials and Methods: We have determined the PK of trabectedin in patients affected by advanced STS unift to receive standard chemotherapy that were treated with intravenous trabectedin 1.3−1.5 mg/m² given as a 24-h infusion every three weeks until disease progression or development of unacceptable toxicity. CrCl was calculated as by Cockcroft-Gault equation. Blood samples were collected before infusion and 4, 8, 23.5, 24 hour during infusion and 0.5, 1, 4, 8 and 24 hour post infusion. Plasma levels of trabectedin were determined by liquid chromatography coupled to tandem-mass spectrometry. Area under the curve of the drug plasma concentration vs time (AUC) and the derived PK parameters were calculated by the software NCPKA v. 2.4.

Results: The PK study was performed in 16 patients with median age 87 years (range 65−91) and with median CrCI 49 mL/min (range 32–123), during the first cycle of trabectedin treatment. Results showed acceptable inter-patient variability of the trabectedin plasma concentration and of PK parameters. During and approximately at the end of the 24-h infusion, trabectedin achieved a steady state concentration of 1.55±0.68 ng/mL, then the drug follows a rapid decline of concentration, up to 10 times within 1 h. Mean±SD of AUC, Clp, volume of distribution and elimination half-life were 27.90±8.62 ng/1h·L, 39.84±14.28 L/h, 1452±564 L/min and 26.7±9.1, respectively.

Conclusions: Trabectedin PK data obtained in the present study conducted in elderly, with moderate impairment of the renal function, are in the range of those previously reported in the literature in younger adults with normal renal and hepatic function, suggesting that the doses commonly used in adult patients can be safely administered to elderly or patients affected by mild to moderate reduction of the renal function.

No conflict of interest
Conclusion: Phase 2 paediatric oncology trials that examine a single cancer type, use combination therapies and are based on previous paediatric trials have the highest possibility of achieving success. No preclinical experimental results enhanced the possibility of achieving clinical trial success. 

No conflict of interest.

180  Poster (BoardP006)  
Efficient clinical research infrastructure and trial performance. 
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Background: No studies have evaluated the processes, timelines, and enrollment in early clinical trials, especially from units focused on early phase drug development. Here, we present an overview of the research infrastructure utilized by the Investigational Clinical Therapeutics (ICT) dept. at MD Anderson Cancer Center and assessed trial performance under this model.

Materials and Methods: We assessed the trial development processes within the MD Anderson ICT dept. to identify key elements of the clinical research infrastructure.

Discussion: Clinical trial performance was analyzed from the MD Anderson Clinical Oncology REsearch (CORe) database, a prospectively maintained registry that tracks all clinical studies at MD Anderson. The current analysis was limited to phase II-III trials submitted after January 1, 2004 (year that the ICT dept. was founded). A total of 2,261 trials met study criteria. Each ICT dept. trial was matched to a trial from another department within MD Anderson utilizing 1-to-1 exact matching by trial phase, sponsorship, (industry, institutional, national cooperative group, and external peer review), and year of trial submission. Comparisons of trial development timelines and participant accrual between matched ICT vs. non-ICT dept. trials were conducted via paired T-tests.

Results: We identified 140 trials within the CORe that met the research infrastructure; parallel processing of trial approval steps, a physician-led research team structure, and regular weekly meetings meant to foster an atmosphere of research accountability. Among the 2,261 available trials, 221 ICT dept. trials were included in this analysis. ICT dept. trials were submitted from 2004 to 2014. Separate analyses were conducted to assess matched industry-sponsored (n = 133 ICT and 133 non-ICT) and institutional-sponsored (n = 68 ICT and 68 non-ICT) trials. ICT dept. trials exhibited faster development timelines, including time from IRB approval to trial activation (matched difference industry-sponsored: 1.3, institutional-sponsored: 3.5 mo) and activation to first participant enrolled (matched difference industry-sponsored: 1, institutional-sponsored: 1.2 mo) compared to trials from other depts. (all P <0.05). Furthermore, ICT dept. trials exhibited significantly higher total accrual (matched difference industry-sponsored: 14.2, institutional-sponsored: 5.7, institutional-sponsored: 12.8 participants/year) (all P <0.05).

Conclusions: Utilization of a clinical research-focused infrastructure within a large academic cancer center to conduct research on early phase clinical trials was associated with faster trial development and increased participant accrual. 

No conflict of interest.

182  Poster (BoardP008)  
Analytical validation of comprehensive assays for genomic profiling of cancer from DNA and RNA
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Background: Genomic assays are increasingly used in oncology to guide clinical management and assess tumor responsiveness to novel therapeutics. However, the number of cancer genes related to cancer driver genes continuously expands, necessitating broader mutational profiling of tumors. Here we present the analytical validation of the ACE Cancer Portfolio, which includes the largest cancer gene panel available to date for clinical use and an augmented exome and transcriptome for more complete genomic characterization.

Materials and Methods: Commercially available standards are of limited utility, so we aimed to create standards consisting cancer cell lines in combination with primary cancer samples. Validation was performed on our cancer gene panel for DNA and RNA containing >1600 genes, and on our augmented exome and transcriptome assay containing >20,000 genes, including 8,000 supplemented for more uniform coverage. Validation was accomplished using >30 cancer cell lines and reference standards with known single nucleotide variants (SNVs) and small insertions and deletions (indels). >15 cancer cell lines with known copy number alterations (CNAs), and >17 cell lines with known gene fusions. Tumor heterogeneity was simulated by mixing the cell lines at various ratios, generating variant allele frequencies down to 1%, and emulated reduced tumor purity by mixing cell lines with paired normals at ratios down to 5%. Data were analyzed using cancer bioinformatics pipelines in both tumor-only and tumor-normal modes. The assay was validated for use in different clinical specimen types (formalin fixed paraffin embedded (FFPE), fresh frozen (FF), blood).

Results: Uniform DNA sequencing coverage was achieved at mean alignment depth of >500 and >200 reads for the DNA panel and exome respectively. The sensitivity of the panel assay was 99.7% for SNVs at ≥5% (n = 16,132), 99.4% for small indels (AF ≥10%, n = 207,171), 91.2% for CNAs (n = 54, copy number 0 or >3 in tumor-only cell-lines), and 95.0% for fusion transcripts (n = 20). The specificity of the panel assay was >99% for SNVs and indels. For cancer cell lines and clinical tumor specimens where a matched normal was available, tumor-normal analysis
refined somatic variant calling in comparison to tumor-only analysis. The assay also performed robustly in real clinical specimens, including FFPE. Further data will be presented summarizing the sensitivity and specificity results for the exome and transcriptome assay.

Correlation has developed and validated comprehensive cancer NGS assays, with highly uniform deep coverage, ensuring high sensitivity and specificity for all variant types. This assay represents a versatile tool that can be used to (i) test a core set of clinically actionable genes, (ii) implicate new cancer genes as clinically relevant, (iii) facilitate discovery of novel therapeutic targets.

**Conflict of interest:** Ownership: Financial relationships (such as employment, stock ownership or options, patents) may exist.

183 Poster (Board P009)

**Cancer-related internet use in patients with advanced cancer in a phase I clinical trials clinic**

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**Background:** We examined patterns, correlates and impact of cancer-related internet use among patients with advanced cancer in a clinic for phase I clinical trials of molecularly targeted oncologic agents.

**Material and Methods:** An anonymous questionnaire on internet use for cancer-related purposes, incorporating input from phase I physician oncologists and clinical trial clinic patients, was self-administered by patients (age > 18) in a phase I clinic for targeted therapy. Multivariable modeling was used. Data were analyzed for the overall sample and by generation, defined by year of birth as follows: Millennials (after 1990), Generation X (1965-1989), Baby Boomers (1946-1964), and Greatest/Silent Generation (1945 and prior).

**Results:** Of 291 patients (52% women, 82% non-Hispanic white, 50% < 60 years) seen in a phase I clinic beginning in 2012, 62% were cancer-related internet users (CIUs). Cancer-related internet use was associated with an income of > $60,000 (OR = 2.42, p = 0.004). CIUs used the internet more to learn about cancer, drugs, and treatment/clinical trials rather than for emotional support. The hospital website (70% of CIUs) was most frequently used to learn about clinical trials, followed by ClinicalTrials.gov (42%), and search engines (41%). CIUs reported informational gains from the internet about their cancer (85%), side effects of treatment (85%), clinical trials (52%), new alternative treatments for their cancer (42%), and management of symptoms (41%). Emotional impact of internet-derived cancer information on CIUs varied: 56% felt empowered, 34% anxious, 29% collaborated, and 25% was not influenced. Cancer-related internet information may alter 42% of Millennials/Generation X/Y CIUs and women compared to < 25% of CIUs from older generations (born 1964 and prior). Most CIUs desired more online information about new experimental drugs (91%) and US Food and Drug Administration-approved drugs for cancer (72%). Based on online scores of trust measured on six-point likert scales ranging from 0 (no trust) to 5 (complete trust), trust of online cancer-related information was higher among CIUs than among non-CIUs (2.8 vs. 1.8, p < 0.001). However, CIUs and non-CIUs did not differ in their trust of referring (4.4 vs. 4.5, p > 0.05) or phase I physicians (4.3 vs. 4.2, p > 0.05). CIUs' trust of referring (4.4) and phase 1 physicians (4.3) were higher than CIUs' trust of online cancer-related information (2.8) (p < 0.001 for both).

**Conclusions:** As most phase I patients use the internet for cancer-related purposes, the internet should be leveraged to provide accurate and empowering information to phase I patients. Websites of hospitals that conduct phase I trials should be updated with the latest clinical trial-related information for patients. Given phase 1 patients' trust of physicians, physician-authored or attributed content should be prioritized on hospital websites.

No conflict of interest.

184 Poster (Board P010)

**A robust population-based screening platform, HuScreen™, enables identification of candidates, indications and biomarkers in mouse clinical trial using a large, diverse and fully annotated PDXs**

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**Background:** While patient derived xenograft (PDX) mimics original patient, a large cohort of PDXs reflect heterogeneity of patient populations [1, 2], which enables drug efficacy evaluation in a population-based clinical trial (HuTrial™) to select indication and guide patient stratification [3-5]. Recent works by others have also demonstrated the utility of this type of trial format for screening large panel of candidate agents, enabling prioritization of lead development [6] and reposition of existing drugs.

**Method:** We aim at building a large diverse PDX library and large-scale global industry capacity supporting multiple-center mouse clinical trial (MCT). We analyzed several MCT datasets to assess the impact of different parameters used in trial/screen process, including endpoints, number of models, number of mice per treatment group, etc., which can be further used to guide the trial/screen design and data analysis, so the final data can adequately support conclusions and answer important translational questions.

**Results:** We have built the largest diverse PDX library (> 3,000 HuPrime™) with full annotations (1, 3-5, 7-9) that enables the simultaneous testing of large panel of different agents on hundreds of subject models. A large cohort of live models (hundreds, also called warm test subjects) maintained in our facilities enable speedy trial enrollment and ensure rapid MCT. Piggyback strategy at the global scale with many partners can significantly reduce trial cost and broaden the acceptance. We have generated several MCT datasets, from which we compared the common response or survival endpoints used in human (RECIST, OS, PFS) and mice (TAC, TGI, PFS, OS) and established certain levels of relevance equivalency. We then discussed suitable situations for each endpoint can be used. We have also explored new novel data analysis methods beyond traditional endpoint analysis, and identified new methods sufficiently robust to handle the high variations in tumor growth dynamics in different mice or models during MCT. We also developed new statistic approaches to discover predictive biomarkers (signatures) according to trial data and model annotations.

**Conclusion:** Optimally designed and implemented HuScreen™ platform can be a powerful tool to prioritize candidates, select indications and discover predictive biomarkers, as well as repurpose drugs.

No conflict of interest.

185 Poster (Board P011)

**Suitability of post-diagnostic core needle tumor biopsies for correlative studies of molecular drug action (pharmacodynamics)**

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**Background:** Although key specimens for evaluating tumor pharmacodynamics (PD) response using biomarkers of molecular drug action, core needle biopsies collected during Phase 0/1 clinical trials from various metastatic lesions and historical origins present with heterogeneous tumor and variable tumor content. We have found that a significant percentage of biopsies are unsuitable for PD evaluation across multiple assay platforms, and new approaches, sampling methods and procedures are needed to improve the percentage of evaluable biopsies.

**Materials and Methods:** For microscopy-based measurements, biopsy pairs are collected using image guidance are snap-frozen, thawed under fixative, and embedded in parallel. The likelihood of finding optimal regions for biomarker analyses is optimized by preparing a series of 35 sections, flanking slides are stained with H&E and annotated by an anatomic pathologist who determines whether tumor content is sufficient to meet assay requirements.

**Results:** From evaluating 87 biopsies for slide based PD analyses across three trials at NCI’s Developmental Therapeutics Clinic (DTC), 65% of biopsies and 53% of biopsy pairs were found to contain sufficient tumor content to be suitable for the intended qIF assay (qIF). Approximately 18% contained tumor content too low to adequately represent the tumor as a whole given the biomarker variability and/or to yield a reliable quantitative measurement using current procedures. Additionally, 16% were found to contain no analyzable tumor cells. Similar incidences of both low- and no-tumor content biopsies have been observed at several other clinical centers.

**Conclusions:** There are different requirements of tumor sampling for diagnosis and for studies of drug mechanism. Improved communication between oncologists and radiologists will provide better understanding of factors that affect the suitability of biopsies for robust PD biomarker analyses. NCI’s DTC has implemented protocol modifications including
additional cores from each patient, and regular case reviews by the Phase 1 team may identify features during image guidance that relate to biopsy suitability. For qIFA, biomarkers that identify carcinoma cells even when expressing the mesenchymal phenotype can aid in segmenting tumor from normal tumor tissue and stroma to facilitate image analyses in low tumor content specimens. While these adjustments increase the percentage of low tumor content biopsies analyzable via qIFA, these biopsies are still inadequate for lyase assays that destroy morphology, and measurements are needed for the content of tumor in extracts. Technologies for "smart" biopsy needles containing sensors for optimizing tumor yield may replace image-guided needle placement, but these devices are currently in development. Funded by NCI Contract No HHSN261200800001E. No conflict of interest.

186 Poster (Board P012)
Electroacupuncture for chemotherapy-induced peripheral neuropathy: A pilot multicenter randomized, patient-assessor blinded, controlled trial
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Background: Chemotherapy-induced peripheral neuropathy (CIPN) is the main dose-limiting side effect of neurotoxic chemotherapeutic agents. CIPN can lead not only to loss of physical function, difficulties in activities of daily living (ADLs), and decreased quality of life, but also to dose reduction or even cessation of treatment. Currently, there are few proven effective treatments for CIPN. This randomized controlled clinical trial is designed to evaluate the effects and safety of electroacupuncture (EA) for patients with CIPN.

Methods and Design: This is a multicenter, two-armed, parallel-design, patient-assessor blinded, randomized, sham-controlled clinical trial. Forty eligible patients with CIPN will be randomized in a ratio of 1:1 to the EA or sham EA arms. During the treatment phase, patients will undergo 8 sessions of verum EA or sham EA twice weekly for 4 weeks, and then will be followed-up for 8 weeks. Electrical stimulation in the EA group will consist of a mixed frequency of 2/120 Hz and 80% of bearable intensity. Sham EA will be applied to non-acupoints, with shallow needle insertion and no current. All outcomes and analyses of results will be assessed by researchers blinded to treatment allocation. The effects of EA on CIPN will be evaluated according to both subjective and objective outcome measures. The primary outcome measure will be the European Organization for Research and Treatment of Cancer (EORTC) quality of life questionnaire to assess CIPN (QLQ-CIPN20). The secondary outcome measures will be the results on the numerical rating scale, the Semmes-Weinstein monofilament test, the nerve conduction study, and the EORTC QLC-C30, as well as the patient's global impression of change and adverse events. Safety will be assessed at each visit.

Results: Currently, all 40 participants have been recruited and clinical trial has been finished. After coding the participants' data, the result of this study will be analyzed before and after treatment, and at 4-week/8-week of flu after the end of treatment according to the method described in the protocol, and will be shown on the presentation.

Conclusions: The results of this on-going study will provide clinical evidence for the effects and safety of EA for CIPN compared with sham EA. No conflict of interest.

Drug Delivery

187 Poster (Board P013)
Tumor-specific PI3K inhibition by targeted delivery in head and neck squamous cell carcinoma
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Background: Mutations of PIK3CA, the gene coding for the isoform α of the phosphatidylinositol 3-kinase p110 catalytic subunit (PI3Kα), are frequent in head and neck squamous cell carcinoma (HNSCC). Specific inhibitors of PI3Kα have shown promising activity in PIK3CA-mutant HNSCC patients and recent data suggest that they can act as radiosensitizers in this disease. However, the clinical use of PI3K and AKT inhibitors is limited by a relatively narrow therapeutic window that inevitably leads to emergence of adverse effects such as hyperglycemia, skin rash, diarrhea and fatigue.

Material and Methods: In this study we explored the efficacy, specificity and safety of targeted delivery of BYL719, a potent alpha-specific PI3K inhibitor, using nanoparticles that selectively target the cell adhesion molecule P-selectin in the microvasculature of PIK3CA-mutated HNSCC. We used both cell line-based and patient-derived xenograft models to measure the specificity of drug delivery and the consequent antitumor activity of BYL719.

Results: We found that BYL719 encapsulated in nanoparticle (FIBY) selectively accumulated in both subcutaneous and orthotopically implanted xenografts after administration of a single dose of FIBY. P-selectin targeted delivery of 50 mg/kg/week of FIBY resulted in tumor growth arrest that was comparable to the standard drug administration of 350 mg/kg/week (50 mg/kg/day). Moreover, even though the treatment with FIBY was 7-fold lower in absolute amount of inhibitor, it showed the same radiosensitizing properties as standard BYL719 dosage. Pharmacodynamic studies revealed that systemic treatment with BYL719 elicited a strong, albeit transient, inhibition of the PI3K/AKT/mTOR pathway in either cell line- or patient-derived xenografts. This was compatible with the short half-life of this compound in the plasma. Conversely, a single administration of FIBY719 resulted in complete and durable suppression of the pathway, consistent with accumulation of FIBY in the tumor microenvironment. When labeled with a fluorescent dye, FIBY was found to localize exclusively in the tumor tissue.

In order to confirm that normal tissue was not exposed to FIBY, we measured glyceremia and insulinemia, the most frequent adverse events upon PI3K inhibition, in animals treated with this agent. While blood glucose and insulin levels were spiking soon after standard oral BYL719 administration, these parameters were virtually unchanged upon FIBY treatment.

Conclusions: P-selectin-targeted delivery of BYL719 resulted in potent inhibition of the PI3K/AKT/mTOR pathway sufficient to elicit durable antitumor activity and radiosensitize several HNSCC animal models. This novel targeting strategy could be used to treat HNSCC patients with PIK3CA-mutant tumors avoiding most of the systemic adverse effects consequent to PI3K/AKT/mTOR inhibition.

No conflict of interest.

188 Poster (Board P014)
Elastin-like polypeptide for improved delivery of small molecule drugs and therapeutic peptides for anticancer therapy
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Background: Current anticancer treatments are limited by poor drug solubility, poor pharmacokinetic parameters, and non-specificity resulting in severe toxicity from systemic administration of antineoplastic agents.

Material and Methods: To address these current shortcomings in cancer therapies, we developed a drug delivery system based on thermally responsive Elastin-like Polypeptide (ELP), which can be targeted to a tumor-specific site. Our local drug delivery strategy was modified by adding a cell penetrating peptide (CPP) to facilitate cell entry and polypeptide delivery to the tumor tissue. ELP delivery system was conjugated with peptides targeting the Hedgehog (Hh) signaling pathway–reported to be aberrantly activated in cancer, or with an anthracycline derivative of doxorubicin, which is commonly used in treating a wide range of cancers.

Results: The anti-proliferative activity of CPP-Hh-ELP was examined in three breast cancer cell lines; MCF7, MDA-MB-231, and SKBR-3. Cells treated with 20 μM of peptide for 2 days had 40% inhibition of cell proliferation, which was increased two-fold by application of hyperthermia. Treatment of the breast cancer cells with CPP-Hh-ELP reduced levels of GLI-1, which is a downstream target in Hh pathway, indicating that the cytotoxicity is based on hedgehog pathway inhibition. Moreover, treatment of SKBR-3 cells with CPP-Hh-ELP significantly reduced the formation of mammospheres, indicating that ELP-based Hedgehog inhibitory peptides may be an effective, promising treatment strategy against breast cancer stem cells.

To show that ELP can be used for the delivery of small molecule drugs, a doxorubicin derivative was conjugated to ELP through a cleavable linker enabling doxorubicin release in the targeted low pH environment (acid sensitive linker or amino acid sequence (Gly-Phe-Leu-Gly)--a substrate of membrane P-glycoprotein). Doxorubicin (Paclitaxel) has greater cytotoxicity than doxorubicin with non-cleavable linker (ncDox) in MCF7 and in the drug resistant cell line, MCF7-ADR. Confocal microscopy experiments have shown that while constructs with cDox were concentrated in the nucleus, the construct with ncDox had a perinuclear localization. Flow
cytometry results indicated 2–3 fold higher uptake in drug sensitive MCF 7 cells compared to resistant MCF7/ADR cells. These results indicate that the ELP drug complex is capable of inhibiting cancer cell proliferation and overcoming drug resistance.

Conclusion: The developed drug delivery system may provide a method for targeted delivery of therapeutic peptides and small molecule drugs to tumor cells. Also, depending on the molecular target, these drugs or peptides may eradicate cancer stem cells and overcome drug resistance.

No conflict of interest

189 Poster (Board P015)

Nanoparticle-encapsulated piperlongumine modulates metastatic processes in triple-negative breast cancer cells

J. GhassemiRad 1, D.W. Hoskin 1,2,3.

Background: Metastatic disease is the major cause of morbidity and mortality among breast cancer patients in spite of recent advances in cancer treatment. Novel therapeutics are urgently needed to decrease breast cancer mortality by preventing epithelial-to-mesenchymal transition (EMT)-associated metastasis. Transcription factors such as β-catenin, ZEB1, and Slug, along with epigenetic machinery including histone deacetylases (HDAC) and DNA methyltransferases (DNMT) are well-studied regulators of EMT. Piperlongumine, a major alkaloid in pepper spices, inhibits breast cancer cell growth in vivo and in vitro; however, its lipophilicity has restricted possible clinical application. The purpose of this study was to increase the water solubility of piperlongumine using a nanoparticle as drug carrier and investigate the anti-metastatic potential of piperlongumine-nanoparticles in the context of EMT regulation in triple-negative breast cancer cells.

Material and Methods: The thin-film hydration method was used to encapsulate piperlongumine into biodegradable methoxy poly(ethylene glycol)-b-poly(D,L-lactic-co-glycolic) acid (mPEG-PLGA) copolymer. Colorimetric MTT and Annexin-V–FLUOS/propidium iodide staining assays were performed on MDA-MB-231 and MDA-MB-468 triple-negative breast cancer cells to determine the effect of piperlongumine-nanoparticles on cell growth and viability. The in vitro studies used to determine the effect of piperlongumine-nanoparticles on cell growth and viability. The in vitro studies were complemented with cancer stem cell assays. The expression of EMT markers, β-catenin, Slug, and ZEB1, along with the expression of E-cadherin and NDRG1, inhibitors of EMT and metastasis, in MDA-MB-231 cells. Furthermore, piperlongumine decreased the expression of HDAC1 and NDRG1, both of which are known transcriptional suppressors of E-cadherin and NDRG1.

Conclusions: These results demonstrate that piperlongumine inhibits metastatic properties of MDA-MB-231 breast cancer cells through epigenetic changes and inhibition of EMT-associated transcription factors. Ultimately, these findings indicate the potential use of nanoparticles as phytochemical carriers for future in vivo studies to improve the bioavailability and serum solubility of piperlongumine.

No conflict of interest

191 Poster (Board P017)

Gemcitabine impairs tumor perfusion in stroma rich murine endogenous PDAC


Background: The ELP drug complex is capable of inhibiting cancer cell proliferation and overcoming drug resistance.

Conclusions: The developed drug delivery system may provide a method for targeted delivery of therapeutic peptides and small molecule drugs to tumor cells. Also, depending on the molecular target, these drugs or peptides may eradicate cancer stem cells and overcome drug resistance.

No conflict of interest

190 Poster (Board P016)

Efficacy of the MDM2 inhibitor SAR405838 in PDX models of GBM is limited by active efflux at the BBB


Background: SAR405838, an inhibitor targeting the MDM2-p53 interaction, has been shown to have significant anticancer activity in solid tumors. Given that CNS delivery of SAR405838 will be critically important in treating invasive brain tumors, the objective of the current study was to examine the brain distributional kinetics of SAR405838, and correlate the observed changes in delivery with efficacy in glioblastoma (GBM) models.

Methods: A PDX model overexpressing MDM2 (G108) was modified with lentiviral transduction with either empty vector (G108-VEGF) or vector containing VEGFA transcript (G108-VEGF). VEGFA was measured by ELISA. In vivo efficacy studies were performed with heterotopic and orthotopic xenografts of G108. Mice were treated with placebo or SAR405838 (50 mg/kg/d). Texas Red dextran (3kD) images were obtained to examine the integrity of the BBB. Plasma and brain samples were harvested after a single oral dose of SAR405838 in wild-type (WT) and transgenic FVB mice; including Mdr1a/b−/− (PKO), Bcrp1−/− (BKO), and Mdr1a/b−/− Bcrp1−/− (TKO). Steady-state plasma and brain samples were harvested after a 48-hour infusion using an osmotic pump. The concentrations in plasma and brain were analyzed using LC-MS/MS.

Results: VEGFA expression in G108 cell lines with VEGFA overexpression (G108-VEGF) was orders of magnitude greater than empty vector (G108-VE). The tumor distribution of SAR405838 was greater and more homogeneous in G108-VEGF tumors, based on the results of MALDI-Mass Spectrometry Imaging. Efficacy in orthotopic PDX models showed a significant survival benefit in G108-VEGF tumor-bearing mice over the G108-VE group. Texas Red images showed that the integrity of BBB was disrupted in G108-VEGF tumors. Pharmacokinetic parameters and partition coefficients of brains were determined by concentration-time course analysis. The half-lives in PKO and TKO were longer than in WT and BKO. Partitioning into brain (calculated by AUChuman/AUCplasma ratio) and brain/plasma concentration ratio acquired from the steady-state experiment showed that the accumulation of SAR405838 in the brain was significantly greater in PKO and TKO mice compared to WT and BKO mice.

Conclusions: Brain delivery of SAR405838 is limited due to p-glycoprotein-mediated active efflux at the BBB. The survival studies conducted in orthotopic mouse models show that SAR405838 has greater efficacy in GBM if the drug is more available in the tumor, as in VEGFA overexpressed models lacking an intact BBB. Delivery of SAR405838 to invasive areas of the PDX GBM model across the BBB is critical to achieve efficacy in intracranial tumors and efflux transporters at BBB play a significant role in limiting brain delivery of SAR405838. This class of molecularly-targeted agents (MDM2 inhibitors) should be explored in treating GBM if drug delivery hurdles can be overcome.

No conflict of interest
**Drug Design**

192  Poster (Board P018)

**Diagnosis and therapy of aggressive breast cancers by targeting urokinase receptor**

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**Background:** Triple negative Breast Cancer (TNBC) is a highly aggressive BC subtype, with an increased likelihood of distant recurrence and death compared with other types of BC. Patients diagnosed with TNBC lack the estrogen and progesterone receptor, the human epidermal growth factor 2 and do not respond well to current therapies. Targeted therapies such as epidermal growth factor receptor inhibitors, showed variable response rates but no survival benefit. One hallmark of aggressive cancers is increased urokinase receptor (uPAR) expression. The over-expression of urokinase plasminogen activator (uPA) and its receptor (uPAR) have been found to contribute to the aggressive phenotype in a number of cancers and is found uniformly in many metastases. uPAR ubiquitousness in TNBC makes it an attractive target for uPAR-directed therapies. It participates in many protein/protein interactions, which leads to pericellular proteolysis and signaling that is mediated by specific integrins. Therefore, inhibition of one or more of these interactions would progressively contribute to a reduction in aggressive behavior.

**Material and Methods:** Six inhibitory anti-uPAR Antibodies (Abs) were identified. Two of those Abs, referred to as 2G10 and 3C6, obstruct protein interactions between uPAR and uPA or beta 1 integrin, respectively. The Abs were tested in vitro and in vivo TNBC models and showed diagnostic and therapeutic potential. In a mouse model of TNBC the Abs targeting two distinct subdomains of slowed or blocked tumor growth. Moreover, treatment of TNBC cell lines in vitro with a combination of 2G10 and 3C6 demonstrated synergy, suggesting that blocking multiple uPAR effector functions simultaneously may provide a dramatically enhanced response.

**Results:** The anti uPAR Abs, have been assembled using a modular platform to give bi-specific Abs based on DNA linkers. The platform enables a library of heterofunctional molecules to be made with precise geometries, valencies, and rigidities. Basic linear construct combining 3C6 and 2G10, have been tested for uPAR recognition ability and therapeutic effect in vitro in TNBC cell lines.

**Conclusions:** Our results indicate that at fixed total protein concentration, the scaffolded Abs outperform the unscathed Abs in blocking MDA-MB-231 cell invasion.

**Conflict of interest:** Acknowledgments: This work was supported by Komen PDF1303204. Reagents quantities of aldehydic tag Fabs were provided by Catatyal Pharma Solutions.

193  Poster (Board P019)

**Aryl group modification in novel anti-cancer agents based on α-3,17,18-epoxyicosapentaenoic acid**

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**Background:** Dietary and experimental studies have shown that α-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) inhibit the development of certain cancers. The CYP-derived 17,18-epoxode EPA ((α-3,17,18-epoxy-EPA) and its saturated analogues impair tumour cell growth and activate apoptosis (Ciù et al., 2012; Dyari et al., 2014). We recently developed a stable mimic of α-3,17,18-epoxy-EPA termed CTU that rapidly killed tumour cells in vitro and in vivo in mouse xenograft models. In this study, the structural requirements for tumour cell killing by synthetic CTU analogues were evaluated.

**Materials and Methods:** The nature of the aromatic system in CTU was modified to produce 12 new analogues for the evaluation of steric and electronic factors in anti-tumour activity. The viability of MDA-MB-231 breast cancer cells was assessed by ATP production, apoptosis by caspase-3 activity and cell cycle kinetics by flow cytometry.

**Results:** Three di-substituted CTU analogues that carried electron-withdrawing groups were active. NK14, NK24 and NK18 decreased ATP production to 50±3%, 48±1% and 81±3% of control (10 µM, 48 hr) and NK14 and NK24, but not NK18, also increased caspase-3 activity to 141±6% and 137±10% of control (10 µM, 48 hr); these changes were more pronounced at higher concentrations. NK14 in particular markedly increased the proportion of cells in sub-G1 phase (31±3% versus 4.4±0.4% in control; 10 µM, 48 hr), and decreased G0/G1 and G2/M populations. In contrast, CTU analogues carrying bulky aromatic or heteroaromatic substituents or that contained weakly electron-withdrawing groups were inactive.

**Conclusions:** Like CTU, NK14 decreased the viability of MDA-MB-231 cells by activating apoptosis, impairing energy metabolism and disrupting cell cycle progression. CTU and several other α-3,17,18-epoxy-EPA mimics show promise as potential anti-tumor agents.

**References**


**No conflict of interest.**
Pharmacology, Ulm, Germany

Aim of this study was to investigate the effects of positively and negatively diverser tasks, which ranged for example from proinflammatory (M1 subset) macrophages may differentiate into distinct subsets exerting a variety of functions. Yet, the mechanisms underlying these effects have been partially elucidated. In spite of biomedical research partly for toxicological reasons, but increasingly also for their potential application in therapy and diagnosis. In spite of biomedical research partly for toxicological reasons, but increasingly also for their potential application in therapy and diagnosis. In spite of biomedical research partly for toxicological reasons, but increasingly also for their potential application in therapy and diagnosis. In spite of biomedical research partly for toxicological reasons, but increasingly also for their potential application in therapy and diagnosis. In spite of biomedical research partly for toxicological reasons, but increasingly also for their potential application in therapy and diagnosis. In spite of biomedical research partly for toxicological reasons, but increasingly also for their potential application in therapy and diagnosis.

Background: In the last few years, nanomaterials moved into focus of biomedical research partly for toxicological reasons, but increasingly also for their potential application in therapy and diagnosis. In spite of their potential medical usage, nanomaterials might induce non-specific "adverse effects" in the human body. Macrophages play an important role in the immunological defense of the human body. Because they guard all entry sites of the body including the circulation, macrophages are among the first cells to encounter infiltrating nanoparticles. Yet, the mechanisms underlying these effects have been partially elucidated. In spite of biomedical research partly for toxicological reasons, but increasingly also for their potential application in therapy and diagnosis. In spite of biomedical research partly for toxicological reasons, but increasingly also for their potential application in therapy and diagnosis. In spite of biomedical research partly for toxicological reasons, but increasingly also for their potential application in therapy and diagnosis. In spite of biomedical research partly for toxicological reasons, but increasingly also for their potential application in therapy and diagnosis. In spite of biomedical research partly for toxicological reasons, but increasingly also for their potential application in therapy and diagnosis. In spite of biomedical research partly for toxicological reasons, but increasingly also for their potential application in therapy and diagnosis.

Material and Methods: Human peripheral blood monocytes were differentiated in vitro into M1 and M2 macrophages and the impact of amino or carboxyl functionalized polystyrene nanoparticle exposure on cell viability and polarization profiles was investigated. Proinflammatory M1 and anti-inflammatory M2 macrophage subtypes were characterized by morphology, protein markers, cytokine secretion profiles and functional differences.

Results: In vitro differentiated M1 and M2 macrophages took up, both, positively and negatively charged polystyrene nanoparticles. Viability was not compromised by nanoparticle exposure for 3 days; however, negatively charged nanoparticles increased the metabolic activity of both macrophage subsets. Regardless of the surface charge, nanoparticles inhibited polarization towards anti-inflammatory M2 macrophages as seen by decreased expression of CD163 and CD200R, and inhibition of IL-10 secretion.

Conclusions: Despite intensive investigations of nanomaterials for biomedical applications, there are still important unresolved questions regarding their toxicity that need to be addressed. In our study, we found that nanoparticles greatly inhibit macrophage polarization towards the M2 phenotype. As part of the innate immune system, macrophages strongly control inflammatory processes. Disregulation of macrophage polarization might lead to dysregulated inflammation, however, controlled manipulation of M1 and M2 macrophage polarization could also be exploited to shape immune responses.

No conflict of interest.

Immunoconjugates as novel anti-cancer therapeutics

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Background: Antibodies and related products are among the fastest-growing therapeutic agents for a broad range of indications including cancer treatment. Next-generation antibody therapeutics such as bispecific antibodies offer opportunities for differentiated mechanisms of action and allow for potentially increased efficacy and reduced side effects.

Materials and Methods: An immunoglobulin light chain-bridged single chain bispecific antibody platform has been developed at the Development Center for Biotechnology, Taiwan. Using this platform, with either lambda (Cλ) or kappa constant (Cκ) as the bridging domain and with different combination of the target domains, we have successfully constructed several single chain bispecific antibodies. Here, a Cκ-bridged HER2/CD3-bispecific antibody designated LbscbHER2-CD3 was characterized for its in vitro and in vivo properties and functions.

Results: Immunoglobulin light chain-bridged single chain bispecific antibodies could be expressed with high yield in mammalian expression systems. Noteworthy, the bridging domain has made the purification process more efficient by lambda- or kappa-capturing affinity chromatography. Binding affinity assays revealed that the LbscbHER2-CD3 is able to independently and simultaneously bind to HER2+ tumor cells and CD3+ T cells at low nanomolar concentrations. In co-culture experiments, the addition of LbscbHER2-CD3 resulted in a significant "target cell-dependent" increase in cytokine secretion of human peripheral blood mononuclear cells (PBMCs), indicating the activation of T cell subset. Also, the co-engagement of either IL-2 primed or un-primed human T cells and HER2+ tumor cells by LbscbHER2-CD3 facilitated the killing of tumor cells from breast, colorectal and pancreatic cancers. When administered in vivo, the LbscbHER2-CD3 showed strong tumor growth suppression in SCID

xorangefoot mouse models with implanted HER2+ SW480 and HT29 human colorectal cancer cells as well as Capan-1 human pancreatic cancer cells. Conclusions: The LbscbHER2-CD3 bispecific antibody has strong affinity for both the cancer cell-associated marker and the T cell antigen which leads to T cell engagement and cancer cell elimination as shown in vitro and in vivo. These results suggest that LbscbHER2-CD3 may have therapeutic potential for drug development in the treatment of human cancers.

No conflict of interest.

Whole-Cell SELEX method modification for generation of nucleotide-modified RNA-aptamers to the cell surface: Application in Burkitt lymphoma versus non-malignant lymphoblastoid cells model

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Background: The SELEX technology for the generation of aptamers of the interest from the random sequences of combinatorial libraries was first described in 1990 by Tuerk & Gold and Ellington & Szostak. Applications of RNA-aptamers as therapeutics and diagnostics became possible since the nucleic acids that protect from nuclease cleavage have been introduced. We have elaborated the Whole-Cell SELEX method modification (Cell-SELEX-FA) for generation of nucleotide-modified RNA-aptamers to the cell-surface molecules with the aim to isolate nmRNA-aptamers that can distinguish between the Burkitt lymphoma (BL) and non-malignant lymphoblastoid (LCL) B-cells, and to investigate these nmRNA-aptamers for their ability to block BL cell proliferation.

The experimental model: The whole formaldehyde-fixed cells of the high-malignant EBV-positive BL cell line RAJI (Positive Selection) and the non-malignant LCL B-cell line, which was established by EBV infection of normal B-cells (Negative Selection). Two selection protocols were applied: with yeast RNA as a competitor and with the IT-Block protein competitor.

Results: Using elaborated Cell-SELEX-FA, we have collected two nmRNA-aptamers pools that were selected to the cell surface molecules of BL Raji and LCL cells. These nmRNA-aptamers were 5'-labeled with FITC and their specificity was shown by fluorescent microscopy. Two individual nmRNA-aptamers (Apt4 and Apt5) were examined for the cell-proliferation growth-blocking effect by MT assay, using 4 BL, 2 T-cell lymphoma, 1 LCL cell lines, and healthy donor lymphocytes. The MT test revealed inhibition of cells proliferation at 72 hours after the aptamer input, by Apt4 (4.0 μM): 16.2% for BL Raji and 28.6% for MUTI I, but 48.3% for LCL, 51.7% for BL Jijoye P79, and 63.1% for BL41/95; by Apt5 (4.0 μM): 42.3% for BL Raji, 65% for BL Jijoye P79, and 90% for BL41/95, but 26.6% for LCL and 28.1% for MUTI II. Both aptamers were inactive for the T-cell lymphoma cell lines (Jurkat and MT-2) and for the lymphocytes of healthy donor.

Conclusions: We have elaborated the Whole-Cell SELEX method modification (Cell-SELEX-FA) and, using it, we have selected two generated individual nucleotide-modified RNA-aptamers that reveal Burkitt lymphoma cells-proliferation-blocking property. These nmRNA-aptamers should be further studied on the panel of B-cell lymphoma cell lines, and, as the potential therapeutics, should be tested in vivo by using experimental animal model.

No conflict of interest.

Design and characterization of a high affinity and specificity of bicyclic peptide binder to MT1-MMP for development of a treatment for solid tumours

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Background: A proprietary phage display and cyclic peptide technology (Bicycle® technology) was utilized to identify high-affinity binding peptides to membrane type 1-matrix metalloproteinase (MT1-MMP/P1MMP14), MT1-MMP (MT1) is a cell surface membrane protease normally involved in tissue remodelling which is over-expressed in many solid tumors. Overexpression of MT1 has been linked to cancer invasiveness and poor prognosis. While attempts to target the proteolytic activity of MT1 and other MMPs in cancer were unsuccessful in clinical trials largely due to toxicity caused by insufficient selectivity, MT1-MMP remains an attractive cancer target for targeted cytotoxic delivery approaches.
Materials and Methods: Diverse phage libraries containing 10^11 to 10^13 unique peptide sequences, post-translationally cyclized with thiol-reactive scaffolds were used to identify small (1.5–2 kDa) constrained bicyclic peptide binders (Bicycles®) to the hemopexin domain of MT1. Affinity was measured by fluorescence polarization assays. Initial binders were subjected to affinity maturation and stabilization by chemical optimization. Internalization of fluorescent Bicycles® conjugates into MT1 expressing cells was evaluated by confocal microscopy.

Results: A bicyclic constrained peptide binder (Bicycle®) was identified that binds to the hemopexin domain of MT1 with an apparent Kd of approximately 2nM. The Bicycle® peptide (N241) binds with similar affinity to the entire ectodomain of the protease but shows no binding to the catalytic domain. N241 shows good selectivity against closely related MMP family members (MMP15, MMP16, MMP24, MMP1, MMP2). Characterization of the pharmacological effect of N241 on MT1 in vivo shows that the peptide has no direct impact on the catalytic activity of MT1 or related proteases, nor on cell migration or invasion of MT1 expressing cells. However, binding of fluorescently-tagged N241 to MT1 on HT1080 fibrosarcoma cells results in the rapid internalization and subsequent lysosomal localization of the compound. In addition, the Bicycle® binder demonstrates rapid tumor localization when injected IV into mice bearing MT1-positive tumor xenografts, as shown by PET imaging, achieving levels as high as 15–20% injected dose per gram of tumor in less than 60 minutes. These properties suggest that N241 may be a good delivery vehicle for cytotoxic payloads targeting MT1-positive tumor cells. Bicycle drug conjugates (BDCs) with a variety of linkers and cytotoxic payloads were prepared which retained binding affinity to MT1. The anti-tumor activity of select BDCs was demonstrated in MT1-positive human tumor cell lines exposed to N241 in vitro.

Conclusion: A specific peptide binder to MT1 hemopexin domain was identified which can bind to MT1-positive tumor cells and deliver cytotoxic payloads resulting in tumor regression in mouse models.

No conflict of interest.
least in part by reducing NCOA3 expression, the critical ER coactivator in endocrine resistant breast cancer cells. No conflict of interest.

202 Poster (Board P028)
Mitochondria-targeted doxorubicin: A new therapeutic strategy against drug-resistant osteosarcoma
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Background: Doxorubicin (Dox) is one of the leader drugs for osteosarcoma standard chemotherapy. 40−45% of osteosarcoma patients are unresponsive to Dox, due to the overexpression of the drug efflux transporter ABCB1/P-glycoprotein (Pgp). Mitochondria metabolism is critical for the survival of drug resistant cells.

Methods and Methods: We used a chemically modified Dox containing the anthracylone scaffold conjugated with a mitochondria-targeted peptide (mtDox), against human Dox-sensitive U2OS and Saos-2 osteosarcoma cells and the corresponding variants (DX30, DX100, DX580) with progressively higher Dox-resistance and Dox-resistant osteosarcoma.

Results: Differently from Dox, which had a nuclear accumulation, mtDox was selectively delivered into mitochondria where its retention increased progressively with the increase of resistance. While Dox was not cytotoxic in resistant variants, mtDox still induced either cell necrosis or immunogenic death. Interestingly, Dox resistance increase was paralleled by the up-regulation of 111 genes controlling mitobiogenensis and mitochondria energy metabolism, mtDox, but not Dox, down-regulated more than 2-fold 59 of these genes, decreased mitobiogenesis, mitochondrial transport of proteins and metabolites, mitochondrial energy metabolic pathways and ATP synthesis, while it increased ROS, mitochondrial depolarization and caspase 9/3 activation in resistant cells. mtDox, but not Dox, reduced the growth of Dox-resistant/Pgp overexpressing osteosarcomas implanted in syngenic BALB/c mice, by killing tumor cells, increasing intratumor apoptosis and raising a proper anti-tumor response by the host immune system. Differently from Dox, mtDox did not display signs of systemic toxicity and was not toxic for not-transformed osteoblasts. Furthermore, the indexes of cardiotoxicity did not differ between untreated animals and mtDox-treated animals.

Conclusions: We propose a new and effective chemotherapeutic strategy for Dox-resistant osteosarcomas, by using a mitochondria-targeted Dox that exploits the metabolic signature typical of resistant cells – i.e. the hyperactive mitochondrial functions – and hits the energy pathways crucial for Dox-resistant osteosarcoma. mtDox was effective also in Pgp-overexpressing tumors and was not cardiotoxic, overcoming the main limitation of Dox-resistant osteosarcoma therapy. Our work may pave the way to the potential use of mtDox in clinical settings, in particular for patients with Pgp-positive osteosarcomas or as a possible second-line treatment for relapsed patients.

No conflict of interest.

203 Poster (Board P029)
C/EBP-β-LIP turnover decides cancer chemotherapy outcome
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Background: Chemotherapy often induces endoplasmic reticulum (ER) stress. ER stress activates molecular networks which first promote cell survival; if this attempt fails, cell death is induced. CAAT/enhancer binding protein-β (C/EBP-β) plays a key role in determining cell fate under ER stress. It has two isoforms: the pro-survival C/EBP-βL and the pro-apoptotic C/EBP-βI. LAP/LIP ratio is correlated with chemotherpay response because it regulates the expression of multidrug resistant protein (Pgp) (Pgp), which causes chemoresistance. The regulation of LAP/LIP turnover in cancer cells and its role in chemotherpay response is not known.

Material and Methods: We compared basal, chemotherpay- and ER-stress-induced LAP/LIP ratio in ER stress-sensitive/chemosensitive and ER stress-resistant/chemoresistant cancer cells. We investigated the mechanism of LAP/LIP turnover, which was dictated by the rate of the reciprocal degradation, and the impact of altering LAP/LIP degradation on chemotherpay response.

Results: Differently from ER stress-sensitive/chemosensitive cells, ER stress-resistant/chemoresistant cells had high LAP/LIP ratio and did not express LIP in response to ER stress or chemotherapy. This was due to the constitutive LIP ubiquitination followed by lysosomal/proteosomal degradation in resistant cells. The inducible intratumor over-expression of LIP, as well as the prevention of LIP degradation by lysosomes and proteasome inhibitors, improved chemotherpay outcome, by reducing Pgp levels and restoring chemotherpay- and ER stress-mediated cell death. Constitutive LIP degradation was detected in primary solid and hematological tumors unresponsive to chemotherpay.

Conclusions: Our work demonstrates that the constitutive ubiquitination and degradation of LIP induces resistance to ER stress- and chemotherpay-induced cell death. LIP ubiquitination level may be considereed a predictive biomarker of chemotherpay response. Lowering LAP/LIP ratio by preventing LIP ubiquitination and degradation represents a new approach to treat chemoresistant tumors.

No conflict of interest.

205 Poster (Board P031)
Pharmacokinetics and metabolite identification study of flavonoid dimer FD18: A potent P-glycoprotein moderator in reversing cancer drug resistance
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Background: Overexpression of P-glycoprotein (P-gp) is one of the leading causes of multidrug resistance in chemotherapy (PD150) of around 140 nM in reversing paclitaxel (PTX) resistance. FD18 can also reverse P-gp-mediated PTX resistance in human breast cancer xenograft model in vivo. Here, we report the PK profile and metabolite identification of FD18, and subsequently in vitro and in vivo P-gp modulating evaluation of FD18 metabolites.

Methods: PK study of FD18 was conducted in SD rat. Metabolism of FD18 was evaluated in rat and human liver microsome assay in vitro and SD rat in vivo. Metabolite identification of FD18 was done by UPLC-MSMS QTOF and authenticated using pure, synthetic compounds. P-gp modulating activities of the metabolites were evaluated with various anticancer drugs on LCCSMADR cells in vitro and subsequently in breast cancer xenograft model in vivo.

Results: IV administration of FD18 resulted in a first order kinetics and a non-linear plasma PK profile. IP administration of 45 mg/kg FD18 resulted in a mean residence time (MRT) of approximately 600 minutes. Three major metabolites of FD18 (M1, M2 and M3) were identified in vitro and in vivo. Metabolites identities were authenticated using pure, synthetic compounds. The P-gp modulating activity of M1, M2 and M3 (in reversing PTX resistance) was determined to be 305±35 nM, 70±26 nM and no activity, respectively. Surprisingly, M2 is also a potent P-gp modulator towards vincristine, vinorelbine, doxorubicin, mitoxantrone and daunorubicin with EC50 of 53±13 nM, 64±17 nM and 0±1.7 nM respectively. Hydrochloride salt of synthetic M2 demonstrated in vivo efficacy in reversing PTX resistance in breast cancer xenograft model. P-gp-overexpressing xenograft was treated with 72 injections of M2 (28 mg/kg, IP) and PTX (12 mg/kg, IV) every other day (q1d×12). On day 30, tumor size of animals treated with M2 and PTX was 773±114 mm3 (n=8) while animals in the solvent control group was 1759±455 mm3 (n=6). Tumor size of animals treated with PTX (12 mg/kg, IV) alone was 1208±66 mm3 (n=8).

Conclusion: Flavonoid dimer is a new class of safe and potent P-gp modulators. The proposed metabolism pathway of FD18 is via N-dealkylation and oxidative demamination. M2 is a metabolite of FD18 with potent in vitro and in vivo P-gp modulating activity.

Conflict of interest: Other Substantive Relationships: Part of the project involves a technology licensed to Athenex Ltd.

206 Poster (Board P032)
Targeting ErbB3 activation in drug-resistant ovarian carcinoma cells over-expressing the receptor tyrosine kinase Axl
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Ovarian carcinoma is the most common gynecological cancer and a major cause of cancer-related death in women. The high lethality of this cancer is mainly due to late diagnosis and treatment failure. In fact, the efficacy of
the platinum drug-based therapy of ovarian carcinoma is often limited by the occurrence of drug resistance. Because the Receptor Tyrosine kinase (RTK) Axl can be deregulated in ovarian carcinoma and can play a role in maintaining tumor cell survival, the aim of this study was to examine the gene expression of ovarian carcinoma cells over-expressing Axl undergoing molecular targeting of Axl.

Preclinical pharmacology approaches were employed including growth inhibition assays, western blot analyses, antibody arrays, gene knockdown by siRNA transfection, quantitative Real-time PCR, invasion and migration assays. The drug interaction was analysed using the Chou and Talalay method.

We examined the effect of molecular targeting of Axl in different ovarian carcinoma cell lines including the cisplatin-resistant variant IGROV-1/Pt1, which over-expressed Axl and exhibited enhanced invasive potential as compared to parental cells. When the IGROV-1/Pt1 cells were transfected with Axl-tailored siRNAs, a marked and persistent reduction of mRNA/protein levels was obtained. Axl-silenced cells displayed reduced growth and invasive/migratory capabilities compared to control siRNA-transfected cells, in the absence of changes in cisplatin sensitivity. Axl silencing resulted in increased activation of ErbB3 in IGROV-1/Pt1 cells. Such cells displayed reduced sensitivity to AZD8931, a small molecule which inhibits ErbB3, besides EGF receptor and ErbB2, as compared to the parental IGROV-1 cell line. In keeping with this behavior, increased phosphorylation of ErbB3 at Tyr1289 was detected in IGROV-1/Pt1 cells. When exploring the possible advantage of the combination of cisplatin and AZD8931 in IGROV-1/Pt1 cells, a favourable drug interaction was observed in in vitro assays with AZD8931 before exposure to cisplatin. The analysis of the synergistic interaction after Axl silencing showed a reduced efficacy of the drug combination.

Our findings indicate that compensatory survival pathways involving ErbB3 can be upregulated upon Axl silencing in ovarian carcinoma cells. The activation of this RTK, associated with drug resistance of ovarian carcinoma cells, can be counteracted by treatment with a specific inhibitor which displays a synergistic effect in combination with cisplatin. The evidence of increased activation of ErbB3 in platinum-resistant cell endowed with reduced sensitivity to other target-specific agents suggests the need to simultaneously target multiple survival factors to overcome drug resistance.

No conflict of interest.

207 Poster (Board P033)
Enzastaurin inhibits ABCB1-mediated drug efflux independently of effects on protein kinase C signalling and the cellular p53 status
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Background: Enzastaurin is a PKCδ inhibitor that has been tested in clinical trials. Here, enzastaurin was tested in neuroblastoma and rhabdomyosarcoma cell lines.

Material and Methods: Cell viability in response to enzastaurin was tested in the cell lines, their vincristine-resistant sub-lines, primary neuroblastoma cells, ABCB1-transduced, ABCC2-transduced, and p53-depleted cells. Pathway activation was determined through detection of protein phosphorylation. ABC transporter function was studied using cytotoxic and fluorescent substrates, specific inhibitors, determination of ABC transporter ATPase activity. The interaction of enzastaurin and ABCB1 was studied by in silico docking studies.

Results: Enzastaurin IC50s ranged from 3.3 to 9.5 μM in cell lines and primary cells independently of the ABCB1, ABCG2, or p53 status. Enzastaurin 0.3125 μM interfered with ABCB1-mediated drug transport. PKCα and PKCγ may phosphorylate and activate ABCB1 under the control of p53. However, enzastaurin exerted similar effects on ABCB1 in the presence or absence of functional p53. Also, enzastaurin inhibited PKC signalling only in concentrations ≥1.25 μM. The investigated cell lines did not express PKCγ. PKCγ depletion reduced PKC signalling but did not affect ABCB1 activity. Intracellular levels of the fluorescent ABCB1 substrate rhodamine 123 rapidly decreased after wash-out of extracellular enzastaurin, and enzastaurin induced ABCB1 ATPase activity resembling the ABCB1 substrate verapamil. Computational docking experiments did not directly interact of enzastaurin and ABCB1. These data suggest that enzastaurin directly interferes with ABCB1 function. Enzastaurin further inhibited ABCC2-mediated drug transport but by a different mechanism since it reduced ABCC2, but not ABCB1, ATPase activity.

Conclusions: The interaction of enzastaurin with ABCB1 transporters needs to be considered for the further development of therapies combining enzastaurin with ABC transporter substrates.

No conflict of interest.
210 Poster (Board P036)

SLUG transcription factor promotes cell proliferation and predicts outcome of patients with gastrointestinal stromal tumor

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Background: Approximately 85% of gastrointestinal stromal tumors (GISTs) harbor a kinase activating mutations of KIT or platelet-derived growth factor receptor alpha (PDGFRα) receptors. SLUG is a transcription factor that is linked with several cancers, drug resistance and the KIT signaling pathway. Therefore, we investigated whether it has a role in GIST tumorigenesis and patient outcome prediction.

Material and Methods: SLUG expression was investigated in two clinical GIST sample series consisting of samples from patients treated with surgery (n = 187) and patients treated with surgery and adjuvant imatinib (n = 313). In addition, the effects of SLUG on cell proliferation and imatinib sensitivity was investigated in two GIST cell lines.

Results: SLUG was expressed in 25% out of the 500 clinical GIST samples. Its expression was associated with poor GIST-specific and overall survival in imatinibe-naïve patient series (HR = 3.30, 95% CI = 1.85–5.89, P < 0.001; and HR = 1.88, 95% CI = 1.20–2.93, P = 0.006, respectively), and with poor recurrence-free survival among patients treated with adjuvant imatinib (HR = 1.83, 95% CI = 1.29–2.60, P = 0.001). Inhibition of SLUG by using siRNA decreased GIST cell proliferation and sensitized one of the 2 GIST cell lines investigated for imatinib-induced apoptosis.

Conclusion: The data indicate that SLUG expression in GIST is associated with poor outcome when patients are treated with surgery alone or with surgery and adjuvant imatinib. SLUG may enforce pro-survival signaling in some GISTs.

No conflict of interest.

212 Poster (Board P038)

Impact of intratumoral heterogeneity of renal cancer on drug response and development of resistance in patient derived xenografts

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Background: Patients with advanced renal cell carcinoma (RCC) have a poor prognosis due to fast onset of resistance towards Standard of Care (SoC) drugs. Recent work has shown pronounced intratumoral heterogeneity in RCC as a potential cause for treatment resistance. We developed a large panel of patient derived xenografts (PDX) from RCC, including a subset of these PDX established by transplanting tumor material from several different regions within individual renal tumors. Chemosensitivity of these PDX models was evaluated to better understand correlations between inter- and intratumoral heterogeneity and SoC treatment response.

Methods: Specimens from primary and metastatic RCCs were collected from consenting patients and transplanted to immunodeficient mice within 24 h. Initial tumor engraftment was monitored for up to 4 months. Successfully engrafted patient-derived tumors were subsequently passaged. Tumor sections were examined histopathologically to assess correlation between different tumor regions and PDX model. Stable-growing xenografts were tested for sensitivity towards SoC drugs, namely Sunitinib, Sorafenib, Bevacizumab and Everolimus. PDX models were analyzed for global gene expression using Affymetrix microarrays as well as for sequence variations using the Illumina TruSeq Amplicon Cancer Panel.

Results: More than 200 samples from primary and metastatic renal cancers were transplanted resulting in 34 newly established PDX models. Among these, several models were derived from distinct regions of individual tumors. Altogether, 13 PDX models were obtained from heterogeneous tumor regions of three patients with advanced disease. This characterization all PDX models regarding their sensitivity towards SoC treatment. Those established from distinct regions of individual tumors were further examined. Here, we were able to identify one out of eight regions from one particularly aggressive RCC that clearly differentiates from the other regions of the same tumor with regard to SoC treatment response. Genomic analysis further revealed that this region is different from the other regions in its global gene expression and sequence variation pattern. In addition to a common MET mutation, this region exhibits a variation in the HRAS oncogene. In summary, we found 34 sequence variations in 20 genes, including ATM, MET, TP53 and VHL.

Conclusions: Distinct regions within one individual tumor exhibit differences in SoC treatment response as well as genetic profile. These differences and their correlation to their molecular heterogeneity is subject of ongoing investigations to explain and treat resistance. Altogether, we have made available a large panel of RCC PDX for translational research and for preclinical testing of novel drug candidates.

No conflict of interest.

213 Poster (Board P039)

Targeting the HER family with Pan-HER effectively overcomes resistance to cetuximab

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Cetuximab, an antibody against the Epidermal Growth Factor Receptor (EGFR) has shown efficacy in treating head and neck squamous cell carcinoma (HNSCC), metastatic colorectal cancer and non-small cell lung cancer (NSCLC). Despite the clinical success of cetuximab, many patients do not respond to cetuximab. Furthermore, virtually all patients who do initially respond become refractory, highlighting both intrinsic and acquired resistance to cetuximab as significant clinical problems. To understand mechanistically how cancerous cells acquire resistance, we previously developed models of acquired resistance using the H226 NSCLC and UM-SCC1 HNSCC cell lines. Cetuximab-resistant clones showed a robust upregulation and dependency on the HER family receptors EGFR, HER2 and HER3. Here, we examined Pan-HER, a mixture of six antibodies targeting these receptors on cetuximab-resistant clones. In cells exhibiting acquired or intrinsic resistance to cetuximab, Pan-HER treatment led to degradation of all three receptors and down-regulation of the AKT and MAPK signaling pathways. This resulted in inhibition of cell proliferation. To determine whether Pan-HER had a therapeutic benefit in vivo, we established de novo cetuximab-resistant mouse xenografts and treated resistant tumors with Pan-HER. This regimen resulted in a superior growth delay of cetuximab-resistant xenografts compared to mice continued on cetuximab. Furthermore, intrinsically resistant HNSCC patient-derived xenografts were treated with Pan-HER which exhibited significant growth delay compared to vehicle/cetuximab controls. These results suggest that targeting HER family receptors simultaneously with Pan-HER is a promising treatment strategy for tumors displaying intrinsic or acquired resistance to cetuximab.

Conflict of interest: Corporate-sponsored Research: J. Lantto, I.D. Horak, and M. Kragh are employed by Symphogen A/S. D.L. Wheeler holds a laboratory research agreement with Symphogen A/S.

214 Poster (Board P040)

Identifying kinases and phosphatases regulating STAT3 with potential dual anti-cancer effects

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STAT3 is a critical immuno-oncology regulator acting both as an oncogene promoting tumor progression and drug resistance, and regulating immunomodulatory processes in the tumor as well as stromal cells. Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer that is characterized by a strong immunosuppressive and fibrotic microenvironment. A striking genetic feature of PDAC is the early emergence of KRAS mutations. Interestingly, STAT3 promotes the mutant KRAS-driven tumor progression by increasing myofibroblast fibers, and tissue tension. Simultaneously, STAT3 signaling in myeloid cells promotes development of immunosuppressive tumor microenvironment. Therefore, targeting STAT3 signaling in PDAC is a promising approach to both make the cancer susceptible to chemotherapy and immunotherapy.
STAT3 activity is regulated through protein phosphorylation. In PDAC cells, STAT3 phosphorylation appears to be independent of the JAK-family kinases. To better understand how STAT3 is regulated in PDAC and how it may be targeted, we set out to identify protein kinases and phosphatases that regulate STAT3 activity. Using C. elegans expressing ST3(3x3k) or hyperactivated STAT3(Y640F) and a STAT3-driven transcriptional luciferase reporter we screened them against a protein kinase and phosphatase siRNA library. After primary and validation siRNA screens we found 8 genes whose silencing inhibited the activity of STAT3 and one that activated STAT3 mutants. Several of the hits were previously known to be activated (i.e. CSNK2A1) or impaired (CDK8, CSK) in different cancers. Surprisingly, no major differences were found between regulation of wild type and the STAT3 mutant other than the effect of CSK knockdown. No JAK-family kinases were found as validating hits, but other previously described regulators such as casein kinase 2 (CSNK2A1) were. Together these data suggest that inhibition of the hits may provide new strategies for repression of tumorigenesis and reverting STAT3 driven drug resistance in PDAC.

No conflict of interest.

215 Poster (Board P041)
SETBP1-1/SET/PP2A/p-ERK cascade is involved in type II interleukin 1 receptor associated regorafenib resistance that is overcome by MEK inhibitors in human colorectal cancer cells
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We have previously shown that enhanced expression of type II interleukin 1 receptor (IL1R2), an IL-1 decoy receptor, was closely associated with regorafenib resistance in human colorectal cancer (CRC) cells. Regorafenib, a newly approved multi-kinase inhibitor by US FDA, has been demonstrated to have overall survival benefits in CRC patients at the late-stage cancer. We also found that IL1R2 expression was associated with poor prognosis and 5-year survival of CRC patients. In this study, we designed experiments to elucidate the role of IL1R2 in regorafenib resistance and to investigate the treatment regimen to improve the therapeutic outcome. We first adopted shRNA to silence the expression of IL1R2 and ectopic expression to enhance the IL1R2 levels in various CRC cells. Our results revealed that the protein levels of IL1R2 were closely associated with regorafenib resistance. Similar findings were observed in regorafenib-resistant DLD-1 cells (DLD-1-R) that were established by long-term culturing DLD-1 cells in the presence of regorafenib. We have previously found that IL1R2 may function together with c-Fos to activate several AP-1 downstream genes. In the present study, we further demonstrated that IL1R2 mediated through AP-1 element to enhance the expression of SETBP1, which complexes with SET and PP2A and hence inhibited PP2A activity. Since PP2A was a negative regulator of ERK, we observed that p-ERK was significantly enhanced in CRC cells which IL1R2 was overexpressed and PP2A activity was reduced. We further found that pretreatment of H22, IL1R2-overexpressing HCT116, and DLD-1-R cells with MEK/ERK inhibitors significantly overcame their regorafenib resistance in vitro and in vivo systems. Accordingly, the present study reveals the involvement of IL1R2 in regorafenib resistance and the use of MEK/ERK inhibitor to overcome regorafenib resistance. These findings may be helpful to improve the therapeutic outcome of CRC patients.

No conflict of interest.

216 Poster (Board P042)
Role of non-coding RNAs in resistance to targeted therapies in cutaneous melanoma
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Background: Activating BRAF mutations are effectively targeted by specific inhibitors, such as vemurafenib, which have shown important clinical responses in advanced cutaneous melanoma (CM). However, their clinical effectiveness is impaired by the emergence of an early drug resistance. Non coding RNAs (ncRNAs) are of increasing biologic and therapeutic relevance considering their role in modulating gene expression. Among these transcripts lacking coding potential, long ncRNAs (IncRNAs) are emerging as important causal factors to CM initiation and progression. Furthermore, recent evidence indicates that IncRNAs may be involved in the induction of drug resistance in other tumors. Accordingly, elucidating the role that IncRNAs may play in BRAF inhibitors (BRAFi) resistance would be helpful for understanding the pathogenic mechanism as well as for developing new therapeutic strategies in CM.

Material and Methods: Sequential adaptation to increasing concentrations of the BRAFi vemurafenib was used to raise resistant (VR) isogenic cell cultures from BRAF V600-mutant CM cell lines established in our institution. RNA sequencing identified IncRNAs modulated following acquisition of drug resistance. The paired-end reads were aligned on reference track GRCh37, obtained from ENSEMBL, using the bioinformatics tool STAR (version 2.5.0a) with the standard parameters, while the Bioconductor package DESeq2 was used to normalize the data and then to perform the differential expression analysis. Gene ontology analysis was performed on IncRNAs using Co-LinkRNA tool. The expression of selected genes was evaluated by quantitative RT-PCR analysis.

Results: RNA sequencing identified about 230 mapped IncRNAs significantly differentially expressed between VR-resistant and -sensitive CM cell lines, thus indicating a difference in the IncRNA expression profiles. Gene ontology analysis revealed that the top of the neighbor coding gene function of differentially expressed IncRNAs involved apoptosis and cellular component movement, including the TGF-β pathway genes. Consistent with this data, VR-resistant cells appear more aggressive, with a phenotype that is reminiscent of a de-differentiation and activation of an epithelial mesenchymal transition-like program.

Conclusions: Though additional studies are required, our findings suggest that IncRNAs may be involved in BRAFi resistance by regulating the anti-apoptotic and tumor-progressing aspects of TGF-β signaling in CM.

No conflict of interest.

217 Poster (Board P043)
Synergy in reversing platinum resistance by combined inhibition of EZH2 and EMT1/2
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Background: EZH2 and EMT1/2 catalyse the maintenance of repressive H3K27me3 and H3K9me3 histone marks respectively. HKMTI-1–005 is a dual EZH2/EMT2 inhibitor.

Materials and Methods: Cell viability was measured using the MTT assay following treatment of ovarian cancer A2780/cp70, PEO4 and PEO23 cell lines with HKMTI-1-005 (dual EZH2 and EMT1/2 inhibitor), GSK343 (EZH2 inhibitor), UNC0638 (EMT1/2 inhibitor) or decitabine (DNA demethylating agent). Drug synergy was analysed by isobologram analysis. Gene expression was analysed by qRT-PCR and Western, histone marks by Chip PCR and DNA methylation by pyrosequencing. The RIKIP gene was knocked out in PEO4 and ectopically expressed in the PEO4, using the CRISPR System.

Results: Combined treatment using the DNA demethylating agent Decitabine with the dual EZH2/EMT1/2 inhibitor HKMTI-1–005 results in synergistic sensitisation of cisplatin-resistant ovarian tumour cells, compared to either compound alone or combined treatment of Decitabine with selective EZH2 or EMT2 inhibitor. Genes upregulated by the combination are not necessarily DNA methylated at their promoter, although do show changes in repressive H3K27Me3 and H3K9Me3 marks. One such example is the RIKIP gene, whose expression is associated with progression-free survival in ovarian cancer patients. Activation or knockdown of RIKIP gene expression in ovarian cancer showed, a direct correlation between RIKIP gene expression and cisplatin resistance.

Conclusions: Combined DNA demethylation and inhibition of EZH2 and EMT1/2 that maintain repressive histone marks can synergistically overcome cisplatin resistance, with RIKIP expression being a potential stratification and pharmacodynamic biomarker for future clinical development of this combination.

No conflict of interest.

218 Poster (Board P044)
Next-generation sequencing results of brain metastasis in NSCLC patients with acquired EGFR-TKI resistance other than T790M mutation
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Background: Mechanisms of acquired resistance for EGFR tyrosine kinase inhibitor (TKI) in non-small cell lung cancer (NSCLC) has been studied broadly, and T790M mutation, bypass singaling, and phenotype change have been suggested. However, questions remain in terms of the
selection of sub-clones according to the sites during EGFR TKI therapy, especially in brain due to blood-brain barrier.

Four metastatic NSCLC patients harboring sensitive EGFR mutation were treated with EGFT TKI with good response but became refractory and symptomatic brain oligo-metastasis occurred thus metastectomy and next-generation sequencing were done.

Methods: Fresh brain metastasis tissue was obtained during surgery and frozen in liquid nitrogen. Amplicon sequencing (Thunderbolt) was performed. Library preparation and quality control sequencing amplicon libraries were prepared using ThunderBolts Cancer Panel, according to the manufacturer's instructions. The ThunderBolts Cancer Panel uses 23A microarray-based bioreductive assays and the IncuCyte ZOOM Protein Expression and phosphorylation were assessed using Western blot analysis. Transcript expression was quantified using Canine 2.0 microarray analysis and RT-qPCR.

Results: Whole exome analysis of 13 samples identified 29 genes shown to be drivers or suppressors in human cancer. The most common deleterious mutation was an activating V to E mutation in BRAF identified in 7 samples. Analysis of 33 additional canine bladder samples identified BRAF V to E in 67% of samples. BRAF mutant canine lines tested for sensitivity to Vemurafenib had IC50 values >10μM while the IC50 for sensitive A375 human melanoma cells was 94 nM. A 2125 bp BRAF transcript was amplified from Bliley canine TCC cells and sequenced, confirming heterozygous expression of the V548 to E mutant form of BRAF. The predicted protein (AA 10–715 of XP_013975364.1) exhibited 99% homology to human BRAF AA53–763 (NP_0043242.4). BRAF protein expression was confirmed by Western blot in canine cells. Vemurafenib (16μM and 24 hrs) reduced Erk1/2 phosphorylation in the BRAF mutant canine cells, but completely blocked phosphorylation in A375 cells. The MEK1/2 inhibitor, Selumetinib, also reduced but did not eliminate ERK1/2 phosphorylation in the BRAF mutant canine cell lines. Transcript expression of downstream target ETV1 was reduced by Vemurafenib in A375 cells, but not the canine cells which expressed high levels of ETV1. Copy number variation (CNV) of ETV1 was assessed and increases were observed in both samples. CNV was also assessed for receptor tyrosine kinase and significant amplification was observed in: MET (6 samples), EGFR (2 samples), FGFR2 (5 samples), and ALK1 (10 samples). In canine TCC cells relative to the Raf inhibitor, Sorafenib, correlated with sensitivity to Vemurafenib while Crozotinib sensitivity correlated to MET/ALK expression.

Conclusions: These data indicate that although constitutively active BRAF is expressed in canine TCC, other factors may contribute to pathogenesis and resistance to Vemurafenib.

No conflict of interest.

223 Poster (Board P049)
Different degree of epithelial–mesenchymal transition phenotype in docetaxel and cabazitaxel castration-resistant prostate cancer cells
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Background: The transition (EMT) process has been described to play a role in resistance to D therapy in metastatic CRPC. In this study we investigated the differences between C and D resistance related to the EMT phenotype acquisition.

Material and Methods: Sensitivity to D and C was analyzed in both D- and C-resistant derivatives from DU-145 and PC-3 cell lines by MTS. Gene expression of EM markers CDH1, VIM and ZEB1 were checked by qRT-PCR and protein levels by Western Blot (WB). Effect of D or C exposure on EM gene expression markers in a dose-response manner was analyzed by qRT-PCR. Cell migration was assessed using the CellFlex cell migration kit (Trevena). ZEB1 was inhibited by siRNA transfection to PC-3 and PC-3CZR and after C treatment effect was evaluated by MTS.

Results: DU-145 and PC-3 CRPC cell lines were converted to D-resistant cell lines (DU-145DR and PC-3DR, respectively) and C-resistant cell lines (DU-145CZR and PC-3CZR) in a previous work of our group. Gene expression and protein levels of D- and C-resistant cell lines were also resistant to C and D, respectively. Gene expression analysis showed that EM markers were differentially expressed in D-resistant cell lines with their parental cells. These differences were also detected, although less pronounced, in C-resistant cells. WB confirmed these results. Dose-response experiments exhibit that both D and C exposure increased similarly the expression of VIM and ZEB1 in PC-3 and PC-3CZR. EMT
phenotype in D-resistant cell lines was not modified with this short-term treatment. Moreover, the migration level of both D- and CZ-resistant cells was higher compared with parental cells, being the migration of CZ-resistant cells lower than in the D-resistant. In a previous work in our group, ZEB1 inhibition restored the sensitivity to D in D-resistant cells. Conversely, here we show that ZEB1 inhibition in PC-3ZR did not restore the sensitivity to CZ.

Conclusions: These data reveal a different grade of EMT phenotype in D vs. CZ CRPC cell lines, being more pronounced in D-resistant cells. The found differences could explain the activity of CZ in D-resistant cells. Further functional studies should be performed in order to confirm these results.

No conflict of interest.

224 Molecular determinants of resistance to CDK4/6 inhibition in ER+ breast cancer

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Approval of the first CDK4/6 inhibitor (palbociclib) marks an exciting breakthrough in treatment. Palbociclib in combination with endocrine therapy is the new standard of care for advanced ER+ breast cancer patients. Molecular predictors of response to CDK4/6 inhibition, however, have been sparse so far. While RB loss is a bona fide intrinsic resistance mechanism, other biomarkers of response have faltered in the clinic. For example, loss of p16 expression or CCND1 amplification predict sensitivity to palbociclib in vitro, but failed to do so in patients. In order to better treat patients with advanced ER+ breast cancer and to preserve the efficacy of CDK4/6 inhibitors, we must understand the landscape of resistance to these targeted agents.

To this end, we have generated cultured to resistance (CTR) models and conducted genome-wide open reading frame (ORF) screens. Whereas CDK4/6 inhibition downregulates phosphorylated RB and CCNE2 in sensitive ER+ breast cancer cells, these markers are maintained in CTR cells. CRISPR RB1 knockout breast cancer cells are also more resistant to palbociclib than abemaciclib. Since RB is the main gatekeeper of G1/S cell cycle progression, our results suggest that abemaciclib may have relevant off-target activity in ER+ breast cancer. Furthermore, it is unclear how estrogen may impact resistance to CDK4/6 inhibition or whether acquired resistance may differ between inhibitors. Thus, we have conducted novel genome-wide open reading frame (ORF) screens to uncover the molecular determinants of resistance to palbociclib and abemaciclib. ER+ breast cancer cells were infected with Broad Institute’s 17,255 ORF collection and dispensed for 10-12 generations. The selected single clones were then cultured in the presence of D and D-resistant. In a previous work in our group, ZEB1 inhibition in PC-3ZR did not restore the sensitivity to CZ. In D-resistant cell lines, ZEB1 inhibition restored the sensitivity to D in D-resistant cells.

Conclusions: These data reveal a different grade of EMT phenotype in D vs. CZ CRPC cell lines, being more pronounced in D-resistant cells. The found differences could explain the activity of CZ in D-resistant cells. Further functional studies should be performed in order to confirm these results.

No conflict of interest.

Drug Screening

225 Imatinib (IM) discontinuation in chronic myeloid leukemia (CML): A pharmacogenetic score for patients with durable complete molecular response (CMR)

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Background: After more than 15 years of IM in CML patients, the focus is now on IM discontinuation in patients (pts) with stable CMR. The pharmacokinetics of IM is influenced by many factors, such as single nucleotide polymorphisms (SNPs) in CYP3A4, CYP2C19, CYP2D6, CYP1A2. Genetic analysis between groups used two-tailed Student’s t test. Genotype frequencies were analysed by the y2-test for trend and the odds ratio was calculated using a dominant model. A score assigned +1 to variants that increased IM bioavailability and −1 to those that reduced it (Table). Discrimination of IM susceptibility with a receiver operating characteristic (ROC) curve analysis based on the score. Bonferroni correction was applied.

Results: We enrolled 59 consecutive pts. After 24 months 8 pts changed therapy because of treatment failure, 23 obtained a major MR (group 1), 18 a CMR. Among those 18, 15 had a stable CMR for more than 2 years and discontinued IM. 6 lost CMR and resumed therapy, 9 maintained CMR (group 2). Pts with an unstable CMR or that relapsed after IM suspension were pooled in group 2. The only significant difference is the higher female proportion in group 2 and 3 compared with group 1 (p < 0.005). Pooled genotypes provided a score of 3.2 ± 1.2 for group 3, 1.1 ± 1.4 for group 1 (p = 0.0014) and 0.5 ± 1.2 for group 2 (p = 0.0027). The ROC curve of the arbitrary score had an area under the curve of 0.9074 (0.7496 to 1.065) (P = 0.003). Using the cut-off value of 1.5, a stable CMR to IM could be predicted with a specificity of 89% and a sensitivity of 89%, with a positive likelihood ratio of 8.00.

Conclusions: As of today, there is not a predictive marker for IM CMR before starting therapy. Using multiple genes we reduced potential interaction and created a score with clinical implications, related to stable CMR, that could be applied to IM discontinuation.

No conflict of interest.

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proteomic profiling showed mechanistic differences among the MTH1 inhibitors, we investigated whether overexpression of human MTH1 rescues cells from MTH1 inhibitor-induced cell death. Contrary to our expectations, overexpression of MTH1 did not rescue cells from MTH1 inhibitor-induced cell death. Furthermore, siRNA-mediated knockdown of MTH1 did not suppress cancer cell proliferation.

**Conclusions:** Taken together, we conclude that the cytotoxicity of MTH1 inhibitors is attributable to off-target effects and that MTH1 is not essential for cancer cell survival.

**No conflict of interest.**

227 Poster (Board P053)

Development of high-throughput screening assay to identify inhibitors of diacylglycerol kinases (DGKs): Utilization of DGK assay panel for the selectivity profiling of inhibitors

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**Background:** Lipids play important roles in a wide variety of signal transduction pathways, and diacylglycerol kinases (DGK) are thought to be important molecules to maintain the balance between lipids (diacylglycerols) and phospholipids by phosphorylation. Therefore, DGKs are considered to be attractive targets in drug discovery research.

To date, ten mammalian DGK isozymes have been identified, but only few DGK inhibitors have been reported so far probably due to low-throughput and complex assay system using radiotracers. To identify potent and selective DGK inhibitors, we have developed a high-throughput assay system using 10 DGK isozymes using non-radioactive methods.

**Material and Methods:** Ten human DGK isozymes were cloned as fusion proteins with a tag consisting of a biotin acceptor peptide and DYDDDDDK-tag at the N-terminal, and were expressed in S21 insect cells. The extract cells were purified by DDDDK-tagged protein purification gel to obtain DGK proteins. The assay conditions for each DGK enzyme were established by modifying substrates and detergents to maximize enzyme activities. The detection of enzyme reaction was performed by measuring ADP production.

**Results:** We successfully developed a panel of 10 DGK isozymes assay, and screened commercially available DGK inhibitors. Interestingly, an EGFR kinase inhibitor was identified as pan-DGK inhibitor, which would be a useful tool for DGK inhibitor research.

In summary, the validation study demonstrated that our DGK assay panel would be a powerful tool for the drug discovery for novel selective DGK inhibitors.

**No conflict of interest.**

228 Poster (Board P054)

Validation of human, rat and mouse intestinal organoid models as preclinical screens to assess GI toxicity in novel oncology drug development

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Gastrointestinal (GI) toxicity is a common and severe dose limiting side effect of chemotherapy. Symptoms include diarrhoea, dehydration and ulceration leading to increased susceptibility to infection, due in part to the damage or loss of crypt and/or villi structures in the small intestine. As improvements in oncology therapeutics are pursued to acquire more efficacious agents, assessment of their potential GI toxicity is therefore crucial.

The mouse small intestinal in vitro organoid model was first described by Sato et al in 2009. We have further developed this model and have assessed its suitability as a screening tool within three species: mouse, rat and human, in order to predict test article induced GI toxicity or mucosal regeneration potential. The in vivo translatability of these models was also assessed.

The intestinal organoid culture conditions were designed to mimic the stem cell niche allowing cell differentiation and proliferation to occur, with each species requiring different conditions. All intestinal lineages were present in the organoids derived from each species and the epithelial hierarchy closely resembled that observed in vivo. The response of organoids to cytotoxic insult via treatment with common colorectal chemotherapy drugs correlated with their known responses in vivo. The organoid branches, which represent the crypts and crypt lineage of the proliferative cells, were first lost upon treatment before complete organoid death presumably due to the loss of stem cells within the branches. Organoid viability and branching percentages decreased in a time and dose-dependent manner upon this treatment. The level of toxicity associated with these chemotherapeutics agents was determined by calculation of IC50 values, which were then compared to published in vivo and clinical data. Correlation in drug sensitivity within the GI was observed in all species. For example, rats treated with 5FU experienced more severe diarrhoea and earlier occurrence of mortality than mice. The 5FU IC50 value within the mouse organoid assay was higher than that within the rat assay. Conversely, mice were more sensitive to CPT11 than rats, although both were equally sensitive to the active metabolite, SN38. Organoid human toxicity correlated well with the incidence of late onset diarrhoea. For example, human organoids were more sensitive to CPT11 than oxaliplatin, which are associated with a 20–40% and 4% incidence of grade 3/4 diarrhoea respectively.

In summary, we conclude that these are predictive preclinical models to help identify any potential off-target, off-tissue GI toxicities induced by novel oncology therapeutics. Toxicity, mechanism of action and dose schedule can all be addressed in vitro to potentially reduce in vivo experimentation (and associated costs and timelines), via a more informed experimental design.

**No conflict of interest.**

229 Poster (Board P055)

The screening of sorafenib analogs for the treatment of hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is one of the most common and lethal cancers, yet there is only one pharmacological therapy approved for advanced stage treatment, the multi-kinase inhibitor sorafenib (BAY 43–9006, Nexavar). Since sorafenib's approval in 2007, many drugs have failed to improve upon its small but significant survival advantage. A significant challenge in improving upon sorafenib is that the mechanism of this drug, in particular the functional basis for efficacy and toxicity, remains poorly understood in HCC. To better understand the mechanism of sorafenib's efficacy, a library of sorafenib analogs (sorafeligs) was developed with small to broad chemical changes in its structure to screen on HCC cell lines, for which sorafenib is effective at high micromolar doses. The half maximal effective dose (EC50) of each compound was determined using Alamar blue cell viability. A fraction of the sorafeligs converged upon an EC50 value, and very few sorafeligs improved upon sorafenib's EC50 dose in cell lines suggesting sorafenib's selectivity for HCC is retained on the cell lines. The most active compounds were secondarily screened using clonogenic assays on both the HCC cell lines and a normal hepatocyte cell line, THLE5B, to determine impact on replicative capacity as well as gauge therapeutic window. One compound was the most effective on most of the HCC cell lines, up to 100 fold more active than sorafenib, while having a larger therapeutic window. The compound was screened using KNativ, an in situ kinase profiling platform, which reveal possible clinically relevant and novel targets in the treatment of HCC. In conclusion, the use of a library of sorafeligs on HCC cell lines was able to isolate a compound, with increased efficacy and possibly lower toxicity in the context of HCC. Validation and detailed understanding of this compound's mechanism of action are ongoing, as well as in vivo HCC mouse models to determine clinical application of the compound as a treatment for HCC.

**No conflict of interest.**

230 Poster (Board P056)

High-throughput screening for small chemical inhibitors: Investigation and intervention in tartrate-resistant acid phosphatase’s role during cancer progression

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**Background:** Tartrate-resistant acid phosphatase (TRAP/ACP5), a metallocenzyme that is a characteristic master for osteoclasts and bone resorption has recently gained considerable attention as a driver of cancer progression. TRAP expression has been associated with clinically relevant parameters for tumor aggressiveness and its upregulation in several types of cancers was i.a. associated with reduced survival and the development of metastases. Interestingly, we found that TRAP overexpression in breast cancer cells promoted an epithelial–mesenchymal transition signature, which was mediated via an upregulation of Transforming growth factor-β signaling, a pathway pivotally involved and often permutated in development processes and tissue homeostasis. TRAP is found in two isofoms that display differences regarding cellular localization and expression patterns. Importantly, the isoform TRAP 5b, which derives from cleaving of the monomeric TRAP 5a precursor was suggested as a serum marker for bone metastasis. Recently, we have characterized the small
Adult and pediatric sarcoma cell line screen findings in Notch, DNA repair and cell cycle gene and miR expression and compound response


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Background: Sarcomas are a group of heterogeneous tumor types that are exceptionally challenging to diagnose. Thus, a novel and precise diagnostic tool with practical utility for cancer pathologists is needed.

Methods: We developed a cancer diagnostic software, called HuDiagnostics™, to help pathologists to accurately diagnose cancers, presently covering 33 major types. The software predicts each patient sample as either in the training set or a validation set with 2:1 ratio, maintaining same proportions for the diseases types. A random forest classifier was developed on the training set based on results from our previous analysis on cancer classification [1], and was tested on the validation set.

Results: HuDiagnostics™ achieves ~97% accuracy in the training set via out-of-bag (OOB) estimation, and performs equally accurate in the validation set, therefore, does not suffer overfitting. We also observed: (1) colon and rectum adenocarcinoma are indistinguishable by our classifier, in accordance with our previous results [1]; (2) ~20% esophageal adenocarcinoma samples are classified as gastric adenocarcinoma, and vice versa; (3) ~20% cholangiocarcinoma and hepatocellular carcinoma are classified into another type; and (4) ~10% samples of 3 kidney cancers, namely kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, and kidney chromophobe renal cell carcinoma, are classified into another type. The result suggested that colon and rectum adenocarcinoma are essentially indistinguishable diseases (1 above); while the misclassified cancer pairs or trio (2, 3, 4 above) are either in the same organ or anatomically neighboring and related. We next tested our classifier on several large RNAseq datasets on tumor tissues from Gene Expression Omnibus [3], including GSE76987 and GSE66729 where raw data were not identically processed, and found it performs well but with somewhat reduced accuracy, accordingly due to variations introduced in sequencing and data analysis from the TCGA dataset. Classifiers individually retrained on these datasets demonstrated comparable performance to ones on the TCGA data. In addition, we are continuing to further expand the software that is able to sub-classify different cancer types.

Conclusions: HuDiagnostics™ is a good assistant for cancer pathologists to precisely diagnosing cancer diseases that are available on the web.

No conflict of interest.
lines or vesicles made from cell lines transfected with transporters known to mediate clinically relevant DDI. Risk for clinical DDI was evaluated per regulatory guidelines.

**Results:** GDC-0810 was found to competitively inhibit CYP1A2, 2B6, 2C8, 2C19, 2D6 and CYP3A4/5 with IC50 values of 0.0364, 0.68, 0.60, 16 and 14 μM. The relative CYP mediated contribution to total metabolism of GDC-0810 appears to be negligible. UGTs are the major contributors to the metabolism of GDC-0810 in HH and in HLM in the presence of UDPGA (co-factor for UGT). In the absence of UDPGA and therefore glucuronidation, the CYP enzymes involved in the metabolism of GDC-0810 are CYP2C8, and to a lesser extent, CYP2C19. GDC-0810 was an inhibitor of CYP1A2, 2B6, and 3A4 at concentrations up to 10 μM. GDC-0810 was not a substrate of P-gp, BCRP, OATP1B1, OATP1B3, OAT1, and OAT3. The IC50 of GDC-0810 inhibition of P-gp, BCRP, BSEP, MRP2, OATP1B1, OATP1B3 and OAT3 were 6.52, 9.10, 10.5, 49.4, -0.3, 0.913, and 2.97 μM, respectively. GDC-0810 was not an inhibitor of OAT1.

**Conclusions:** The risk for metabolism and transporter mediated DDI resulting in alterations in GDC-0810 exposure is low. In context of the clinical dose and exposure, the mechanistic-static model with a conservative fraction bound of 0.01, suggests the potential for GDC-0810 to increase the exposure of drugs that are substrates of CY2C, 1A2, 2B6, and low potential to increase exposure of CYP2D6 and 3A4 substrates. GDC-0810 inhibition of OATP1B1/3 suggests a potential for DDI. During the drug development of GDC-0810, guided by these findings, clinical DDI will be further assessed.

**Conflict of interest:** Corporate-Sponsored Research: All authors are employees and shareholders of Roche/Genentech. Genentech is developing GDC-0810.

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**234** Poster (Board P060) A large-scale siRNA kinome screen identifies novel synthetic lethal combinations in colorectal cancer


**Background:** Targeted therapy for metastatic colorectal cancer is largely limited to the anti-EGFR antibodies cetuximab and panitumumab, which are beneficial for some patients with wild-type (non-mutated) KRAS/NRAS. However, for those patients who do not respond, and for patients with resistant KRAS/NRAS mutant tumours, treatment options are restricted to chemotherapy (FOLFOX or FOLFIRI). As such, there is an unmet clinical need for targeted therapeutic agents to treat these patient subgroups.

In order to identify synthetic lethal combinations and potential targets for therapy, we have performed a high-throughput simultaneous siRNA screen of the human kinome across a panel of 33 colorectal cancer cell lines. The screen utilised inhibitors of EGFR, MEK and PI3K in combination with the respective siRNA library to identify synergistic gene interaction events. Significant combinations were ranked, and confirmed through deconvolution of the siRNA pools.

We identified 8 combinations that were validated in siRNA pools. These siRNAs were used to screen an siRNA library to identify synergistic gene interaction events. Significant combinations were ranked, and confirmed through deconvolution of the siRNA pools. The activity and selectivity of kinase inhibitors is typically determined by measuring their inhibitory potency (IC50) in enzyme activity assays. These assays are closed systems, which is different from living systems, where drug concentrations are not constant and target residence time (t) may be a more important determinant of activity than IC50 or affinity (Kd). Surface plasmon ressonance allows measurement of target residence time of compounds in an open system.

**Material and Methods:** We have developed kinase binding assays on Biacore T200 for forty-four human kinases using amino-terminally biotinylated kinases expressed in insect cells.

**Results:** Here we provide the Keq and t of ninety-five kinase-inhibitor interactors, including approved tyrosine kinase targeting drugs, Aurora kinase and PI3K inhibitors. By surface plasmon resonance we could follow in real time, the irreversible kinase-inhibitor binding of the EGFR targeting drug afatinib, the BTK targeting drug ibritinib and the FGFR4 inhibitor BLU9931. Whereas response graphs of reversible inhibitors followed a simple 1:1 stoichiometry, the binding of irreversible inhibitors corresponded to an induced fit, two-state model. These studies suggest that Biacore assays can be used to identify irreversible kinase inhibitors. For several reversible kinase inhibitors we compared kinetic parameters with IC50 in enzyme assays. For the multikinase inhibitor ponatinib, which was tested on ten kinases, comparison of t and IC50 provided a strikingly different view on its selectivity. Furthermore, we observed that some reportedly selective Aurora A or pan-Aurora kinase inhibitors had a much longer target residence on Aurora B, despite having comparable Keq for Aurora A and B. Finally, we show that the investigational (γ)-isoform selective PI3K inhibitor duvelisib differentiates from the PI3K targeting drug idelalisib by having slow off-rates from its targets γ and δ, and higher selectivity over μ.

**Conclusion:** Our study demonstrates the added value of kinetic experiments in kinase drug discovery.

**No conflict of interest.**
237 Poster (Board P063) Rational molecular assessment and innovative drug selection (RAIDs): Pharmacological profiling of 20 cervical cancer cell lines

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Background: Aiming to enlarge cancer treatment options, large-scale pharmacological screenings of cancer cell lines were set up: The NCI-60 DTP human tumor cell panel intended to screen 3000 compounds per year on a set of 60 different human tumor cell lines. The Broad-Novartis Cancer Cell Line Encyclopedia aimed at integrating genomic and pharmacological results on a panel of 673 cancer cell lines. Only 6 cervical cancer cell (CC) lines are included in this Encyclopedia.

Methods: We performed pharmacological profiling of a panel of 20 original cervical cancer cell lines and attempt to correlate drug responsiveness with genomic markers. 43 different drugs have been tested, singly or in combination with Paclitaxel and Carboplatin for their ability to induce cell death. Full exome sequencing has been completed on all cell lines.

Results: The number of “druggable” mutations detected in each of the 20 cell lines varied between 5 and 21 while the range of genetic variants identified by full exome sequencing was 500 and 2000. Pharmacological profiling showed some cell lines to be highly sensitive and others highly resistant to many drugs. 5 CC cell lines were tested for (a) their sensitivity or resistance to a family of drugs affecting the spindle assembly checkpoint (SAC), as well as (b) a control drug, methotrexate belonging to an independent family of drugs. Focusing on one highly resistant –IC3, and one highly sensitive -IC5 cell line, the comparison with molecular data showed the resistant cell lines to carry 7 relevant mutations. The sensitive cell line had 5 relevant mutations which included BRCA. Similarly, in patient treatment, BRCA mutated tumors are highly sensitive to chemotherapy. The search for correlations between bioinformatics analysis with targeted drug sensitivity as a function of genomic markers is still ongoing.

Full exome sequencing from the first 48 BioRAIDs patient tumors (>365 patients included) revealed predominant mutations in many pathways including the PI3K pathway, chromatin remodeling and loss of function mutations of the suppressor gene FBXW7. Using an atlas for cancer signaling network (ACSN), 5 clusters could be defined. All 20 cell lines were contained in clusters 1 and 4, which (according to ingenuity pathway analysis) were enriched in dysfunctions of oxidative phosphorylation and mitochondrial energy production.

Discussion: A strategy envisioning to elegantly cancer treatment options will need to take into account: (1) correlations between mutational data and drug response in cell line screening, (2) selection of the patients who need alternative therapeutic options most (by (docking) molecular markers of drug resistance/incomplete response (from BioRAIDs) and (3) drug availability both for laboratory screening and for patient treatment.

No conflict of interest.

238 Poster (Board P064) Performance of docking strategies in the enrichment of fragment-like inhibitors of indoleamine 2,3-dioxygenase

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Background: Virtual screening of compound libraries by molecular docking can help narrow down a large list of compounds to a more manageable size for testing. Fitness functions within molecular docking programme’s software calculate how a compound fits the target site of the protein and assigns a numerical value quantifying how well it fits. Different molecules can be compared and ranked based on these values. As fitness functions calculate “fitness” based on different parameters and perform differently depending on the properties of the target site, the success of a virtual screen can depend on the choice of the fitness function and the configuration of the target protein used for docking. We evaluated Goldscore, AsteX Statistical Potential (ASP), Chemscore, ChemPLP fitness functions within the GOLD molecular docking suite to find the combination of fitness functions that performs the best for enrichment of inhibitors of the immunoregulatory enzyme indoleamine 2,3-dioxygenase (IDO1).

Methods: IDO1 inhibitory compounds were identified from a fragment library by sequential screening using differential scanning fluorimetry followed by enzyme inhibition assays. This same library was docked into three conformations of the IDO1 active site and rescored with combinations of the four fitness functions in the GOLD suite. The performance of the different scoring function combinations was evaluated by comparison to the empirical screen.

Results: For binding mode calculations, all fitness functions in GOLD, except ASP, reproduced the known binding mode of 4-phenylimidazole to within 1.0 Å RMSD. A larger active site conformation was important in reproducing known binding of the inhibitor Armg-1. With respect to early enrichment of inhibitors, the Chemscore fitness function performed the best when used to rank compounds. The top 5% of Chemscore-ranked libraries contained >25% of the inhibitors in the library. Only 5–15% of the inhibitors were found in the top 5% of Goldscore-ranked libraries. We also noted that different fitness functions selected different types of compounds. Chemscore ranked inhibitory naphthalene compounds highly, but failed to rank inhibitory benzoxazole and some benzothiazole compounds favourably. In contrast Goldscore ranks inhibitory naphthalene compounds inconsistently, but performs well with benzoxazole and benzothiazole inhibitors. Although apparently worse than Chemscore overall in this study, Goldscore is valuable as it is able to capture inhibitory compounds unidentified by Chemscore. The active site conformation had little effect on enrichment of fragments.

Conclusions: A small pilot screen, such as the one presented here, can be useful for deciding on the docking parameters before embarking on more extensive screens.

No conflict of interest.

239 Poster (Board P065) Differential drug sensitivity score (DSS) for indolent and aggressive prostate cancer cell lines

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Background: Prostate cancer (PC) is the most common malignancy in men and the second leading cause of cancer-related deaths. The majority of the PCs are classified as adenocarcinomas characterized by the expression of androgen receptor (AR) and prostate-specific antigen (PSA). Two of the most commonly used cell lines are LNCaP and PC-3 cells, derived from lymph node and bone metastases, respectively. It has been well established that LNCaP cells represent the conventional indolent form of PC expressing AR and PSA and are androgen-dependent. PC-3 cells, on the other hand, do not express AR and PSA, are androgen-independent, and represent the highly aggressive form.

Material and Methods: The drug sensitivity of the cell lines was assessed by applying a large panel of drugs covering both cancer chemotherapeutics and many clinically available and emerging molecularly targeted drugs including conventional chemotherapy, kinase inhibitors, metabolic modifiers, rapalogs, differentiating/epigenetic modifiers, kinesin inhibitors, apoptotic modulators, NSAIIs, hormone therapy, immunomodulators and HSP inhibitors. A panel of 460 compounds was tested in five concentrations covering a 10,000-fold drug-relevant concentration range in 384-well format. Cells were seeded to pre-drugged plates, followed by cell viability measurements (CellTiter-Glo) after 72 hours. Maximal and minimal responses to drugs were analyzed, the EC50 values were calculated and Drug Sensitivity Score (DSS) was calculated for each drug as a measure of reduced viability. A selective Drug Sensitivity Score (sDSS) was calculated to identify the selective drug response pattern between the cancer cell lines and controls.

Results: As expected, the results indicate that LNCaP cells in general were more sensitive to drugs of different categories than PC-3 cells. Both LNCaP and PC-3 cells showed sensitivity to PI3K, mitotic, HDAC, mTOR and CDK inhibitors, but LNCaP cells were more sensitive to conventional chemotherapy and kinase inhibitors.

Conclusions: We conclude that the cell-based compound screening combined with DSS analysis provides a possibility to profile cellular responses to an extensive collection of anti-cancer compounds enabling repurposing of existing drugs to new indications, identifying vulnerabilities in different types of cancer cells and functionally investigating cellular pathways behind drug sensitivity or resistance.

No conflict of interest.
Target occupancy studies of XPO-1 were carried out in different cancer relevant cell lines xenografted in mice treated with the Selective Inhibitor of Nuclear Export (SINE) compound Selinexor (KPT-330), currently in advanced clinical trials in patients with hematological and solid cancers. We used a fluorescently labeled tracer (Leptomycin B–Dy647) to quantify the loading of XPO-1 with Selinexor and confirmed target specificity of the signal using a fluorescent labeled antibody detecting native XPO1 protein. The amount of Antibody-XPO1-tracer complex was determined by FCCS. Occupancy of XPO-1 target with Selinexor decreases the amount of tracer-target complexes in a dose and time dependent manner. Target occupancy studies of XPO-1 were carried out in different cancer relevant cell lines xenografted in mice treated with treated animals. As such the approach can be applied to study target occupancy in vitro and in vivo.

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

Conflict of interest: Ownership: Dr. Becker and Dr. Hannus are founders and major shareholders of Intanta Bioscience GmbH. Corporate-sponsored Research: Part of this study was sponsored by Karyopharm Therapeutics Inc.
on ‘unseen’ data. Then, by comparing predicted and real drug response values, we obtain the accuracy measures of the prediction, i.e. RMSE (root mean squared error) and R² (explained variance).

We tested models for 7 anti-cancer drugs which are present in all three datasets: Erlotinib, Paclitaxel, Lapatinib, Nilotinib, Nutlin-3, PLX4720, Sorafenib. We found that we generally can predict AUC and viability at 1μM better than IC50: for models with 200 variables, the average R² values across all drugs and datasets are 0.10 for IC50, 0.175 for AUC and 0.16 for viability at 1μM. Results for individual drugs are heterogeneous – the drugs with highest predictability are PLX4720 (average R² = 0.215), Lapatinib (R² = 0.205), and Nutlin-3 (R² = 0.17). Sorafenib showed the lowest average R² = 0.046.

We also plan to evaluate predictive performance of stratified models built on cell lines’ subsets according to tissue type. For validation of the method on the real tumors, we plan to use data from TCGA and DKFZ-HIPO (Heidelberg Center for Personalised Oncology).

References

No conflict of interest.

244 Poster (Board P070)

Genomics of Drug Sensitivity in Cancer (GDSC): A resource for biomarker discovery in cancer cells

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Background: The Genomics of Drug Sensitivity in Cancer (GDSC; www.cancerRxgene.org) resource facilitates development of targeted cancer therapies through pre-clinical identification of therapeutic biomarkers.

Materials and Methods: We are using pharmacogenomic profiling in a collection of 1,000 highly annotated cancer cell lines as a biomarker discovery platform by systematically linking pharmacological data with genomic information. The GDSC database can be used to guide the pre-clinical development of new therapies and to identify opportunities for repurposing of existing molecules.

Results: Established in 2012, the GDSC database has recently undergone a significant upgrade to increase functionality for mining data, and through the GDSC drug sensitivity platform by systematically linking pharmacological data with genomic information. The GDSC database can be used to guide the pre-clinical development of new therapies and to identify opportunities for repurposing of existing molecules.

Conclusions: Analysis of GDSC data is a web portal based on queries of specific anti-cancer drugs, cancer alterations, or cell lines of interest. Pharmacological and genomic dataset for our cell line collection now contains drug sensitivity data for over 210,000 experiments, describing response to 265 anti-cancer drugs across over 900 cancer cell lines. This includes drug sensitivity data for targeted agents and chemotherapeutics, incorporating clinically approved drugs, drugs in clinical development, and experimental compounds to diverse cancer-associated targets. To identify clinically relevant pharmacogenomic markers, we have derived a set of genomics alterations, including somatic mutations in cancer genes and recurrent copy number alterations, from the analysis of over 11,000 patient tumours. These alterations have been mapped onto our cell lines and integrated with drug sensitivity data using cancer-type specific and pan-cancer analyses.

No conflict of interest.

245 Poster (Board P071)

A high-throughput drug screen identifies new therapeutic vulnerabilities in non-small cell lung cancers (NSCLC) with overexpression of the EMT-associated receptor tyrosine kinase AXL

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We previously used a patient derived pan-cancer epithelial to mesenchymal transition (EMT) signature to identify therapeutic vulnerabilities across multiple cancer types. Common potential therapeutic targets in tumors that have undergone EMT included AXL, PDGFRB, and MMP2 (r > 0.6, n > 9 tumor types). Several AXL inhibitors are currently in clinical development for the treatment of lung cancer and other malignancies. Given the association of AXL with frequent driver of therapeutic resistance and the ongoing clinical development of AXL inhibitors, we performed integrated analyses to identify therapeutic vulnerabilities in NSCLC with high AXL expression and biomarkers of response to AXL inhibition. AXL mRNA and protein expression were correlated with in vitro drug sensitivity (IC50) in a panel of NSCLC cell lines from the MD Anderson Lung Moonshot. Supervised analysis was then applied to identify markers of response to AXL inhibition (BGB324) and next-generation EGFR inhibitors using reverse phase protein array.

Our analysis identified 26 drugs (of 1216) for which AXL gene expression was significantly associated with sensitivity (Spearman r = 0.44–0.68, p < 0.05). Among these, several drugs had common targets; primarily identified using an in-house curated drug target database. As expected from prior studies in NSCLC, cell lines with higher AXL protein were relatively resistant to drugs targeting EGFR and PI3K. In addition to predicting resistance to first generation EGFR inhibitors (erlotinib, gefitinib), high AXL expression was also associated with resistance to second generation EGFR/ErbB family inhibitors, afatinib and dacomitinib (r = 0.86 p < 0.001; r = 0.67 p = 0.048 respectively).

Drugs with greater activity in AXL-high cell lines included those targeting MEK1 and MEK2. For example, selumetinib (FDA approved for melanoma) sensitivity was significantly associated with higher AXL expression (r = 0.51, p = 0.013), suggesting a new population within NSCLC for which selumetinib may have activity.

AXL expression did not predict sensitivity to BGB324. However, comparing proteomic profiles of sensitive (n = 5) and resistant (n = 6) cell lines, we identified other biomarkers of sensitivity and resistance (p < 0.05 by t-test). Top markers of sensitivity included overexpression of PARP1 (p = 0.012), and other DNA damage response (DDR) proteins, suggesting a role for AXL in DDR and supporting further investigation of AXL-DRR combinations. Expression of the AXL-associated receptor tyrosine kinase AXL in NSCLC cell lines was associated with sensitivity to MEK inhibitors, but resistance to both first and second generation EGFR inhibitors. This data provide a rationale for stratifying patients with high AXL protein to specific targeted therapies, including a possible role for MEK inhibitors in those with high AXL expression.

Conflict of interest: Advisory Board: Dr. Heymach serves on the advisory board of AstraZeneca, AbbVie, and Novartis. Dr. Byers serves on the advisory board of AstraZeneca. Corporate-sponsored Research: Dr. Heymach has received funding support from AstraZeneca, GlaxoSmithKline and Bayer; Dr. Byers has received funding support from Millennium Pharmaceuticals. Other Substantive Relationships: Dr. Byers is Principal Investigator on a BergenBio clinical trial of BGB324 in NSCLC, and has been a consultant for Eli Lilly, BioMarin and Medication.

246 Poster (Board P072)

Novel adrenergic receptor inhibitors and their inhibition of stress-induced metastasis of breast cancer

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Background: Cancer survival rates decrease with metastasis formation. Laboratory cancer models [1] reveal a biological role for the stress hormone norepinephrine (NE) in the metastasis of breast cancer, mediated by adrenergic receptors. Epidemiological studies suggest a reduction in mortality in breast cancer patients coincidently prescribed β-blockers [2], but our recent studies suggest effectiveness may differ according to β-blocker design [3,4]. This study aims to explore this hypothesis through the design and synthesis of novel β blockers with the objective of finding an adjuvant therapeutic for the retardation of breast cancer metastasis.

Materials and Methods: A library of 34 compounds have been designed by: (i) using ICI-118,551 (a β selective antagonist) as a template to design structural analogues and (ii) using in silico techniques to design novel molecular scaffolds. All compounds were subjected to a tiered screening programme to assess their influence on cell migration, invasion, proliferation and adhesion in MDA-MB-231, MDA-MB-435, MDA-MB-231 and MCF-7 breast cancer cell lines. Novel compound activity was compared to ICI-118,551 and the non-selective β-blocker propranolol controls.

Results: Effects of norepinephrine and control β-blockers: NE alone was found to have no effect on the proliferation of MDA-MB-231, MDA-MB-435, and MCF-7 breast cancer cell lines.
Serotonergic antagonists target breast tumor-initiating cells in mouse models of human breast cancer

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Despite improvements in diagnosis and treatment, the worldwide incidence of breast cancer is increasing, particularly in developing countries. Unfortunately, conventional therapies such as radiotherapy and cytotoxic chemotherapies rarely achieve durable cancer remissions. The therapy-resistance of breast tumors may be due to an infrequent breast tumor cell population, termed cancer stem cells or breast tumor-initiating cells (BTC), which share properties with mammary epithelial stem cells. Recent studies demonstrate that BTC fuel tumorigrowth, seed metastasis, and resist both radiotherapy and cytotoxic chemotherapies. Whereas the cells of origin of BTC are not known, new data demonstrates that BTC can arise from non-tumorigenic tumor cells by induction of an epithelial to mesenchymal transition, raising the possibility that the abundant non-tumorigenic breast tumor cells might provide a reservoir for BTC. Collectively, these findings suggest that drugs targeting both the infrequent BTC subpopulation and the abundant non-tumorigenic tumor cell population are required to eradicate tumors and their metastases.

Our goal is to identify drugs that target BTC, which can be used in combination with existing cytotoxic anticancer therapies to achieve durable breast cancer remissions. To the latter end we performed a high-throughput screen of small molecules, including FDA-approved drugs, using a sensitive cell viability assay with BTC-enriched mouse mammary tumor cells. We thus identified antagonists of the serotonergic system, which are commonly used to treat mood disorders. In short, structurally diverse compounds targeting independent elements of the serotonergic system irreversibly reduced BTC-activity as determined by functional sphere-forming assays and the initiation of tumor formation as determined by transplant of viable, drug-exposed tumor cells into syngeneic mice. Moreover, a selective serotonin reuptake inhibitor synergized with docetaxel (Taxotere) to shrink mouse mammary tumors in vivo. The serotonergic antagonists similarly reduced BTC activity in human breast tumor cell lines in vitro and in breast tumor xenografts in vivo. Collectively these observations suggest that drugs targeting the serotonergic system might be repurposed to treat breast cancer patients who afford more durable breast cancer remissions than are currently achieved.

No conflict of interest.

247 Poster (Board P073)

Phenylbenzene sulfonyl hydrazides: Selective indoleamine 2,3-dioxygenase inhibitors with potent in vivo pharmacodynamic activity and antitumor efficacy

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Background: Indoleamine 2,3-dioxygenase has been identified to play a key role in local tryptophan metabolism along the kynurenine pathway, and has emerged as a therapeutic target for cancer immunotherapy. Several pharmaceutical companies have discovered indoleamine 2,3-dioxygenase inhibitors that utilized as anti-tumor agents in recent years. Our prior study identified a phenyl benzenesulfonyl hydrazide indoleamine 2,3-dioxygenase inhibitor that had potent in vitro activity but was inactive in vivo. Lead optimization to improve the physicochemical properties of compounds and resulted in several in vivo active compounds.

Material and Methods: Chemistry: All commercial solvents and chemicals were of reagent grade and used without further purification unless otherwise stated. All reactions were carried out under an atmosphere of dry nitrogen or Argon. IDO enzyme activity assay: The IDO activity of test compounds was measured in the assay buffer (pH 6.5) containing, ascorbic acid, methylene blue, catalase, L-tryptophan, purified IDO enzyme and the test compounds in 96-well black plates. The reactions were further incubated with NaOH to hydrolyze N-formylkynurenine to kynurenine. The amount of kynurenine produced was determined in the emission of fluorescence [λ(ex) = 355 nm, λ(em) = 460 nm].

Drug Synthesis

248 Poster (Board P074)

Novel palladiums alone and in combination with phytochemicals in search of affordable chemotherapy

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Background: Objective of the study is to synthesize novel tumour active palladium complexes and overcome drug resistance through combination between palladiums with selected phytochemicals.

Materials and Methods: Synthesis of palladium compounds, characterization (x-ray crystallography, microanalysis, IR, NMR and Mass), MTT cell proliferation assay, Synergy calibration by Chou-Talalay method, DNA damage by agarose gel electrophoresis.

Results: Five novel palladium complexes: NH2 [(tris(benzimidazole)-monochloro palladium(I)] chloride, NH3 [bis(1,8-quinoxalino) palladium(II)], NH4 [tetraakis(1,2-α)imidazolpyridine]palladium(II)], NH5 [bis(1,2-α)-imidazolpyridine] dichloro palladium(II)], NH6 [tetraakis(1,2-α)imidazolopyridine][palladium(II)]. 4H,0.5(1,2-α)imidazolopyridine] have been synthesized. IC50 values obtained from MTT assay are given in Table 1.

Table 1. IC50 values for different cancer cell lines a

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2780</td>
<td>A2780cisR</td>
</tr>
<tr>
<td>Oxaplatin</td>
<td>0.41</td>
</tr>
<tr>
<td>NH3</td>
<td>0.107</td>
</tr>
<tr>
<td>NH4</td>
<td>19.39</td>
</tr>
<tr>
<td>NH6</td>
<td>10.14</td>
</tr>
<tr>
<td>NH7</td>
<td>148.22</td>
</tr>
<tr>
<td>NH8</td>
<td>317.63</td>
</tr>
<tr>
<td>Patulin</td>
<td>1.47</td>
</tr>
<tr>
<td>Emetine</td>
<td>0.023</td>
</tr>
<tr>
<td>ECGG</td>
<td>6.87</td>
</tr>
<tr>
<td>Curcumin</td>
<td>6.80</td>
</tr>
</tbody>
</table>

* Oxaplatin was used as a reference; -, not done.

Combination between emetine and oxaplatin is mostly antagonistic in Caco-2 and HT29/219 cell lines. Synergism is observed for combinations of NH3 with curcumin and ECGG against tested cell lines. DNA damage study indicates the cytotoxicity of platinum and designed palladiums is related to binding with DNA.

Conclusion: Palladium complex NH3 has a great potential for development as a novel anticancer drug candidate if confirmed in vivo.

No conflict of interest.

249 Poster (Board P075)

Phenyl benzenesulfonyl hydrazides: Selective indoleamine 2,3-dioxygenase inhibitors with potent in vivo pharmacodynamic activity and antitumor efficacy

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Background: Indoleamine 2,3-dioxygenase has been identified to play a key role in local tryptophan metabolism along the kynurenine pathway, and has emerged as a therapeutic target for cancer immunotherapy. Several pharmaceutical companies have discovered indoleamine 2,3-dioxygenase inhibitors that utilized as anti-tumor agents in recent years. Our prior study identified a phenyl benzenesulfonyl hydrazide indoleamine 2,3-dioxygenase inhibitor that had potent in vitro activity but was inactive in vivo. Lead optimization to improve the physicochemical properties of compounds and resulted in several in vivo active compounds.

Material and Methods: Chemistry: All commercial solvents and chemicals were of reagent grade and used without further purification unless otherwise stated. All reactions were carried out under an atmosphere of dry nitrogen or Argon. IDO enzyme activity assay: The IDO activity of test compounds was measured in the assay buffer (pH 6.5) containing, ascorbic acid, methylene blue, catalase, L-tryptophan, purified IDO enzyme and the test compounds in 96-well black plates. The reactions were further incubated with NaOH to hydrolyze N-formylkynurenine to kynurenine. The amount of kynurenine produced was determined in the emission of fluorescence [λ(ex) = 355 nm, λ(em) = 460 nm].
IDO cellular assay: Human IFN-γ and compounds in a culture medium containing L-tryptophan were added to the HeLa cells seeded in 96-well culture plates. After incubation for 24 hours, the supernatant was mixed with 6.1 N of trichloroacetic acid in acetic acid and measured at OD480 nm. In vivo pharmacodynamic assay: Compounds at a dose of 50 mg/kg were administered by oral gavage to C57BL/6 mice (n = 4 animals/group). The 3′-O-demethylation of IDO (kyn) and tryptophan (trp) were measured and the kyn/trp ratios were calculated.

In vivo tumor growth inhibition: The murine CT-26 colorectal syngeneic model was used to evaluate the in vivo tumor growth inhibition for test compounds. Results: The resulting N′-(4-bromophenyl)-2-oxo-2,3-dihydro-1H-indole-5-sulfonil hydrizide, which exhibited IDO IC₅₀ = 36 nM and EC₅₀ = 68 nM, demonstrated 56% of oral bioavailability with favorable oral PK. The compound was well tolerated in the epithelial-to-mesenchymal transition and cell cycle progression (2700 mg/mL h) and 73% of tumor growth delay in a murine CT26 syngeneic model, after oral administration of 400 mg/kg, without apparent body weight loss. Conclusion: Phenyl benzensulfonylhydrizides have been identified as selective IDO inhibitors with potent enzymatic and cellular activities. Lead optimization resulted in the N′-(4-bromophenyl)-2-oxo-2,3-dihydro-1H-indole-5-sulfonil hydrizide as a potent in vivo IDO inhibitor. Accordingly, this compound is proposed as a drug lead for advanced preclinical evaluation.

No conflict of interest.

250 Poster (Board P076) Docosahexaenoic acid-acetylated phosphoridizin selectively kills mammary carcinoma cells and inhibits metastasis in vitro and in vivo

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Background: Flavonoids possess wide-ranging disease-fighting properties. However, their clinical applications are restricted by their poor cellular uptake and bioavailability. PZ-DHA combines PZ, a flavonoid abundantly found in apple peels, and DHA, an omega-3 fatty acid through an acylation uptake and bioavailability. PZ-DHA inhibits mammary carcinoma cells (MDA-MB-231, MDA-MB-468, 4T1, MCF-7 and T-47D) of Annexin-V-FLUOS/propidium iodide (PI)-stained MCF-10A cells and immortalized MCF-10A cells using MTS assays. Flow cytometric analysis of Annexin-V-FLUC/propidium iodide (Pi)−stained MCF-10A cells and human dermal fibroblasts compared to MDA-MB-231 cells was performed to confirm the selective cytotoxicity of PZ-DHA for mammary carcinoma cells. The antiproliferative effect of PZ-DHA was tested using flow cytometry of MDA-MB-231 cells stained with Oregon Green 488, FITC-labeled anti-Ki-67 antibody and PI, followed by cell cycle analysis. Wound-healing and transwell cell migration assays were performed using MDA-MB-231 and 4T1 cells to test the in vitro antitumor properties of PZ-DHA. The effect of PZ-DHA on the expression of proteins involved in the epithelial-to-mesenchymal transition and cell cycle progression was demonstrated by western blot analysis. To show the in vivo anti-metastatic activity, PZ-DHA was administered to 4T1 tumor-bearing female BALB/c mice and MDA-MB-231 tumor-bearing female non obese diabetic severe combined immune-deficient (NOD-SCID) mice by intraperitoneal injection. Potential PZ-DHA metabolites were identified by incubating PZ-DHA with human liver microsomes at 37°C and UPLC/MS analysis of the supernatants.

Results: PZ-DHA selectively killed breast cancer cells in comparison to normal human cells. Sub-cytotoxic doses of PZ-DHA arrested MDA-MB-231 cell proliferation at G2/M, causing down-regulation of cyclin B1 and CDK1. PZ-DHA inhibited the migration of MDA-MB-231 and 4T1 cells in vitro. Furthermore, 4T1 tumor-bearing female BALB/c mice and MDA-MB-231 tumor-bearing female NOD-SCID mice that received intraperitoneal injections of PZ-DHA showed a significant reduction in primary tumor volume at the mammary fat pad and fewer metastatic lesions in the lungs compared to control mice. Our preliminary in vitro pharmacokinetic studies showed that PZ-DHA undergoes mainly phase I methylation, hydroxylation and epoxidation.

Conclusions: The findings of this study reveal that PZ-DHA suppresses mammary carcinoma cell proliferation and metastasis, suggesting a potential clinical application to prevent breast cancer progression in patients. Future studies will explore the effects of PZ-DHA metabolites on breast cancer cells.

No conflict of interest.

Epigenetic modulators

251 Poster (Board P077) The BET bromodomain inhibitor (BET-i) BAY 1238097: Mechanism of action and pre-clinical activity in diffuse large B-cell lymphoma (DLBCL)

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Background: BAY 1238097 is a novel BET-i active in lymphoma models (ENA2015). Here, we characterize its mechanism of action and report further in vitro and in vivo activity data in DLBCL.

Materials and Methods: NOD-Scid mice were sc engrafted with germinall center B cell (GCB) SU-DHL-8 or activated B cell-like (ABC) OCI-Ly3 DLBCL cells. When tumors reached 100–150 mm³ volume, BAY 1238097 was given; 15 mg/kg, qd, PO, 14 days (SU-DHL-8); 45 mg/kg, qo, PO, 28 days (OCI-Ly3). Gene expression profiling (GEP) (Illumina HumanHT-12ExpressionBeChips) was done on GCB-DLBC DoHH2 treated with DMSO or 500 nM BAY 1238097 for 8–12–24 h, and GSEA-analyzed. Synergy was assessed with Chou-Talalay combination index (CI) on 72 h MT assay data in cell lines exposed to increasing doses of BAY 1238097 with GSK126 or everolimus: synergism (<0.9), additive effect (0.9–1.1).

Results: BAY 1238097 affected the growth of both GCB and ABC DLBCL xenografts: treated tumors resulted 6–8 fold smaller in volume respect to controls. At GEP, BAY 1238097 decreased target genes of Myc, Notch and E2F, members of the NFKB/MYD88 and mTOR/akt signaling. The upregulated transcripts were mainly represented by histones. The GEP signatures highly overlapped with the signatures obtained with another BET Bromodomain inhibitors and partially overlapped with HDAC-i, mTOR-i and demethylating agents. BTK, CTCB86, CND2, CCLR1, CD19/27, CXCL9/10, CXCR7, FAIM, FGR, KIF1, IL12A, IL7R, IRAK1, MANK1/3/4/5, MLKL, MYB, MYC, PDE4B, PTPN6/22, PTPRO, PVRIG, STAT5A, TNFRSF13B/17, TNFRSF8, VPREB3 were among the GSEA top ranked downregulated genes. Beside histone coding genes, the GSEA top ranked upregulated genes included BRD8, CCL3/5, CD99, CKDN2C/D, GADD45B, JUN, MTK1A/2A, MKK1X2, YPEL5, MCL1, NFKBIZ, PIM1, SESN3.

Based on similarity with mTOR-i GEP signatures and our previous data with the BET-i OTX015 (CCR 2015), BAY 1238097 was combined with the mTOR-i everolimus, leading to synergism in 2/2 ABC- (U2932, CI = 0.47; TM6D, 0.89) and in 5/6 GCB-DLBC (KARPAS422, 0.46; SU-DHL-L, 0.6; DoHH2, 0.77; Toledo, 0.88), and additive in the remaining GCB WSU-DLCL.

Finally, based on our previous observation of BAY 1238097-induced EZH2 protein down-regulation (ENA2015), we performed chromium immunoprecipitation showing reduced BRD4 binding on EZH2 regulatory region after BAY 1238097. The latter with was also synergistic with the EZH2-i GSK126 in 3/3 murine CD2 GCB (SU-DLCL2, CI = 0.85; KARPAS422, 0.3; SU-DHL-L, 0.6) and additive in 3/3 murine CD2 GCB (Toledo, 1; DoHH2/SU-DHL-L, 1.1).

Conclusions: BAY 1238097 has anti lymphoma in vivo activity, and is able to interfere with pathways relevant for lymphoma cells and is synergistic with EZH2-i and mTOR-i. Additional combinations blocking PIM1, MCL1 or NFKB, or with HDAC-i and demethylating agents could be designed based on this GEP results.

EZH2 mediates lidamycin-induced cellular senescence through regulating p21 expression in human colon cancer cells

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Background: Lidamycin (LDM) is a novel member of the enediyne antibiotics identified in China with potent antitumor activity, and enters phase II clinical study in China. However, it remains unclear whether LDM has potential molecular targets that may affect its antitumor activity. Emerson et al. (2012) revealed that EZH2 functions as a histone lysine methyltransferase and mediates trimethylation of histone 3 lysine 27 (H3K27me3). High EZH2 level is found to be positively correlated with the aggressiveness, metastasis and poor prognosis of cancer. Here, we aim to study the role of EZH2 in LDM-induced senescence as well as in the cytotoxicity of LDM in human colon cancer cells.

Material and Methods: Cytotoxicity of LDM was assessed by MTT method in a panel of human colon cancer cells. Senescence phenotypes were examined by cell cycle arrest with FACS analysis and senescence-associated β-galactosidase staining. Immunoblotting and RT-PCR was used to analyze the molecular changes at protein and mRNA levels, respectively. Chi-square test was used to analyze the enrichment of EZH2 and H3K27me3 levels in the p21 promoter.

Results: LDM is relatively more potent in inhibiting the colon cancer cells (HCT116 and SW620 cell lines) harboring high EZH2 expression, and induces robust senescence-like cellular senescence at IC50 level. More importantly, LDM is found to markedly inhibit EZH2 expression at both protein and mRNA levels upon the induction of p21 and cellular senescence. Knockdown of EZH2 with siRNAs abolishes LDM-induced senescence, while EZH2 knockdown markedly increases p21 expression and cellular senescence phenotype. Enrichment of both EZH2 and H3K27me3 levels in the p21 promoter region is reduced upon LDM exposure. Moreover, EZH2 overexpression reduces p21 expression and cellular senescence phenotypes upon LDM exposure. LDM also demonstrates potent antitumor efficacy in xenografted animal models in vivo.

Conclusion: Our work provides first demonstration that EZH2 may mediate, at least partially, the senescence-inducing effects of LDM by regulating p21 expression. Thus, EZH2 may serve as a potential biomarker to indicate the clinical efficacy of the potent enediyne antibiotic drug.

No conflict of interest.

Assessment of apoptosis and senescence in acute myeloid leukemia

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EGCG (epigallocatechin-3-gallate) is the most abundant and the most biologically active green tea (Camellia sinensis) catechin, which among many other activities has been shown to be an epigenetic modifier. Meanwhile, BIX-01294 is a specific inhibitor of the G9a histone methyltransferase. Epigenetic modifiers are an important tool for cancer treatment. Chemical agent capability to cause cellular senescence would increase cancer treatment effectiveness as it limits cancer progression. In the present study we investigated capacity of these two chemical agents to induce epigenetic changes as well cellular senescence in acute myeloid leukemia NB-4 cells. Both EGCG and BIX-01294 inhibited NB-4 cell proliferation and survival as determined by trypan blue exclusion test. Cell cycle analysis was performed using standard Propidium iodide staining procedure and flow cytometry. We showed that both chemical agents arrested cell cycle at phase G1/G0. Cell staining with Annexin V and Propidium Iodide revealed induction of apoptosis after treatment with EGCG and BIX-01294 independent of agents’ capacity to induce cellular senescence was evaluated by gene expression analysis and by staining cells for SA-β-galactosidase activity determination. RT-qPCR analysis demonstrated elevated levels of p53, p21, BcL, ATM, HMGA gene expression after treatment with EGCG. However, treatment with BIX-01294 increased only p21 gene expression. Further, SA-β-galactosidase staining supported observation of EGCG ability to induce cellular senescence but no SA-β-galactosidase positive cell staining was observed after treatment with BIX-01294. We evaluated both protein modifications and expression of EZH2 and H3K9me3 modification level. In addition, EZH2 and SUZ12 – the components of Polycomb repressive complex 2 (PRC2) – protein levels also declined in NB-4 cells after treatment with EGCG and BIX-01294 independently.

No conflict of interest.

Transcription control by the ENL YEATS domain in acute leukemia

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Background: Recurrent chromosomal translocations involving the mixed lineage leukemia gene (MLL) give rise to highly aggressive acute leukemia associated with poor clinical outcomes. The preferential involvement of chromatin-associated factors in MLL rearrangements belies a dependency on transcriptional regulators.

Materials and Methods: To identify new targets for therapeutic development in MLL, we performed a genome-scale CRISPR-Cas9 knockout screen in MLL-AF4 leukemia. Among validated targets, we identified the transcriptional regulator, ENL, as an unrecognized dependency particularly indispensable for proliferation. To explain the mechanistic role for ENL in leukemia pathogenesis and the dynamic role in transcription control, we pursued a chemical genetic strategy utilizing targeted protein degradation.

References

No conflict of interest.
Results: ENL loss suppresses transcription initiation and elongation genome-wide, with pronounced effects at genes featuring disproportionate ENL load. Importantly, ENL-dependent leukemic growth was contingent upon an intact YEATS epigenomic reader domain.

Conflict of interest: Other Substantive Relationships: J.E.B. is now an employee, shareholder, and executive of Novartis Pharmaceuticals.

256 Poster (Board P082)
Dual inhibition of BET and mutant BRAF in BRAF-mutant colon cancer cells suppresses oncogenic pathways and synergistically inhibits their growth

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Background: Bromodomain and extra-terminal (BET) inhibitors show strong cytotoxicity against several tumors by disrupting super-enhancers, defined as a large cluster of enhancer regions. Furthermore, recent studies have suggested the potential of overcoming the resistance to targeted agents by down-regulating oncogenic pathways. In colorectal cancer (CRC), however, it is not fully understood how BET inhibitors influence cell proliferation and the sensitivity to targeted agents. The aim of our research is to characterize CRC-specific super-enhancers and to explore the potentially synergistic effects of dual inhibition of any oncogenic pathway and oncogenic super-enhancers.

Material and Methods: CRC-specific super-enhancers were identified by comparing ChIP-sequencing data of H3K27 acetylation between four CRC cell lines (RKO, Caco2, SW48, and SW620) and normal colon epithelial cells. The sensitivity to a BET inhibitor JQ1 was assessed using cell viability assay in fourteen CRC cell lines. The efficacy of the combined treatment of JQ1 with the BRAF inhibitor vemurafenib was estimated using cell viability assay, cell cycle analysis, and apoptosis assay in three BRAFV600E-mutant CRC cell lines (RKO, Colo205, and HT29). The effect of JQ1 in addition to vemurafenib on oncogenic pathways was evaluated by western blotting and gene expression microarray analysis.

Results: CRC specific super-enhancers were associated with driver oncogenic pathways, such as MAPK signaling pathways and cell cycle. Eight of 14 CRC cell lines showed sensitivity to JQ1 (IC50 <1μM), including BRAF or PIK3CA mutant ones, along with down-regulating super-enhancer associated genes. We selected BRAF mutation as a target of dual inhibition strategy. Combined treatment with JQ1 and vemurafenib synergistically suppressed cell growth (combination index: RKO, 0.42; Colo205, 0.49; and HT29, 0.47 at IC50) with induction of G1/S arrest and increased caspase-3/7 activity. The combination also suppressed phosphorylation of ERK and AKT in RKO and Colo205. Expression microarray analysis showed that the combined treatment additively inhibited the expression of super-enhancer associated oncogenes, such as MAPK3 and CCND1, in the three cell lines. GSEA analysis revealed that the addition of JQ1 significantly down-regulated not only MYC and EZH2 target gene sets, but also the ones associated with additional oncogenic pathways, including NOTCH, MTORC1, KRAS, and Wnt(-)-catenin signaling pathways (FDR q-value <0.25).

Conclusions: Combined treatment of JQ1 with vemurafenib synergistically induced cell cycle arrest, and apoptosis, along with suppression of reactivation of MAPK signaling and down-regulation of additional oncogenic pathways in BRAFV600E-mutant CRC cell lines. Our data provide the proof-of-principle for combined BRAF and BET inhibition in patients with BRAFV600E-mutant CRC.

No conflict of interest.

257 Poster (Board P083)
Development of novel DNA demethylating agents with greater stability and less toxicity

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Background: Applications of epigenetic therapy are expanding from hematologic to solid tumors. However, two approved DNA demethylating agents, 5-azacytidine (5-Aza-CdR, dacarbazine), and hydroxycytidine (5-Aza-CdR, dacarbazine), suffer from rapid degradation by cytidine deaminase and hydroxycytidine ring-cleavage of the base moieties. This makes it challenging to determine the optimal dose for individual patients. Here, we aim to develop novel and stable DNA demethylating agents.

Materials and Methods: Derivatives of 5-Aza and 5-Aza-CdR were chemically synthesized. Demethylation was quantified in vitro by the activity of luciferase gene driven by a methylated promoter CpG island of an endogenous gene [Okochi-Takada et al, manuscript in preparation]. Methylation level of the methylated CpG island was analyzed by qPCR. Demethylation-specific PCR. Cell viability in vitro provided a measure of cell growth. DNMT1 expression was assessed by western blotting. In vivo drug tolerability of twice-a-week administration schedule was evaluated by body weight, moribidity, leukocyte count, and pathological analysis in athymic nu/nu mice.

Results: Fifty-seven derivatives of 5-Aza and 5-Aza-CdR were synthesized to determine the most resistant to degradation by cytidine deaminase and hydroxycytidine ring-cleavage. Five compounds in OP-20 series (including OP-21) and three compounds in OP-10 series (including OP-12) showed luminescence equal to or stronger than that shown by 1 μM and 10 μM SGI-110, respectively; SGI-110 is a clinically promising stable demethylating agent. In a colon cancer cell line, OP-21 treatment suppressed cell growth (IC50 = 1 μM) and caused 50% demethylation of the marker CpG island, similar to SGI-110. Interestingly, OP-12 also induced strong demethylation (90%) similar to that induced by SGI-110, but showed only moderate suppression of cell growth (IC50 = 100 μM). Complete depletion of DNMT1 protein was observed in cells treated with OP-12 and OP-21. Both were better tolerated using this 3x on non-cancerous tissues remains unclear. Maximum tolerated doses of 22.8 μg/kg for OP-12, 7.4 μg/kg for OP-21, and 6.35 μg/kg for SGI-110.

Conclusions: Two novel DNA demethylating agents with strong demethylating activity and less toxicity were developed.

258 Poster (Board P084)
TPC-144, a novel reversible LSD1 inhibitor, exhibited strong antitumor activity in preclinical models of AML and SCLC

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Background: Lysine-specific demethylase 1A (LSD1/KDM1A) is a flavin adenine dinucleotide (FAD)-dependent histone demethylase which specifically modifies histone H3 lysine 4 and lysine 9. LSD1 activity is implicated in the pathogenesis of several human cancers and recent studies indicated that the inhibition of LSD1 is a promising therapeutic strategy for acute myeloid leukemia (AML) and small cell lung cancer (SCLC). Most of current LSD1 inhibitors are of the same class of compounds which covalently modify FAD and their impact on non-cancerous tissues remains unclear. Therefore, a potent and selective LSD1 inhibitor which is active without modifying FAD may provide an alternative strategy for treating AML and SCLC.

Material and Methods: The inhibitory activity against LSD1 enzyme was determined by TR-FRET assay. In vitro studies on growth inhibition and apoptosis induction were conducted by measuring cellular ATP present (CellTiter-Glo, Promega) and sub-G1 population, respectively. Expression changes of surface markers were analyzed by ELISA or flow cytometry. mRNA levels of LSD1 target genes were quantitated by real-time PCR. In vivo efficacy was evaluated in human AML and SCLC mouse xenograft models.

Results: TPC-144 reversibly inhibited LSD1 in a histone peptide-competitive manner and did not form a covalent adduct with FAD. TPC-144 inhibited LSD1 with an IC50 of 1.5 nM and had selectivity over other histone demethylases and methyltransferases. TPC-144 also showed excellent selectivity compared to off-target enzymes in the LeadProfilingScreen (Eurofins Panlabaws Tanwa, Ltd.).

In several human AML cell lines, TPC-144 induced cell differentiation, as indicated by derepression of hematopoietic transcription factor GF1; increased expression of cell surface markers such as CD86 and CD11b; and potently inhibited growth of AML cells (e.g., Kasumi-1, IC50 = 1.3 nM; MV4−11, IC50 = 2.4 nM; HEL, IC50 = 7.1 nM) by inducing apoptosis and cell cycle arrest. In human SCLC cell lines, TPC-144 downregulated the expression of several neuroendocrine factors, including the lineage-specific transcription factors ASCL1 and NKO2-2, leading to strong inhibition of SCLC cell growth (e.g., NCI-H1417, IC50 = 9.4 nM; NCI-H146, IC50 = 12.2 nM). These results suggest that TPC-144 exerts its effects by altering the differentiation status of SCLC cells.

Conclusions: TPC-144 is a novel, potent and selective LSD1 inhibitor with a reversible inhibition mechanism that has antitumor activity in several
human AML and SCLC cell lines and xenograft models. CPC-144 may provide clinical benefits and a favorable safety profile for the treatment of AML and SCLC patients.

No conflict of interest.

259  Poster (Board P085)
Characterization of a novel KDM1A/LSD1 inhibitor DDP_38003 in preclinical models of human and murine acute myeloid leukemia
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KDM1A (LSD1) is a flavine adenine dinucleotide dependent histone demethylase, responsible for the demethylation of lysine 4 on histone H3. As a part of various transcriptional co-repressor complexes, it is known to interact with co-repressor complex CoREST and histone deacetylase 1 and 2. KDM1A is an essential gene, with important roles in different biological relevant processes, including hematopoietic cell lineage differentiation. KDM1A has been reported to be over-expressed in a number of hematologic malignancies and lymphoid neoplasms. Specifically in acute myeloid leukemia (AML) KDM1A demonstrated to sustain in vivo leukemogenic potential of MLL-AF9 expressing leukemia stem cells, thus supporting its determinant role in AML.

Here we describe the anti-tumor effect of a novel KDM1A inhibitor (DDP_38003) tested in AML in vitro and in vivo models. DDP_38003 was a potent, selective, oral bioavailable irreversible inhibitor of KDM1A. The cellular activity of the compound, by measuring its ability to inhibit the colony formation of acute myeloid leukemia cells has been investigated. Potent growth inhibition was observed on AML blast colony forming ability of cells lines representative of different AML subtypes. Treatment of AML cells with the compound promoted the induction of myeloid differentiation markers and a differentiated phenotype. The in vivo efficacy of DDP_38003 was determined in mouse models of acute promyelocytic leukemia (APL), MLL-AF9 driven leukemia and on patient derived xenograft (PDX) AML model. Oral administration of DDP_38003 significantly increases the survival of mice harboring human APL xenografts in NOD-SCID and in MLL-AF9 leukemia models. On MLL-AF9 model, the in vivo activity correlated with target inhibition, with a strong and sustained reduction of circulating leukemic blasts, and it was significantly better when compared to standard combined chemotherapy treatment. In addition, an increased survival has also been observed in a PDX (MLL-AF9) disseminated leukemia model. Importantly, in vivo efficacy could be achieved with different dosing regimens of DDP_38003. Collectively, these data support the advancement of DDP-38003 toward preclinical development.

No conflict of interest.

260  Poster (Board P086)
New reversible inhibitors of histone lysine demethylase (KDM1A/LSD1). From high throughput screening to the identification of low nanomolar inhibitors with cellular activity
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Background: Lysine specific demethylase 1 KDM1A (LSD1) is an epigenetic eraser that regulates histone methylation at residues K4 and K9 and plays an important role in mediating the expression of genes involved in cancer and non-cancer diseases. Its overexpression in fact has been demonstrated in several tumor types. The first KDM1A inhibitor from Oryzon entered clinical trials for the treatment of AML in 2014, subsequently GSK2879552 was admitted to clinical investigation also for solid tumor. Both the clinical candidates are tranylcypromine derivatives and are characterized by an irreversible mechanism of action. The identification of reversible inhibitors of the enzyme instead has not led to any clinical candidate so far, in particular the lack of compounds demonstrating in vivo the mechanism represents a challenge to face.

Material and Methods and Results: We report on a high throughput screening (HTS) campaign, performed with a time resolved fluorescence resonance energy transfer (TR-FRET) technology, on a subset of our chemical collection, representative of almost 200000 compounds. We identified several chemical series able to reversibly inhibit KDM1A at micromolar concentration. Among them, we prioritized the N-phenyl-4H-thieno[3,2-b]pyrrole-5-carboxamide for which we obtained X-ray structure of the most potent hit in complex with the enzyme. To our knowledge no other co-crystal X-ray structure has been reported for a reversible inhibitor in complex to KDM1A. An exhaustive chemical expansion, Structure Based Drug Design (SBDD) driven, thanks to several structures solved, led to the obtained compounds active in single digit nanomolar range, able to show target modulation in cells. The medicinal chemistry modifications applied on the scaffolds subsequently brought us towards the identification of new chemical classes, even more potent than the originators.

Conclusions: With an HTS campaign several potent reversible inhibitors of KDM1A, belonging to different chemical series have been identified. Further optimization SBDD driven brought to low nanomolar compounds active in cells, which have been selected for further development.

No conflict of interest.

261  Poster (Board P087)
Resminostat sensitizes hepatocellular carcinoma cells to sorafenib-induced cell death
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Background: Currently, the only approved systemic therapy in advanced hepatocellular carcinoma (HCC) is a multikinase inhibitor, sorafenib. However, patients suffer adverse effects and are often either primarily resistant or develop resistance during therapy. Resminostat, a novel inhibitor of class I, IIb, and IV of histone deacetylases, was studied in advanced HCC patients after relapse to sorafenib (SHELTER study). In this phase I/II clinical trial the combination of sorafenib and resminostat in HCC patients was safe and showed early signs of efficacy [1]. However, the molecular mechanisms that would explain this potential synergism have not been explored yet. In previous works from our group, we described that lack of response to sorafenib in HCC correlates with a mesenchymal phenotype and the expression of the stem cell marker CD44 [2]. In this work we have performed in vitro experiments to understand whether resminostat regulates epithelial–mesenchymal and stemness phenotype in HCC, which would sensitize cells to sorafenib.

Material and Methods: A panel of epithelial and mesenchymal HCC cell lines was treated with resminostat and sorafenib alone or in combination. Cell growth was analysed by crystal violet staining, cell death by incorporation of propidium iodine (PI). The expression of EMT and stemness related genes was analysed by qRT-PCR. Analysis of protein expression by immunofluorescence.

Results: Resminostat decreases cell growth in all HCC cell lines tested in a dose and time dependent manner. However in the mesenchymal (more invasive) HCC cells resminostat induced significantly higher cell death when compared to the epithelial HCC cells. Mesenchymal HCC cell lines were resistant to sorafenib-induced apoptosis; however, a synergistic effect between resminostat and low, non-cytotoxic, concentrations and sorafenib was observed in terms of cell death. These same low concentrations of resminostat induced a decrease in vimentin expression (marker of mesenchymal cells) and a reorganization of ZO-1 (marker of cell-cell junctions). Moreover, a slight decrease of CD44 after resminostat treatment was observed, which could explain the sensitization to sorafenib treatment.

Conclusions: Resminostat is very effective in inducing apoptosis in mesenchymal HCC cells, usually resistant to sorafenib-induced cell death. Furthermore, low doses of resminostat are sufficient to significantly improve the apoptotic response to sorafenib in these cells, coinciding with a decrease in mesenchymal markers and CD44 expression.

References

No conflict of interest.
Background: Epigenetic mechanisms underlying nuclear chromatin remodeling are increasingly being recognized as crucial factors in hepatocellular carcinoma (HCC). Polycomb group member EZh2 is a key epigenetic regulator of embryonic stem cell identity; however, its role in HCC is poorly understood.

Methods: To investigate the roles of EZH2 and H3K27me3 in HCC, we conducted a review of patients who received surgical resection for HCC from 2009 to 2013. We analyzed EZH2 expression and H3K27me3 methylation status in 67 human HCC samples, and the global RNA expression by RNA-sequencing (RNA-seq) data based on different EZH2 expression. Additionally, we used the EZH2, H3K4me3, and H3K27me3 chromatin immunoprecipitation-sequencing (ChIP-seq) data in ENCODE HepG2 for interpreting our RNA-seq results.

Results: In contrast to our previous results of combined EzH1 and EzH2 loss in mouse experimental data, H3K27me3 expression was significantly related with EZH2 expression in 67 human HCC samples (P=0.05), High EZH2 expression was related with short overall survival (P=0.05), but H3K27me3 expression was unrelated with prognosis. Transcriptome analysis of HCC samples with high EZH2 expression by RNA-seq showed up-regulation of genes related to the cell cycle and DNA replication and down-regulation of estrogen response related genes. ChIP-seq for EZH2, H3K4me3, and H3K27me3 showed that estrogen receptor 1 (ESR1) and early growth response gene-1 (EGR1) were regulated by EZH2 through methyltransferase function.

Conclusions: This study shows that high EZH2 expression is related with poor prognosis in HCC. The EZH2 gene functions as a tumor oncogene by suppressing ESR1 and EGR1 through methyltransferase function in human HCC.

No conflict of interest.
Results: Resminostat exhibited a dose-dependent inhibition of the viability of MyLa and HuT78 cells. High-throughput bead-based ELISA revealed the hyperacetylation of various histone H3 residues upon treating the SzS cells with resminostat. Moreover, the epigenetic modulating small molecule inhibitors upregulated the expression of p38 MAPK while only marginally affecting other cell lines. Treatment of MyLa cells with resminostat reversed the aberrant STAT expression, namely inducing an increase in STAT4 and a decrease in STAT6 expression.

Conclusion: Resminostat displayed conclusive in vitro anti-tumor activities both in MF and SzS cells. The regulation of the aberrant STAT signaling on transcription level suggests a stabilization of the less advanced CTCL stage (Th1) or even a reconversion of the advanced (Th2) to the less advanced Th1 phenotype. Hence, resminostat represents a promising treatment option for indolent as well as aggressive CTCL subtypes, possibly as maintenance therapy. In upcoming functional assays, the impact of resminostat on the immunopathogenesis of CTCL will be analyzed and the anti-tumoral effects confirmed in an in vivo model.

Conflict of interest: Other Substantive Relationships: The authors are employees of 4SC and share. D. Vill is CSO and CDO of 4SC.

Phosphorylation regulates EZH2 neoplastic functions in triple-negative breast cancer

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Background: Triple-negative (estrogen receptor, progesterone receptor, HER2-neu negative) breast cancers (TNBC) comprise 15% of all breast cancers but are responsible for a disproportionately high number of deaths. Overexpression of the histone methyltransferase EZH2 (Enhancer of Zeste Homolog 2) is an independent prognostic biomarker significantly associated with poorly-differentiated TNBCs and poor patient outcome. We previously identified a novel link between EZH2 and the p38 mitogen-activated protein kinase, an important mediator of progression and metastasis of TNBC. We found that EZH2 binds to p38, and that EZH2 and activated p38 were concordantly expressed in the metastases of breast cancer patients. Based on these data and previous in vitro studies, we hypothesized that p38 MAPK may also regulate EZH2 through phosphorylation in breast cancer. We further hypothesized that this phosphorylation event may be important for EZH2 contribution to malignancy.

Methods: In order to test this hypothesis, we performed knockdown rescue experiments in triple-negative breast cancer cell lines MDA-MB-231 and MDA-MB-436. Stable knockdown of EZH2 was achieved using shRNA targeting the 3’UTR. Knockdown of EZH2 was rescued by reintroduction of myc-EZH2 (WT) or a T367A-EZH2 phosphorylation-deficient mutant. Cell lines were then used in functional assays of proliferation, migration, and invasion in vitro. Cells were also used in a murine orthotopic xenograft model in vivo and primary tumor and metastatic growth was monitored by bioluminescence. In order to further interrogate the importance of this phosphorylation event, we developed a phospho-specific EZH2 T367 (pEZH2) antibody and examined the utility of this antibody as a biomarker.

Results: p38-mediated phosphorylation of EZH2 at T367 contributes to the migratory and invasive properties of TNBC in vitro. Phosphorylation at T367 was associated with reduced time to metastasis in vivo. Mechanistically, phosphorylation by p38 does not affect binding to other PRC2 members but may affect EZH2 catalytic activity. In clinical specimens, pEZH2 was upregulated in metastases of breast cancer when compared to matched primary tumors from the same patient.

Conclusions: p38-mediated phosphorylation of EZH2 at T367 contributes to malignancy of triple-negative breast cancers. Our data suggest a new mechanism by which EZH2 is regulated and may offer an additional mechanism by which EZH2 contributes to TNBC progression.

Conflict of interest.

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Conflict of interest.

ODM-207, a novel BET-bromodomain inhibitor as a therapeutic approach for the treatment of patients with castration resistant prostate cancer

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Background: Acetylation of lysine residues on amino-terminal tails of histones has been described to be a hallmark of open chromatin and transcriptional activation, and deregulation of histone acetylation patterns has been linked to aberrant expression of oncogenes resulting in proliferation and tumourigenesis. BET family proteins (Brd2, Brd3, Brd4, and Brd7) bind to acetylated-lysine residues in histones and facilitate the recruitment of transcriptional proteins to chromatin. Pharmacologic inhibition of BET proteins can block cell proliferation in various types of cancer. In many tumour types, the efficacy of BET inhibitors has been attributed to the transcriptional suppression of genes like the MYC. In this study, we evaluated the prostate cancer antitumor activity of ODM-207, a novel, potent and highly selective BET bromodomain inhibitor usin cell lines and in invivo tumor models. Synergy with CDK9 leads to remarkable tumor regressions. HEXIM1 is described as a robust PD biomarker.

Materials and Methods: Prostate cancer cell lines (VaCaP, LNCaP, 22Rv1 and enzalutamide-resistant subtype of VaCaP) were screened for sensitivity to ODM-207 in a 4-day growth inhibition assay. The ability of ODM-207 to downregulate Myc expression was studied in prostate cell lines by immunolabelling Myc in cells. For gene expression analyses, prostate cancer cells were treated with ODM-207 or vehicle and differentially expressed genes were analysed by RNA-sequencing. In the 22Rv1 prostate cancer xenograft model, tumours were established by s.c. inoculation of 22Rv1 cells in male nude mice. Oral treatments with ODM-207 or vehicle control were started when mean tumour size was 122 mm3.

Results: ODM-207 dose-dependently suppressed cell proliferation of prostate cancer cells expressing androgen receptor (AR). Interestingly, ODM-207 also suppresses proliferation enzalutamide-resistant prostate cancer cell lines. Mechanistically, treatment with ODM-207 caused robust transcriptional changes of genes involved in e.g. Myc and AR-dependent transcription without effect on AR protein expression. The potent antiproliferative effects of ODM-207 were associated with cellular senescence. In 22Rv1 prostate cancer xenograft, which expresses both the full-length AR and AR splice variant V7, oral administration of ODM-207 was efficacious in suppressing tumour growth. In this model, ODM-207 inhibited tumour growth at a dose which also inhibits tumour angiogenesis. The observed inhibition of tumour Myc levels correlates with the plasma drug exposures. These results indicate that ODM-207 is able to modulate the expression of BET protein downstream targets in the tumours and that sufficient tumour concentrations can be achieved in vivo.
Conclusions: In summary, ODM-207 is a new generation BET inhibitor for treatment of advanced cancers. It has activity in prostate cancer models that have developed resistance to second generation anti-androgens.


Phase I trial of oral 5-fluoro-2'-deoxyxycytidine with oral tetrahydrouridrouridine in patients with advanced solid tumors


Background: Epigenetic methylation-mediated gene silencing has been implicated in neoplastic transformation. 5-Fluoro-2’-deoxyxycytidine (FdCyd), a fluoropyrimidine nucleoside analog, has a short (10-minute) half-life and is rapidly deaminated in vivo by cytidine deaminase. Co-administration of tetrahydrouridine (THU), an inhibitor of cytidine/deoxycytidine deaminase, has been shown to increase the AUC of FdCyd more than 4-fold. Increased FdCyd exposure allows it to be taken intracellularly and to be incorporated into DNA, inhibiting the action of the enzyme DNA methyltransferase (DNMT). The consequent decrease in DNA methylation can result in the re-expression of tumor suppressor genes. Intravenous (IV) FdCyd+THU has been evaluated in a Phase 1 clinical trial with preliminary evidence of activity. The current trial investigates the oral (PO) administration of both agents, because the intravenous administration schedule was cumbersome. Following achievement of an FdCyd Cmax of 1 μmol/L (DL) or 0.1 μmol/L (DL5) in one patient (pt), consideration was given to increasing the number of days and frequency of FdCyd + THU administration, as this is the level attained following IV administration sufficient to re-express silenced genes in vivo.

Materials and Methods: This is a multicenter 3+3 trial, with NCI as the coordinating center. FdCyd+THU were administered 3–7 daysqwk in 21-day cycles, with THU given PO 30 minutes prior to FdCyd. Main aims of this study were to determine the PK profile of PO FdCyd, the effect of study treatment on re-expression of select genes silenced by methylation and to increase the number of days and frequency of FdCyd + THU administration as this is the level attained following IV administration sufficient to re-express silenced genes in vivo.

Results: A total of 40 pts have been accrued to date on trial. PK data showed the target Cmax for FdCyd was achieved at dose level (DL) 4; following enrollment to DL5, we increased the number of days and frequency of FdCyd + THU administration, as this is the level attained following IV administration sufficient to re-express silenced genes in vivo.

Conclusions: This trial has shown the feasibility of administering FdCyd orally with good drug exposure. Evaluation of disease activity will continue at the MTD. The analysis of gene re-expression in CTCs is ongoing.

No conflict of interest.

Global disruption of productive transcriptional elongation via targeted BET protein degradation

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Background: aberrant gene regulatory processes govern and sustain virtually all aspects and features of human malignancies. Thus, targeting cancer by exploiting non-oncogene addictions to the core transcriptional machinery represents an intriguing therapeutic strategy. However, disruption of oncogenic transcription and cell specification has historically been challenging as transcription factors as well as transcriptional co-activators often lack enzymatically active domains that can be blocked by competitive small molecule inhibitors. To overcome this limitation, we recently devised a strategy for ligand-induced target protein degradation using bifunctional small molecules capable of recruiting the E3 ligase cereblon (CRBN). Focusing our initial efforts on degraders of the BET family of proteins, we observed that they exhibit superior efficacy than competitive BET bromodomain inhibitors through unknown mechanisms.

Materials and Methods: In order to mechanistically explain the differential molecular pharmacology, we used optimized small-molecule degraders and spike-in normalized, kinetic measurements of chromatin structure and state as well as transcriptional output.

Results: We identified a hypersensitivity of T-cell acute lymphoblastic leukemia (T-ALL) cell lines, PDX- and animal models to targeted BET degradation. Mechanistically, we identified that acute loss of BET proteins via targeted degradation leads to a global disruption of transcriptional elongation that features a disproportionally impact on a densely inter-connected, auto-regulatory network of transcription factors central to T-ALL pathogenesis.

Conclusions: Targeted BET degradation enabled us to identify an unrecognized, essential role for BRD4 in global control of transcriptional pause-release and to exploit transcriptional addictions in T-ALL that were intractable using competitive BET bromodomain inhibitors.

Conflict of interest: Other Substantive Relationships: GW is a consultant for C4 Therapeutics; JB is an employee of Novartis Institutes of BioMedical Research as of January 2016; JEB is a Scientific Founder of SHAPE Pharmaceuticals, Syros Pharmaceuticals, Acetyl Pharmaceuticals, Tenisha Therapeutics and C4 Therapeutics and is the inventor on IP licensed to these entities.
Immunotherapy

273 Poster (Board P099)
PD-L1 expression and association with patient outcome in a large pediatric cohort
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Background: Cancers can evade the host immune system by inducing inhibitory signals. Programmed death-1 monoclonal antibody (PD-1 mAb) based therapies were designed to block these signals and have shown promising results in adult clinical trials for the treatment of melanoma and non-small cell lung cancer. It has been proposed that programmed death-ligand 1 (PD-L1) expression represents a potential predictive biomarker of immune checkpoint blockade response in these cancer subtypes. However, literature about the prevalence of PD-L1 expression in the paediatric setting and efficacy of PD-1 mAb therapy in children is lacking. Therefore, we sought to determine the frequency of PD-L1 expression in a large cohort of clinically annotated paediatric tumours with the aim of investigating associations with clinically-pathological features and patient outcome.

Materials and Methods: PD-L1 expression was analysed using immunohistochemistry in 496 paediatric cancer patients (including neuroblastoma, medulloblastoma, low-grade and high-grade glioma, Ewing sarcoma, osteosarcoma and rhabdomyosarcoma) using tissue microarrays. Tumors with >30% cells showing positive membrane staining were considered to have high PD-L1 expression.

Results: PD-L1 expression of any intensity was identified in 12.9% of cases. High PD-L1 expression was found in 3.0% of cases. Neuroblastoma (n=254) showed PD-L1 expression more commonly than any other tumor and, in particular, high PD-L1 expression (4.3%) was significantly associated with risk of relapse (p=0.002). In contrast, low-level PD-L1 expression (26.4%) was significantly associated with longer overall survival (p=0.045). There were no significant associations with patient risk stratification or tumor staging.

Conclusions: High PD-L1 expression level in neuroblastoma patients represents an unfavourable prognostic factor associated with risk of relapse, whereas low PD-L1 expression was associated with better overall survival. This work proposes a novel method to identify neuroblastoma patients with a higher likelihood of cancer recurrence, and suggests that PD-L1 expression may be a predictor of PD-1 mAb therapy efficacy in paediatric cancer patients.

No conflict of interest.

274 Poster (Board P100)
Understanding the mechanisms of immunoresistance in malignant pleural mesothelioma stem cells to find new therapeutic tools
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Malignant pleural mesothelioma (MPM) is a huge medical problem worldwide with a poor prognosis for the poor response to multimodal therapy and the intrinsic immunoresistance. Indeed, MPM creates a strongly immunosuppressive microenvironment. Tumor-derived stem cells (SCs) are responsible for MPM dissemination and progression, but it is not known if they can also exert immunosuppressive properties. Aim of this work was to investigate if MPM-derived stem cells could be responsible for MPM immunoresistance.

From biopsies and pleural effusions of MPM patients we collected and stabilized MPM cell lines. We isolated the SC component by sorting the SOX2*Oct4*Nanog*ALDHhigh cells and checked them for their clonogenicity and self-renewal. High-throughput PCR arrays were used to examine gene expression. Flow-cytometry was used to measure the expression of immune-checkpoints. HMG1B and ATF6 release, and caietretinulin exposure were used as parameters of immunogenic cell death in response to chemotherapy known for inducing immunogenic effects, such as dioxurubin and cisplatin.

MPM SCs had higher endogenous expression of immunosuppressive cytokines such as IL-10 and IL-4, and higher expression of JAK/STAT-related genes such as JAK2-3 and STAT3, than non SCs. Unexpectedly, they had lower expression of the immune-checkpoint ligand PD-L1, resulting poorly responsive to the immunotherapy targeting PD1/PD-L1 system. Chemotherapy induced immunogenic cell death in non SCs MPM cells but not in MPM SCs.

Our study suggests that MPM SCs have immunoevasive properties, resist to the immunogenic cell death induced by chemotherapy and promote tumor-induced immunosuppression by activating the JAK/STAT axis and producing immunosuppressive cytokines. Targeting this axis may open new therapeutic possibilities in overcoming MPM immunoresistance.

No conflict of interest.

275 Poster (Board P101)
Evaluation of immune checkpoint marker co-expression profiles in the tumor microenvironment
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Background: Immunotherapy strategies that target checkpoint pathways have proved promising in recent years. In particular, blocking the immunosuppressive programmed cell death-1 (PD-1) pathway either by targeting PD-1 or one of its ligands, PD-L1, has shown durable efficacy in patients with different cancer types including melanoma and lung cancers. Beyond the PD1/PD-L1 blockade, numerous other checkpoint inhibitors are being developed and various combinatorial approaches are currently being evaluated for clinical efficacy. Thus, measuring biomarkers to predict treatment responses is a desirable ability. While various methods have been employed to measure biomarkers expression, there has been a lack of spatially mapped information at the single-cell level to provide pivotal perception regarding the cellular organization and cell-to-cell interactions in complex tissue has been lacking.

Material and Methods: In this study, we analyzed the tumor microenvironment of 60 archived formalin-fixed embedded (FFPE) non-small cell lung cancer (NSCLC) specimens for single-cell gene expression of immune checkpoint makers by applying the RNAscope® in situ hybridization assay.

Results: The PD-L1 gene expression profile of 56 tumor samples and four adjacent non-tumor tissues presented diverse and heterogeneous expression patterns in both tumor and stromal cells, with PD-L1 positivity in >50% of tumor samples. Co-expression profiles of PD1 or PD-L1 with various other checkpoint markers including PD-L2, LAG3, TIM3, IDO, CTLA4, OX40 revealed complex and differing co-expression profiles in the same cell or tumor environment. Importantly, each tumor sample showed unique co-expression profiles of checkpoint markers.

Conclusions: The findings in this study may provide insight into therapeutic approaches for selecting patients for various different checkpoint inhibitors and combination therapies.

No conflict of interest.

276 Poster (Board P102)
Development of the anti-IL-10 mAb MK-1966 in combination with in situ vaccination of a TLR9 agonist SD-101 for cancer immunotherapy
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MK-1966 is a humanized IgG1/kappa neutralizing monoclonal antibody (mAb) against interleukin-10 (IL-10). IL-10 is an anti-inflammatory cytokine that inhibits secretion of cytokines from activated macrophages, production of CC and CXC chemokines, and a TH1 response, down-regulates MHC and costimulatory molecules on dendritic cells (DCs), and induces regulatory T cells. The immunosuppressive properties of IL-10 support targeting IL-10 in combination with in situ vaccination of Toll-like receptor 9 (TLR9) agonists that induce IL-10 production. SD-101 is a potent TLR9 agonist that is being developed by Dynavax Technologies. SD-101 is a CpG C-class oligodeoxynucleotide, that both activates plasmacytoid DCs (pDCs) and B cells and induces secretion of interferon alpha (IFNα) from pDCs. SD-101 also induces secretion of immunosuppressive molecules including IL-10 that may dampen an anti-tumor immune response. We show in the TC-1 syngeneic mouse bilateral tumor model that SD-101, administered intratumorally into a single tumor in combination with a surrogate anti-mouse IL-10 mAb administered intraperitoneally, resulted in robust anti-tumor activity of not only the injected tumor but the non-injected tumor, demonstrating abscopal effect. The combination also induced gene expression of T cell markers, inflammatory cytokines, and IFNα-inducible genes in both the injected and non-injected tumors in the TC-1 model. SD-101 induced IL-10 and IFNα in human PBMCs, and SD-101 in combination with MK-1966 strongly induced IFNα in human PBMCs. In addition, we have demonstrated that SD-101 induced expression of IFNα-inducible cytokines, cytokines including IL-10, and immune cell activation markers in various human tumor specimens.
using a histoculture platform. Currently, the safety, pharmacokinetics, and pharmacodynamics of treatment with MK-1966 in combination with SD-101 are being evaluated in patients with advanced cancers in a Phase 1 study (NCT02731742).

**Conflict of interest:** Corporate-sponsored Research: All authors are full-time employees of Merck & Co. Inc. Other Substantive Relationships: U. Phan, G. Ayangou, S. Sadekova, T. McClanahan, A. Willingham, R. Raubertas, and R. Kastelein are shareholders of Merck & Co. Inc.

**277** Poster (Board P103)

**PD-L1 targeting peptides identified by bacterial surface display methods as potential drugs for tumor immunotherapy**

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**Background:** Recently, antibodies which could block CTLA-4 or PD-1 (immune checkpoints) have been approved by FDA and are applied in clinic successfully. However, the costs of those antibodies are very high and the effect of those antibodies needs to be improved. In our study, we aimed to obtain peptides which could bind with PD-L1 and block the interaction between PD-1 and PD-L1 effectively. Thus, those peptides could work as alternates for antibodies at the aspect of tumor immunotherapy.

**Materials and Methods:**
1. Screen and identify the PD-L1 binding peptides with bacterial surface display method. 15-mer bacterial peptide library was used for screening the binding peptides to PD-L1. FACS sorting was used to enrich the binding peptide library for PD-L1.
2. Evolve the binding property of peptides with focus peptide library. Through flanking the conserved peptides sequences, focus peptide library was constructed and the sorting procedure was repeated for specific binding peptides to PD-L1.
3. Examine the affinity and specificity of binding peptides to PD-L1. SPR and ELISA methods were used to examine the specificity and affinity of PD-L1 binding peptides.
4. Set up the assay for the PD-L1 binding peptide function in vitro – mixed lymphocytes reaction (MLR). The yield of IFN-γ has the negative relationship with the blockage effect of peptides.
5. Examine the PD-L1 binding peptides biodiversity in vivo. The mixture of the reactive T-cell and H490 cells were into the NOD mice, and the peptides were administrated into the loci of tumors. The volume of tumors and survive period of mice could reflect the therapeutic effect of peptides.

**Results:**
1. The sequences of binding peptides to PD-L1 have been obtained. Fifteen various peptides sequences have been obtained from random library sorting, which could bind with PD-L1.
2. The consensus sequences of PD-L1 binding peptides have been discovered. One consensus sequence (CWCCR) was discovered.
3. The affinity and specificity of PD-L1 binding peptides have been examined. The KD value of one of binding peptides were 94.7 nM, and the peptide could only bind with hPD-L1, not hPD-L2 and mPD-L1.
4. PD-L1 binding peptides have been verified to block the PD-1/PD-L1 biological function, therefore, they can be applied as potential drugs for tumor immunotherapy.

**No conflict of interest.**

**278** Poster (Board P104)

**Development of a new type of chimeric mouse/human models – HuCell™ model for direct evaluation of anti-PD-L1 antibodies**

M. Qiao1, J. Ding2, R. Zhang1, Z. Li1, J. Zheng1, J. Zhang2, Q. Shi1.

**Background:** Therapies that perturb binding of the programmed death-ligand 1 (PD-L1) to its receptor, programmed cell death protein 1 (PD-1), have achieved unprecedented rates of sustained clinical response in patients with various cancer types. Mouse surrogate antibodies were initially evaluated in syngeneic mouse models as a proof of concept for testing the efficacy of anti-PD-L1 therapies. However, there is an urgent need to develop appropriate animal models to directly evaluate anti-human PD-L1 antibodies that are lack of cross-reactivity with mouse PD-L1 before they reach clinical trials.

**Material and Methods:** Here we describe the development of two chimeric mouse/human cell lines, hPD-L1-H22 and hPD-L1-EMT-6 and the corresponding HuCell™ animal models to test anti-human PD-L1 antibodies. PD-L1 expression was profiled by FACS using an anti-mouse PD-L1 antibody, which confirmed expression of PD-L1 in a series of murine cancer cell lines. H22 liver cancer and EMT-6 breast cancer cell lines with moderate expression level of PD-L1 were selected due to its in vivo sensitivity towards anti-PD-1 and anti-PD-L1 agents. The CRISPR/Cas9 system was employed to knock out the murine PD-L1 and then the human counterpart was introduced. Targeted knockdown was confirmed by sequencing, while expression of human PD-L1 was detected by FACS using an antibody that specifically recognizes human PD-L1 and does not cross-react with the mouse isoform. The engineered hPD-L1-H22 or hPD-L1-EMT-6 cells were inoculated subcutaneously into immunocompetent mice to establish an in vivo model with a fully competent murine immune system. The tumor growth was monitored by efficacy evaluation of two benchmark anti-human PD-L1 antibodies (MPDL3280a and MEDI4736).

**Results:** Significant tumor growth inhibition was observed in both benchmark antibodies.

**Conclusion:** Our PD-L1 HuCell™ mouse model may be a valuable tool to evaluate the in vivo activity of anti-human PD-L1 antibody therapies either as a single agent or with combinational strategies. Similar engineering approach may be applied to more murine cell lines (e.g. MC-38, B16F10 and MBT-2, etc) to provide a spectrum of cell lines with different diseases and genetic makeup for immunotherapy strategies involving anti-hPD-L1 antibodies.

**Conflict of interest:** Ownership: Crown Bioscience Inc.

**279** Poster (Board P105)

**TAK-659, a dual SYK/FIT3 inhibitor, leads to complete and sustained tumor regression and immune memory against tumor cells upon combination with anti-PD-1 agent**

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TAK-659 is an inhibitor of Spleen Tyrosine Kinase (SYK) that is being evaluated in hematological malignancies in multiple clinical trials. SYK is a non-receptor cytoplasmic tyrosine kinase that is a common member of various signal transduction cascades in cells of the hematopoietic lineage including those involved in B-cell receptor (BCR) activation. With the recent success of immune checkpoint blockade agents in treating multiple types of cancer, increased attention has been paid in understanding the mechanism of the immune suppressive tumor microenvironment. MDSC (Myeloid Derived Suppressor Cell) mediated immune suppression has been reported in many solid tumors. Both published and in-house studies show that SYK inhibition results in loss of MDSCs and activation of T-cell response both in vitro and in vivo. Although TAK-659 does not inhibit T cells directly, a synergistic activity is expected with a PD-1 inhibitor in combination with TAK-659 in tumors where SYK mediated MDSCs or B cell suppression is active. Our pre-clinical data has shown a decrease in MDSCs or B220+ B-Cells following treatment with TAK-659 in the CT-26 syngeneic colon cancer model. When TAK-659 was administered daily, orally, in combination with anti-PD-1 therapy in the C326 model, complete tumor regressions were achieved in 80% of the mice and the animals were tumor free for over 100 days post-treatment. When these animals were re-challenged with CT-26 tumor cells, they failed to form tumors suggesting vaccine-like memory effects against these cells in these animals. Similar combination efficacy and vaccine memory effect has been observed in other syngeneic models as well. Taken together, TAK-659 treatment in combination with anti-PD-1 resulted in complete tumor growth suppression, prolonged tumor free survival and potential immune memory against tumor cells supporting the rationale for examining the addition of TAK-659 to anti-PD-1 therapy in the clinic.

**No conflict of interest.**
Poster Session – Immunotherapy, Wednesday 29 November 2016

280  Poster (Board P106)
Transgenic antigen-specific, HLA-A*02:01-allo-restricted cytotoxic T cells recognize and kill tumor associated antigen STEAP1+ tumor cells in vivo
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Background: Paediatric cancers, including Ewing sarcoma (ES), are only weakly immunogenic and the tumour-patients immune system often is devoid of effector T cells for tumour elimination. Based on expression profiling technology targetable tumour associated antigens (TAA) are identified and exploited for engineered T cell therapy. Here, the transgenic recognition and lytic potential of transgenic allo-restricted CD8+ T cells directed against the ES-associated antigen STEAP1 was examined. The STEAP1 reactive TCR was derived from isolated STEAP1-specific T cell clones.

Material and Methods: STEAP1 specific transgenic T cells were characterized in vivo via ELISpot, flow cytometry and xCELLigence assay, followed by in vivo analysis using a Rag2−/− c−/− mouse model. Therefore, animals were inoculated with luciferase-expressing ES cells, prior to treatment with specific or unspecific T cells, respectively. Subsequently, tumour growth was monitored via bioluminescent measurement. Resected tumours were further analysed by histological examination for T cell infiltration.

Results: TCR transgenic T cells specifically recognized STEAP1+ pulsed or STEAP1 expressing cells in the context of HLA-A*02:01 with minimal cross-reactivity as determined by specific IFNγ release. Transgenic T cells lysed cells and inhibited growth of HLA-A*02:01+positive ES lines more effectively than HLA-A*02:01negative ES lines. Transferred STEAP1 specific T cells engrafted in blood, spleen and tumour and inhibited tumour growth more effectively than unspecific T cells. Histological examination demonstrated a stronger tumour infiltration of CD3+ T cells in animals treated with STEAP1-specific compared to unspecific T cells.

Conclusion: Our results identify TCRs capable of recognizing and inhibiting growth of STEAP1 expressing ES cells in vitro and in vivo in a highly restricted manner. As STEAP1 is overexpressed in a wide variety of cancers, we anticipate these STEAP1-specific TCRs to be potentially useful for immunotherapy of other STEAP1 expressing tumours.

No conflict of interest.

281  Poster (Board P107)
PET imaging of the PD-1/PD-L1 checkpoint in naive and irradiated tumor-bearing mice
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Background: There is increasing evidence that antibodies blocking the PD-1/PD-L1 checkpoint (either anti-PD-1 or anti-PD-L1) increase in-field anti-tumor responses to ionizing radiation and enhance abscopal effects on non-irradiated metastases. Here, we developed PET tracers based on therapeutic antibodies to visualize whole-body expression of PD-1 and PD-L1 in mice and the biodistribution of the surrogate checkpoint-blocking antibodies.

Materials and Methods: Two novel PET tracers were developed based on anti-PD-1 and anti-PD-L1 checkpoint-blocking antibodies. Non-invasive PET imaging was performed on naive and tumor-bearing mice. Mice bearing s.c. B16 melanomas were treated with hypofractionated radiation therapy (HRT). Combination with CTLA-4 checkpoint blockade specific PET imaging, PD-1 or PD-L1 knockout mice and PD-L1-deficient B16 cells generated using the CRISPR/Cas technology served as specificity controls.

Results: The newly developed PET tracers allowed the highly specific and high-resolution imaging of PD-1 and PD-L1 expression and of the biodistribution of the two therapeutic antibodies in both naive and tumor-bearing mice treated with HRT and CTLA-4 checkpoint blockade. Imaging of the respective knockout mice, blocking experiments with an excess amount of unlabeled antibodies, and the analysis of animals bearing both wild-type B16 melanomas and PD-L1-CRISPR knockout mice demonstrated the high specificity of the two newly developed PET tracers. The in vivo imaging data were confirmed by ex vivo biodistribution analyses. The targets of the PET tracer antibodies were verified by ex vivo flow cytometric analyses. Visualization of immune-related adverse events was also possible.

Conclusion: We developed two innovative PET tracers that allow imaging the expression of the receptor/ligand pair of the important PD-1/PD-L1 checkpoint and the biodistribution of surrogate checkpoint-blocking antibodies in fully immunocompetent mice. This technology also enables whole-body pictures of combination radio/immunotherapies.

No conflict of interest.

282  Poster (Board P108)
Pre-clinical characterization of avelumab (anti-human PD-L1) reveals an enhanced anti-tumor efficacy in hlgG1 isotype
Y. Qu1, W.W. Prior1, Y. Abdiche2, W. Chen2, S. Potluri3, J. Chapaqir-Riggers1, P. Patel1, J. Lin1. 1Pfizer Inc., WIRDORD Rinat, South San Francisco, CA, USA

Background: Antibodies blocking PD-1/PD-L1 axis have been designed as either hlgG4 or as engineered hlgG1 isotypes which has low or no binding to the FcγR (referred as hlgG1 silent). Avelumab is a fully human antibody of the immunoglobulin (IgG) 1 isotype that specifically binds to programmed death ligand 1 (PD-L1). Because PD-L1 can be expressed on activated T cells, in-depth understanding of PD-L1’s expression patterns within the tumor microenvironment is required for optimal iso type selection. Dahan and colleagues recently reported that FcγRs have different impact on anti-PD-L1 antibodies’ activity by using mouse surrogate antibodies and FcγR deficient mouse models. Since avelumab cross-reacts to mouse PD-L1, and has been investigated in multiple clinical trials, we engineered avelumab into a hlgG1 silent isotype, to investigate their mechanism of action (MOA) in preclinical models.

Material and Methods: We generated avelumab hlgG1 silent (hlgG1 D265A/N297A mutant) and compared its efficacy to avelumab hlgG1 in pre-clinical models. CD4, CD8, and NK in vivo depletion studies and tumor infiltrating lymphocyte (TIL) profiling by FACS were conducted to distinguish the MOA between avelumab hlgG1 and hlgG1 silent in vivo. We also conjugated avelumab with fluorophore and used it for immunofluorotyping PD-L1 on TILs isolated from cancer patients.

Results: Avelumab hlgG1 and hlgG1 silent have identical antigen binding affinity based on biosensor analysis. Human IgG1 silent has been confirmed to have minimal detectable binding affinity towards all FcγR subfamilies. In two independent murine syngeneic models, enhanced anti-tumor efficacy was observed with avelumab hlgG1 compared to the hlgG1 silent version. Avelumab hlgG1 treatment did not decrease CD4 or CD8 T cell numbers in either tumor or spleen. We also examined the frequency of different myeloid subpopulations within the tumor, and compared the PD-L1 expression levels on each of the populations. Consistent with a previous report, avelumab hlgG1 decreased the most abundant, PD-L1+ myeloid suppressor populations within the tumor. CD4 and NK in vivo depletion study results confirmed that engagement of NK-mediated ADCC and CD8 T effector cells are critical for anti-PD-L1 antibody’s efficacy. Interestingly, CD4 in vivo depletion study indicates that removal of inhibitory signal between CD4 T cells and myeloid suppressor cells might be another critical contributor towards avelumab hlgG1 activity comparing with its hlgG1 silent.

Conclusions: Pre-clinical characterization of Avelumab confirms the role of ADCC as a critical mechanism driving enhanced anti-tumor efficacy. Interestingly, CD4 T cells and myeloid suppressor cells might be another critical contributor towards avelumab hlgG1 activity comparing with its hlgG1 silent.

Conflict of interest: Ownership: John C. Lin has ownership interest (including patents) in Pfizer Inc. No potential conflicts of interest were disclosed by other authors.

283  Poster (Board P109)
Development of an RNA loaded dendritic cell (DC) immunotherapy starting from tissue obtained via needle biopsy
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Background: AGS-003 is an autologous tumor RNA-loaded dendritic cell-based immunotherapy being tested in advanced renal cell carcinoma (RCC)
patients. In the Phase 3 ADAPT trial, RNA encoding tumor antigens is amplified from 100 mg of primary tumor obtained via nephrectomy. Here we evaluated the feasibility of RNA amplification from smaller tumor masses obtained using a needle biopsy.

Methods: Biopsies of primary RCC were obtained from either resected tumor after nephrectomy or prior to nephrectomy using radiological guidance and placed into a preservative solution, RNAlater. RNA was then extracted and amplified using a modification of the process originally developed for 100 mg of tissue.

Results: Process adjustments were made for RNA extractions from larger (>100 mg) tumor masses that were manipulated with scalpel and forceps to 10 and 25 mg. However, manipulation of fine needle aspirates with forceps was not feasible due to observed loss of integrity for the tissue obtained from biopsy procedures and required further process modifications. Comparison of the mass of tissue prior to placement into RNAlater versus the mass recovered from it resulted in an average loss of 35% of mass (N = 18), possibly due to dehydration and/or tissue integrity losses. Nevertheless, the RNA extraction and amplification for the recovered tissues was successful achieved.

Based on the developed process, a Phase II clinical study was initiated with primary RCC tissue procured via needle biopsy prior to nephrectomy. Interim results from the first three subjects demonstrate the ability to produce 30, 14 and 7 doses of the immunotherapeutics from 7 mg, <10 mg and 51 mg of tissue respectively.

Conclusion: RNA amplification from a needle biopsy is feasible. These findings afford the ability to initiate manufacturing of AGS-003 prior to nephrectomy, thus allowing more rapid dosing after recovery from surgery. In addition, these findings may extend the applicability of AGS-003 to other solid tumor indications where surgical resection is not part of the standard of care.

Conflict of interest: Ownership: IT, JH, AS, GL, CN are employees of Argos Therapeutics, a publicly traded company.

284 Poster (Board P110) Phase I clinical study of intratumoral injection of oncolytic Clostridium novyi-NT spores in patients with advanced cancers F. Janku1, R. Murthy2, A. Wang-Gillam2, D. Shepard2, T. Helgason1, F. Henry3, C. Rudin4, S. Huang4, D. Sakamuri4, S. Solomon5, A. Collins3, B. Kreed1, M. Miller2, S. Saha2, D. Tung3, M. Vartarasan1, L. Zhang4, H. Zhang4, M. Gounder5, T. Wulff1. 1 The University of Texas MD Anderson Cancer Center, Department of Investigational Cancer Therapeutics Phase I Clinical Trials Program, Houston, USA; 2 The University of Texas MD Anderson Cancer Center, Department of Interventional Radiology, Houston, USA; 3 Washington University in St. Louis, Gastrointestinal Oncology Program, St. Louis, USA; 4 Cleveland Clinic, Tausig Cancer Institute Phase I and Sarcoma Programs, Cleveland, USA; 5 Memorial Sloan-Kettering Cancer Center and Weill Cornell Medical College, Department of Interventional Radiology, New York, USA; 6 BioMed Valley Discoveries Inc., BioMed Valley Discoveries Inc., Kansas City, USA

Background: Intratumoral injection of Clostridium novyi-NT (C. novyi-NT), an attenuated strain of Clostridium, induced a macroscopically precise response, which was restricted to tumor tissue in a rat orthotopic brain tumor model, in companion dogs bearing spontaneous solid tumors and in the first patient treated on a Phase I human clinical trial (Roberts et al, Sci Transl Med. 2014). C. novyi-NT lyses malignant cells by secreting lipases, proteases, other hydrolytic enzymes, and recruiting inflammatory cells to tumors eliciting anti-tumor immune responses in animals. Furthermore, intratumoral injection can plausibly induce an immune mediated abscopal effect in non-injected tumor sites.

Material and Methods: The objectives of this Phase 1 study using the 3+3 dose escalation design were to determine the dose limiting toxicities (DLTs), maximum tolerated dose (MTD) and preliminary activity of C. novyi-NT when administered as a single intratumoral injection. Adult patients with advanced cancers and at least one accessible, superficial tumor mass (1–12 cm in size) that did not involve bone, were eligible.

Results: To date, a total of 19 patients (women, n = 10; men, n = 9) with advanced cancers (sarcoma, n = 10; carcinoma, n = 7; melanoma, n = 1; chondroma, n = 1), median age 55 years, have received an intratumoral injection ranging from 1 × 10⁴ to 100 × 10⁴ spores in 5 dose levels. Evidence of C. novyi-NT germination has been noted in all dose levels in 8 (42%) patients and has consisted of pain in the injected tumor, fever, elevated WBC with a left shift, an elevated C-reactive protein, tumor necrosis and gas pockets in the injected tumor on radiographic imaging. One DLT (Grade 4 sepsis) was observed on the dose level 4. Grade 3 treatment-related adverse events included respiratory insufficiency and pathologic fracture of right humerus. Anticancer activity was observed in all 8 patients where C. novyi-NT germination resulted in extensive, in some cases, complete central necrosis of the injected tumor. Two patients had between 22–24%, shrinkage in the injected tumor based on RECIST assessment. Five out of 7 patients who were evaluated at Month 2 had an overall response of stable disease. 2 out of the 7 patients had progressive disease. Moreover, 2 out of the 7 patients experienced stable disease at Month 4. Correlative studies for predictors of immune response included pre and post treatment biopsies from injected and non-injected lesions, serum cytokine analysis, and immunophenotyping of patients’ circulating lymphocytes.

Conclusions: A single dose of intratumoral injection of Clostridium novyi-NT is feasible and has promising anticancer activity. A study of PD1 antibody with C. novyi-NT has been designed to evaluate any combination of effects of PD1 blockade and C. novyi-NT on tumor burden.

Conflict of interest: Corporate-sponsored Research: Filip Janku, Andrea Wang-Gillam, Dale Shepard and Mirnal Gounder received research funding BioMed Valley Discoveries through their institutions to support this study. Other Substantive Relationships: Amanda Collins, Brent Kreider, Maria Miller, Saurabh Saha, David Tung, Mary Vartarasan, Lingping Zhang and Halle Zhang are current or former employees of BioMed Valley Discoveries.
Further, it reduced the expression of immunosuppressive enzymes, IDO1 and ARG1 both in tumor cells and myeloid-derived suppressor cells (MDSCs). Remarkably, 4SC-202 distinguished from comparator HDAC inhibitors as it is not toxic for tumoral immune cells. By enhancing the immunogenicity of tumor cells and sparing the tumoral immune cells, 4SC-202 enables the immune system to attack tumor cells and thereby, provides a well-founded rationale for the combination with checkpoint inhibitors. In a syngeneic colon carcinoma CT26 mouse model, the monotherapy with 4SC-202 inhibited CT26 tumor growth and strongly induced the infiltration of cytotoxic T cells. In a second in vivo experiment, 4SC-202 was combined with anti-PD-1 therapy in a Renca orthotopic model which revealed a markedly anti-tumoral response in the combination treatment whereas the anti-PD-1 monotherapy was not active. These in vivo combination results demonstrated that 4SC-202 is able to sensitize tumors for effective treatment with checkpoint inhibitors in indications where checkpoint inhibitors alone are inactive.

**Conclusion:** The epigenetic modulator 4SC-202 which already displayed well tolerability, objective responses (1 CR, 1 PR) and disease stabilization in several patients in a phase I clinical trial with relapsed or refractory hematological tumors, proved to be an ideal combination partner for checkpoint inhibitors due to its immune priming capacity, the excellent safety profile and the oral formulation allowing convenient application and flexible dosing schedules.

**Conflict of interest:** Other Substantive Relationships: The authors are employees of 4SC and share. D. Vitt is CSO and CDO of 4SC.

**Background:** Immune checkpoint inhibitors are now established therapies in many advanced cancers. Preliminary studies suggest combining immune checkpoint inhibitors with platinum-based chemotherapy may enhance antitumor activity. The primary objective of this multicenter study was to evaluate the safety and tolerability durvalumab (Du), a PD-L1 inhibitor, ± tremelimumab (Tr), a CTLA-4 inhibitor, in combination with one of four standard platinum-doublet regimens (pemetrexed (pem), gemcitabine (gem), etoposide (each with cisplatin) or nab-paclitaxel (nabP, with carboplatin)), in order to establish a recommended phase II dose (RP2D) for each combination.

**Methods:** Patients (pts) with advanced solid tumors, regardless of tumor PDL1 status or number of prior therapies, were enrolled into one of four cohorts. Dose level (DL) 0 added Du 15 mg/kg IV q3wks + Tr 1 mg/kg q6wks + P 100 mg/m² q3wks, DL1 = Du 15 mg/kg q3wks + Tr 1 mg/kg q3wks + P 100 mg/m² q3wks, DL2a = Du 15 mg/kg q3wks + Tr 1 mg/kg q6wks × multiple doses; DL2b = Du 15 mg/kg q3wks + Tr 3 mg/kg q6wks × multiple doses.

**Results:** Seventy-eight pts (median age 60 years, range 30–80; 51% male, 99% ECOG PS ≤1) were enrolled of which 63% were chemonaive. Thus far 285 cycles have been administered. Across dose levels, the majority of drug-related adverse events (AEs) were Grade 2. Most AEs were attributable to chemotherapy though attribution of some AEs could be either chemotheraphy or immune-related (renal, hepatic, skin and pulmonary toxicity). AEs that were considered by the investigator related to either Du or Tr were mainly Grade 2 and manageable, the most common of which were fatigue (45%), nausea (20%), rash (14%) and anorexia (12%). Four patients had possible, but reversible DLTs, including hepatitis [1 each at DLO (nabP) and DL2a (gem)], pneumonitis [1 pt each at DL1 (etoposide) and DL2a (pem)]; Accrual is ongoing and expansion cohorts are planned at the RP2D.

**Conclusions:** In this PD-1 unselected patient population, Du 15 mg/kg q3wks and T 3 mg/kg q6wks has to date been safely combined platinum-doublt chemotherapy. Updated safety data and clinical activity will be presented.

**No conflict of interest.**

289 Poster (Board P115)

A Canadian Cancer Trials Group phase II study of durvalumab with or without tremelimumab ± standard platinum-doublet chemotherapy in patients with advanced, incurable solid malignancies (IND.226)

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**Background:** Immune checkpoint inhibitors are now established therapies in many advanced cancers. Preliminary studies suggest combining immune checkpoint inhibitors with platinum-based chemotherapy may enhance anti-tumor activity. The primary objective of this multicentre study was to evaluate the safety and tolerability durvalumab (Du), a PD-L1 inhibitor, ± tremelimumab (Tr), a CTLA-4 inhibitor, in combination with one of four standard platinum-doublet regimens (pemetrexed (pem), gemcitabine (gem), etoposide (each with cisplatin) or nab-paclitaxel (nabP, with carboplatin)), in order to establish a recommended phase II dose (RP2D) for each combination.

**Methods:** Patients (pts) with advanced solid tumours, regardless of tumour PDL1 status or number of prior therapies, were enrolled into one of four cohorts. Dose level (DL) 0 added Du 15 mg/kg IV q3wks + Tr 1 mg/kg q3wks + P 100 mg/m² q3wks or T 3 mg/kg q6wks × multiple doses; DL2a = Du 15 mg/kg q3wks + Tr 1 mg/kg q3wks + P 100 mg/m² q3wks × multiple doses.

**Results:** Seventy-eight pts (median age 60 years, range 30–80; 51% male, 99% ECOG PS ≤1) were enrolled of which 63% were chemonaive. Thus far 285 cycles have been administered. Across dose levels, the majority of drug-related adverse events (AEs) were ≤ Grade 2. Most AEs were attributable to chemotherapy though attribution of some AEs could be either chemotheraphy or immune-related (renal, hepatic, skin and pulmonary toxicity). AEs that were considered by the investigator related to either Du or Tr were mainly ≤ Grade 2 and manageable, the most common of which were fatigue (45%), nausea (20%), rash (14%) and anorexia (12%). Four patients had possible, but reversible DLTs, including hepatitis [1 each at DLO (nabP) and DL2a (gem)], pneumonitis [1 pt each at DL1 (etoposide) and DL2a (pem)]; Accrual is ongoing and expansion cohorts are planned at the RP2D.

**Conclusions:** In this PD-1 unselected patient population, Du 15 mg/kg q3wks and T 3 mg/kg q6wks has to date been safely combined platinum-doublt chemotherapy. Updated safety data and clinical activity will be presented.

**No conflict of interest.**

290 Poster (Board P116)

Incidence of immune-related adverse events in patients who received immunotherapy and radiographic analysis to predict pneumonitis

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**Background:** Despite the promising results with immunotherapies, immune-related adverse events (irAEs) are a challenge. In the current study, the incidence of irAEs among different immunotherapy trials was investigated. Additionally, radiological features from baseline CT scan to predict the development of pneumonitis were evaluated.

**Methods:** Patients with advanced cancer treated with immunotherapy at the Department of Investigational Cancer Therapeutics at MD Anderson Cancer Center from January 2010 to June 2015 were included. Immunotherapies were categorized into checkpoint inhibitors, cytokines,
Background: Patients diagnosed with glioblastoma multiforme (GBM) have an extremely poor prognosis. Consequently there is an urgent need for new therapeutic options. A deletion variant of the epidermal growth factor receptor (EGFR), EGFRvIII, is described as a highly specific tumor antigen and the high activity of AMG 596 in vitro and in vivo, together with the absence of overt toxicity in a preclinical safety study with a large antigen and the high specificity of AMG 596, we used orthotopic xenograft models in immunodeficient mice. On day 1 glioblastoma cells were injected intracranially and in vitro-expanded human T cells were measured in flow cytometry-based cytotoxicity assays using healthy donor peripheral blood mononuclear cells co-incubated for 48 hours with EGFRvIII expressing cell lines. To investigate in vivo anti-tumor activity, we used orthotopic xenograft models in immunodeficient mice. On day 1, glioblastoma cells were injected intracranially and in vitro-expanded human T cells were injected intraperitoneally on day 5. AMG 596 or vehicle was administered by intravenous (IV) bolus injection into the lateral tail vein once daily for 16 consecutive days starting on day 8 after tumor cell injection. Duration of survival was monitored. To assess the potential for toxicity, we conducted a dose range finding study in healthy cynomolgus monkeys. Here AMG 596 was administered as a continuous IV infusion for seven days at different doses. Tolerability of the treatment was assessed by observation of clinical signs, measurement of cytokine levels and white blood cell counts, and analysis of changes in lymphocyte subpopulations in the peripheral blood.

Results: We demonstrated specificity of AMG 596 for EGFRvIII versus wild type EGFR, HER2, HER3 and HER4 in flow cytometry binding assays. AMG 596 showed high activity in recruiting T cells against EGFRvIII expressing GBM cell lines in vitro with half maximal effective concentration values below 1 µM. In orthotopic xenograft models, AMG 596 significantly prolonged survival of treated mice versus control animals (p = 0.001). Furthermore, no toxicities were observed in cynomolgus monkeys at serum concentrations of up to 7.6 nM.

Conclusions: Given the unique tumor specificity of EGFRvIII as a target antigen and the high activity of AMG 596, we considered potential for AMG 596 for the treatment of EGFRvIII-positive GBM. A first-in-human study of AMG 596 in relapsed EGFR-positive GBM patients is planned.

Conflict of interest: Ownership: All authors: stock of Amgen Inc. Advisory Board: None. Board of Directors: None. Corporate-sponsored Research: Sponsored by Amgen Inc. and subsidiaries. Other Substantive Relationships: All authors: Employment by Amgen Inc. and subsidiaries.

292 Novel small-molecule inhibitors of ecto-nucleotidase CD73: Activation of human CD8+ T cells and effects on tumor growth and immune parameters in experimental tumor models

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Introduction: Extracellular adenosine triphosphate (ATP) in the tumor-micro-environment (TME) is sequentially hydrolyzed to adenosine (ADO) by the ecto-nucleotidases CD39 and CD73. ADO is a potent inhibitor of T-cell activation resulting in an immunosuppressed phenotype. Various anti-CD73 antibodies are being advanced into clinical trials but only a limited number of potent and selective small-molecule CD73 inhibitors, such as those described here, have been described.

Methods: Enzymic assays: To determine the activity of our small-molecule inhibitors, ecto-nucleotidase activity was assessed based on the amount of inorganic phosphate produced after 50-min incubation with 25 nM ATP or ATP, in the presence of varying concentrations of test compound. Various cellular systems were used.

Results: A000830 is a representative of a series of potent and specific small-molecule inhibitors of human and mouse CD73. Using overexpressing cell lines, the IC50 for A000830 against human and mouse CD73 was 1.0 nM and 3 nM, respectively. A000830 blocked AMP to ADO generation by human ovarian cancer cells (SKOV-3) with IC50 = 0.2 nM and >10,000-fold selectivity relative to other ecto-nucleotidases and a large panel of unrelated enzymes, receptors and ion channels. Using primary human and mouse CD8+ T-cells, A000830 robustly reversed ADO-driven inhibition of proliferation, CD25 expression, and IFNγ and granzyme B production.

Conclusion: A000830 is a representative of a series of potent and specific small-molecule inhibitors of human and mouse CD73. Using overexpressing cell lines, the IC50 for A000830 against human and mouse CD73 was 1.0 nM and 3 nM, respectively. A000830 blocked AMP to ADO generation by human ovarian cancer cells (SKOV-3) with IC50 = 0.2 nM and >10,000-fold selectivity relative to other ecto-nucleotidases and a large panel of unrelated enzymes, receptors and ion channels. Using primary human and mouse CD8+ T-cells, A000830 robustly reversed ADO-driven inhibition of proliferation, CD25 expression, and IFNγ and granzyme B production.

Conflict of interest: Corporate-sponsored Research: Arcus Biosciences Inc.
survival was observed with no death observed in case of 4-1BB targeting antibody combined to either anti-PD-1, or anti-CTLA-4 monoclonal antibody and only 14% of death when anti-4-1BB antibody is combined to anti-PD-L1 antibody. Moreover, splenomegaly was observed in mice receiving anti-PD-L1 or anti-CTLA-4 monoclonal antibody alone. Splenomegaly was decreased in mice treated with anti-4-1BB agonist antibody in combination. Due to lethality, it is hard to conclude on efficacy of anti-PD-1, anti-PD-L1 or anti-CTLA-4 single therapy when used alone. In group receiving 4-1BB targeting antibody combined to either anti-PD-1 or anti-CTLA-4 antibody. However, a significant antitumor efficacy was evidenced when 4-1BB targeting antibody was combined to anti-PD-L1 antibody (optimal T/C value of 37% on D30). Despite the increase in survival, no significant efficacy was observed in groups receiving 4-1BB targeting antibody combined to either anti-PD-1 or anti-CTLA-4 antibody. Increased survival allowed analyzing antitumor efficacy of anti-PD-1, anti-PD-L1 or anti-CTLA-4 antibody when combined to anti-4-1BB antibody. No conflict of interest.

294 Poster (Board P120)
Preclinical assessment of anti-tumor activity and immune response in syngeneic tumor models

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Preclinical immuno-oncology (I/O) needs identification and refinement of tumor models that recapitulate relevant biological dynamics. We tested several murine models for their response to checkpoint inhibitors like anti-CTLA-4, anti-PD-L1 and anti-PD-1 antibodies and found sensitive, moderately sensitive and insensitive models. Furthermore, since the application of more sophisticated endpoints is critical to confidently assess drug sensitivities we also evaluated the immune profiles of these models following treatment.

The CT26 colon model was identified as sensitive to a variety of I/O agents. Following treatment with anti-PD-L1 antibody we observed a 40% response rate which could prove useful in a combination setting. To further analyze how treatment with anti-PD-L1 modifies the immune response we utilized flow cytometry to characterize the immune profile of the model. Tumors from treated mice displayed an increased abundance of CD45+ cells and NK cells when compared to isotype control mice. Additionally, MDSCs from treated mice had a more M-MDSC dominant phenotype vs the control mice that had a more G-MDSC dominant phenotype.

The 4T1 mammary carcinoma model when treated with anti-PD-1, anti-PD-L1 or anti-CTLA-4 antibody. Increased survival allowed analyzing antitumor efficacy of anti-PD-1, anti-PD-L1 or anti-CTLA-4 antibody when combined to anti-4-1BB antibody. No conflict of interest.

295 Poster (Board P121)
Arginase inhibitor CB-1158 elicits immune-mediated anti-tumor responses as a single agent and enhances the efficacy of other immunotherapies

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Myeloid derived suppressor cells (MDSCs) and polymorphonuclear cells (PMNs) limit effective anti-tumor immune responses; however there are no approved clinical agents that directly antagonize the activity of these cells. One of the immunosuppressive mechanisms of MDSCs and PMNs is the expression and secretion of the enzyme arginase into the tumor microenvironment resulting in local depletion of the amino acid arginine, a key nutrient required by T-cells and natural killer (NK) cells to proliferate and mount an effective anti-tumor response. To assess the potential of arginase inhibition as a therapeutic strategy, we surveyed the abundance of arginase in tumor and plasma from cancer patients across multiple histologies, a modert with previous reports, we observed that multiple tumor types have substantial arginase-expressing PMN infiltrates and that cancer patients have higher levels of plasma arginase and lower levels of plasma to healthy controls. This study explored the anti-tumor activity of CB-1158 blocked T-cell proliferation. The addition of CB-1158 blocked activated arginase activity, maintained arginase levels, and allowed T-cells to proliferate in the presence of MDSCs/PMNs, highlighting arginase as a prominent immunosuppressive mechanism of these myeloid cells. CB-1158 has high oral bioavailability in mice and rats. Twice-daily oral dosing of CB-1158 produced dose-dependent pharmacodynamic increases in plasma and tumor arginine levels and resulted in single-agent anti-tumor efficacy in several murine syngeneic tumor models including Lewis Lung carcinoma (LLC), Madison-109 lung carcinoma, and B16F10 melanoma. Immunodepletion of either CD8+ T-cells or NK-cells partially antagonized the anti-tumor effect of CB-1158 in the LLC and B16F10 models indicating that the CB-1158 acts by an immune cell-mediated mechanism. Consistent with immune-mediated anti-tumor efficacy, CB-1158 bearing mice resulted in increases in tumor infiltrating CD8+ T-cells, increased levels of tumor Th1 T-cell cytokines, and increased expression of T-cell and NK-cell activation markers. Based on its novel mechanism of action, there is potential for CB-1158 to enhance the activity of other immunotherapies or standard-of-care therapies. We observed improved anti-tumor activity when CB-1158 was combined with either epacadostat or anti-PD-L1, with low dose ionizing radiation in the Madison-109 model, and with gemcitabine in the LLC model. These results support the clinical development of CB-1158, a first-in-class arginase inhibitor, as a novel immuno-oncology agent targeting the immunosuppressive effects of tumor-infiltrating myeloid cells.

Conflict of interest: Ownership: Calithera Biosciences.

296 Poster/Poster in the spotlight (Board P122)
Dose ranging study of monalizumab (IPH2201) in patients with gynecologic malignancies: A trial of the Canadian Cancer Trials Group (CCTG): IND221

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Background: IPH2201 (monalizumab) is a first in class anti-NKG2A antibody; binding to NKG2A on tumour-infiltrating lymphocytes prevents the transduction of inhibitory signals when NKG2A binds to its endogenous ligand, HLA-E. HLA-E is upregulated in many cancer types and is often associated with worse prognosis, including in ovarian cancer. We conducted a dose ranging study to identify the single-agent recommended phase 2 dose (RP2D) for future cohort expansion.

Methods and Materials: Eligibility: Recurrent platinum sensitive or resistant high-grade serous ovarian/fallopian tube or primary peritoneal carcinomas, ECOG PS <2, <3 prior therapies, and availability of FFPE tissue sample.

Randomization was to one of 3 i.v. dose levels of monalizumab (1–40 mg/kg; DL1, 2, 3) q 2 weekly, with 6 patients planned per dose level. Response (RECIST V1.1) was assessed every 8 weeks. The RP2D was determined by toxicity, and PK and PD data.

Results: 18 patients were enrolled; median age was 60 yo and PS was 1. Most had ovarian carcinoma (n = 14). The median number of prior therapies was 2. The median number of prior therapies was 1. Most had ovarian carcinoma (n = 14). The median number of prior therapies was 2. The median number of prior therapies was 1. Most had ovarian carcinoma (n = 14). The median number of prior therapies was 2. The median number of prior therapies was 1. Most had ovarian carcinoma (n = 14). The median number of prior therapies was 2.

Poster Session – Immunotherapy, Wednesday 29 November 2016
Preclinical efficacy in multiple syngeneic models with oral immune checkpoint antagonists targeting PD-L1 and TIM-3

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Background: Antibody-mediated blockade of PD-L1 and PD-L1 has transformed the cancer therapy paradigm by eliciting durable antitumor responses and long-term remissions in a subset of patients with a broad spectrum of cancers. Interestingly, in an endeavor to enhance the response rate, a combination of antibodies targeting PD-L1 and CTLA-4 has resulted in significantly higher patient response rate. However, such combination has suffered from increased immune-related adverse events (irAEs) due to the breaking of immune self-tolerance. Sustained target inhibition as a result of a long half-life (>15−20 days) and >70% target occupancy for months are likely contributing to irAEs observed. Towards addressing these shortcomings, we are developing small molecule agents targeting more than one immune checkpoint pathway to increase the response rate and dosing by oral route with relatively shorter pharmacokinetic exposure for better management of irAEs.

Material and Methods: Herein we report the pharmacological evaluation of the first-in-class small molecule antagonists capable of targeting both PD-L1 and TIM-3 immune checkpoint pathways. The design hypothesis for generating a dual antagonist is primarily based on the pockets of sequence similarity of PDL-1 and TIM-3 proteins. A focused library of compounds mimicking the interaction of checkpoint proteins was designed and synthesized. Screening and analysis of the resulting library led to the identification of hits capable of functional disruption of the PDL-1 and TIM-3 signaling pathways. Further optimization resulted in compounds displaying equipotent antagonism towards PD-L1 and TIM-3 with desirable physicochemical properties and exposure upon oral administration.

Results: Potent functional activity comparable to that obtained with an anti-PD1 or anti-TIM-3 antibody in rescuing lymphocyte proliferation and effector functions were observed with a lead compound, AUPM-327. AUPM-327 showed selectivity against other immune checkpoint proteins including CD18, CD40, CD40L, VISTA and BLTA as well as in a broad panel of receptors, and enzymes. In syngeneic preclinical models of melanoma, breast and colon cancers, AUPM-327 showed significant efficacy in inhibition of both primary tumor growth and metastasis upon once a day oral dosing with excellent tolerability. Anti-tumoral activity correlated well with drug exposure and activation of CD4+ and CD8+ T cells.

Conclusions: The findings demonstrating the dual inhibition of PD-L1 and TIM-3 pathways resulting in activation of T cells and anti-tumor activities support further development of these orally bioavailable agents.

Conflict of interest: Ownership: All authors are employees of Aurigene Discovery Technologies Limited.

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Poster (Board P124)

T cell activation and anti-tumor efficacy of anti-Lag3 mAbs are independent of Lag3–MHC Class II blocking capacity

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Lag3-3 has been shown to act as an inhibitory molecule in the regulation of T cell activation, proliferation and homeostasis. Exhausted T cell populations that evolve in the tumor microenvironment or during chronic viral infections show coordinated expression of Lag3 andPD-1. Lag3-3 has been identified as a protein structurally related to CD4 and which also binds to MHC-II. Anti-Lag3-3 antibodies have shown preclinical efficacy in several disease models in particular when combined with anti-PD-1 antibodies to enhance the binding of Lag3-3 to MHC-II. We have demonstrated that Lag3-3 blockade is efficacious in both CD4+ andCD8+ T cells despite the lack of significant MHCII levels on CD8+ T cells. We describe studies that evaluated if anti-Lag3-3 efficacy is dependent on the ability of the antibody to stabilize the binding of Lag3-3 to MHCII. We have compared, the anti-mLag3-3 antibody (C9B7W) which does not block Lag3-3–MHCII interaction and an in-house generated anti-Lag3-3 antibody (2BG10) that strongly inhibits the interaction of its ligand (MHCII) in a series of in vitro assays. Biophysical characterization confirmed the differences of these antibodies to disrupt Lag3-3–MHCII binding and epitope mapping indicated that this may be associated with the different regions of Lag3-3 that these antibodies bind to. Furthermore, in vivo functional assays performed using TCR transgenic CD4+ T cells, no differences were seen between the two anti-Lag3-3 antibodies to enhance antigen-specific T cell responses. In addition, their ability to synergize with an anti-PD-1 antibody was comparable. To understand if the overall enhancement in CD4+ T cell activation obtained with C9B7W and 2BG10 was achieved through different mechanisms of action, we evaluated generation of IL-2 responses and long-term remissions in a subset of patients with a broad spectrum of cancers. initiative of apoptosis in activated immune cells upon contact with CD95 ligand (CD95L) makes CD95 another putative immune checkpoint. Like other checkpoint ligands, the CD95 ligand is frequently overexpressed on cancers expressing cognate checkpoint ligands in order to escape immune surveillance. The classical death receptor CD95 is a regulator of immune homeostasis and is consequently upregulated following activation of immune cells. Initiation of apoptosis in activated immune cells upon contact with CD95 ligand (CD95L) makes CD95 another putative immune checkpoint ligand. Like other checkpoint ligands, the CD95 ligand is frequently overexpressed on cancers and tumor-associated blood vessels, which possibly facilitates killing or inactivation of immune cells (i.e., a novel counter-attack). Here we examined the mode-of-action of APG101, a drug designed to neutralize CD95L, in protection of immune cells from activation induced cell death and subsequent effects on tumor cell killing.

Methods: Monocytes isolated from healthy donor blood samples were differentiated in vitro into either M1- or M2-type macrophages, which was confirmed by multicolor-flow cytometry for subtype markers. Subsequently, we analyzed the respective M1 and M2-type macrophages regarding apoptosis induction upon CD95L exposure. Analogous experiments were performed using purified T-cells. Analytical FACS was used to monitor apoptosis by appearance of cleaved PARP. Real-time cell analysis was performed on direct co-cultures of activated T-Lymphocytes and monocyte-depleted PBMCs with tumor cell lines to monitor tumor cell killing by immune cells.

Results: Differentiated M1- and M2-type macrophages express specific marker antigens including CD95. Exposure of macrophages to soluble recombinant CD95L induces apoptosis in both populations. Higher sensitivity of M1- compared to M2-macrophages is observed, coinciding with higher expression of CD95 on M1-macrophages. Addition of APG101 dose-dependently protects macrophages from CD95L-induced cell death. Similar results are obtained after treatment of T-cells with CD95L and APG101 in apoptosis-assays. In direct co-culture, purified T-Lymphocytes and monocyte-depleted PBMCs are able to induce apoptosis in several tumor cell lines. Exogenous CD95L does not interfere with chemical-induced activation or stimulation of immune cells by anti-CD3 and -CD28 antibodies and, importantly, tumor cell killing is not impaired.

Conclusion: APG101 is a potent inhibitor of pro-apoptotic CD95/CD95L signaling and protects activated immune cells from AICD by undergoing...
apoptosis through exposure to CD95 ligand. Importantly, APG101 does not interfere with macrophage differentiation or activation of the cytoplasmic functions of T-Lymphocytes and PBMCs in vitro. **Conflict of interest:** Other Substantive Relationships: All authors are employees and/or stock holders of Apogenix AG.

300 Poster (Board P126)

Immune cell activation by novel hexaivalent CD40 agonist APG1233 compared to trimeric formats or agonistic anti-CD40 antibodies

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**Introduction:** The co-stimulatory receptor CD40 is strongly expressed on B cells, monocytes and antigen-presenting cells (APC). By promoting their maturation, activation and survival, CD40 signaling greatly contributes to anti-tumor responses of the immune system. The HERA-Technology developed by Apogenix is a powerful engineering platform for the production of modular fusion proteins targeting the TNFR1-receptor superfamily. Here we compared the efficacy of different CD40 agonist formats, including the novel hexaivalent scCD40L-RBD-Fc (APG1233), and the functional consequences of differential receptor clustering.

**Methods:** Immune cells were isolated from healthy-donor blood samples and profiled by multicolor flow cytometry (MC-FC). Subsequently, immune cells were cultured in growth media containing various forms of CD40 agonists. Upregulation of activation markers on B cells, monocytes and PBMCs (e.g., CD69, HLA-DR) and T cell induced killing of tumor cells in direct co-culture was assessed by MC-FC and employing a real-time cell analysis system (xCELLigence), respectively. Secretion of cytokines in response to CD40 ligation and the pharmacokinetic properties of the fully human APG1233 and the chimeric murine/human APG1274 were determined by ELISA.

**Results:** In vivo stability of APG1233 was demonstrated in a single dose mouse PK study revealing a terminal half-life of 84 hours. The chorionic surrogate molecule APG1274 which binds murine CD40 is eliminated much quicker (1½ of 4 hours) demonstrating the specificity of both compounds. In vivo the hexaivalent APG1233 efficiently stimulated B cells, monocytes and PBMCs. In contrast, neither trimeric CD40L nor an agonistic antibody against CD40 were able to upregulate expression of activation markers. Similarly, the secretion of proinflammatory cytokines such as IL-12, CD86L and IFNγ by PBMCs was only stimulated after exposure to APG1233 and not in the presence of other CD40 agonists. In functional co-culture assays, after exposure to APG1233, in vitro generated M2-macrophages underwent conversion and acquired M1-type surface markers which strongly enhanced co-culture of naive CD4+ T cells. Consistent with these data, only APG1233 efficiently increased direct cytotoxic activity of immune cells against tumor cells measured by a real-time cell analysis assay.

**Conclusion:** The CD40 agonist APG1233 is a member of a novel class of hexaivalent TNFRSF agonists which binds its target with high specificity, exhibits excellent in vivo stability and superior biological activity over other agonistic formats (e.g. agonistic antibodies to CD40).

**Conflict of interest:** Other Substantive Relationships: All authors are employees and/or stock holders of Apogenix AG.

301 Poster (Board P127)

Costimulatory T-cell engagement by PRS-343, a CD137 (4-1BB)/HER2 bispecific, leads to tumor growth inhibition and TIL expansion in a humanized mouse model

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**Background:** VCN-01 is a selective oncolytic adenovirus with hyaluronidase activity in patients with advanced or metastatic cancer

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of G4 liver toxicity that spontaneously solved was evidence at 1E13 vp. Common toxicities after combination with chemotherapy were: neutropenia, thrombocytopenia, and pyrexia (86%). One episode of DLT consisting of febrile neutropenia G4 recovered adequately after 10 days. Twelve of 23 patients showed secondary viremia peaks, all of them receiving 3.3E12 vp and 1E13 vp. Shedding of VCN-01 was observed in all tested biologic fluids mostly until day 8. Preliminary PK calculations show linearity in dose ratio. All analyzed biopsies at day 8 post-injection (4 of 4) were positive for VCN-01 presence. Increased CDS infection was observed within tumor tissue. PET evaluation at 4 weeks after VCN-01 administration indicated metabolic improvement in a majority of patients (4/7) and more than 50% of patients treated with VCN-01 + AG showed PR at 16 weeks by RECIST with 2 out of 3 patients maintaining PR at 32 weeks.

Conclusions: VCN-01 shows evidences of clinical activity at well-tolerated dose levels. Pharmacodynamic observations confirm viral infiltrate in tumor tissue where active replication may modulate intratumor immune response.

Conflict of interest: Ownership: Ramon Alemany, Gabriel Capella and Manel Cascallo own shares of VCN Biosciences. Advisory Board: Ramon Alemany, Gabriel Capella are members of the Advisory Board of VCN Biosciences. Board of Directors: Manel Cascallo is member of the Board of VCN Biosciences. Corporate-sponsored Research: Ramon Alemany and Alba de Martin participate in Corporate-sponsored Research by VCN Biosciences.

303 Poster (Board P129)
Comparison of protein and mRNA immunophenotyping platforms in formalin-fixed paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) and melanoma samples
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The immune repertoire of human tumors from various cancer types, including non-small cell lung cancer (NSCLC) and melanoma, correlates with a positive outcome to certain therapies. Multiple methods exist for profiling the immune contexture of tumors; however, there is no clear gold standard. Here, we evaluated two technologies that assess immune cell populations in archival tumor tissue. The first used the Perkin Elmer multiplex IHC platform which allowed for the evaluation of multiple protein markers on a single slide while also allowing for measurement of spatial context between cell types. The second technology assessed was the NanoString nCounter platform which allows for the direct measurement of up to 800 transcripts from FFPE tissues allowing for broad quantitative characterization of immune related transcripts in clinical samples. NSCLC (n = 12) and melanoma (n = 14) FFPE samples were tested on both immunoprofiling platforms. CD4, CD8, CD68, FoxP3, and PDL1 protein expressions were evaluated using the Perkin Elmer platform on one section from each tumor sample and analyzed using the InForm software to quantitate positive cell counts and cell densities (cell counts/mm²). RNA expression was evaluated using the NanoString PanCancer Immune Profiling Panel and analyzed using nSolver software to normalize raw counts. Both cell counting and cell density from the multiplex IHC results were individually compared to normalized mRNA counts from NanoString for each marker-gene pair. CD8 protein expression was also compared against two published cytotoxic gene pathways on the NanoString platform. Simple linear regression identified a strong correlation for CD8 protein (both cell density and cell counts) and CD8 mRNA expression among melanoma samples (adjusted R² = 0.82, p < 0.001) and a similar trend was seen for FoxP3. In the NSCLC samples, PD-L1 showed the strongest correlation between the two platforms (adjusted R² = 0.80, p < 0.001), with CD4, CD8 and CD68 also displaying a trend towards being significantly correlated. Regularized regression analysis suggests both cytotoxic pathways tested are highly predictive of CD8 protein expression in melanoma samples, but not in NSCLC samples. Direct correlations between protein and mRNA expression are different depending on immune markers and tumor type. This study provides an approach for baseline characterization of immune related transcript in clinical samples. NSCLC of up to 800 transcripts from FFPE tissues allowing for broad quantitative characterization of immune related transcript in clinical samples. NSCLC and melanoma FFPE samples were tested on both platforms. No conflict of interest.

304 Poster (Board P130)
Dual roles for T cell depletion and co-stimulatory signaling in agonistic GITR targeting for tumor immunotherapy
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Background: Agonistic monoclonal antibodies targeting glucocorticoid-induced TNFR family-related protein (GITR) have shown potential therapeutic activities in pre-clinical tumor models. As this approach moves into the clinic, there is need to understand the precise mechanism of action and how such targeting will impact human immune cells. Both depletion and destabilization of the intratumoral Treg population has been previously implicated as the means by which the mouse anti-GITR antibody DTA-1 exerts its tumor-inhibitory effects. Using a Treg fate mapping approach we sought to define precisely the contributions of these two mechanisms. In addition, we assessed the effects of MK-4166, a humanized anti-human GITR agonist mAb that is currently being evaluated in a phase 1 clinical study in patients with cancer, on regulatory T cells.

Material and Methods: To evaluate whether treatment with anti-mouse GITR mAb DTA-1 causes depletion and stabilization of intratumoral Tregs into anti-tumor effector T cell populations, we developed a Treg lineage tracing mouse, Foxp3-GFP-Cre x Rosa26-loxP-stop-loxP-TdTomato. To assess the effects of human anti-GITR mAb MK-4166 on Treg in vivo, we used hu-CD34+ NSG mice bearing SKMEL-5 human melanoma tumors.

Results: Loss of intratumoral Treg was observed in both DTA-1-responsive (MC38) and non-responsive (LL2) syngeneic tumor models in WT mice. Studies in Foxp3-GFP-Cre x Rosa26-loxP-stop-loxP-TdTomato mice showed that intratumoral Treg loss after DTA-1 treatment was due primarily to cell depletion with minimal evidence of Treg conversion to a non-Foxp3-expressing population. Further characterization of persisting Tregs following DTA-1 treatment showed that highly activated subpopulation of CD44hi(CD25hi) intra-tumoral Tregs were preferentially targeted for elimination, with remaining Tregs exhibiting a less suppressive phenotype. Following these changes in the Treg population, intra-tumoral CD8+ T cells acquired a more functional phenotype, as demonstrated by their downregulation of the exhaustion markers PD-1 and LAG-3. This reversal of CD69+ T cell exhaustion was dependent on both agonistic GITR signaling and Treg depletion, as neither of these mechanisms by itself could fully reverse the exhaustion phenotype. These effects of DTA-1 on the tumor model using humanized mice mimicked many effects of DTA-1 in syngeneic tumor models, decreasing both Treg numbers and suppressive phenotype while enhancing effector responsiveness.

Conclusions: We have shown that intra-tumoral Treg loss after DTA-1 treatment is due primarily to cell depletion with minimal evidence of Treg conversion to a non-Foxp3-expressing population. In addition, we have shown that MK-4166 modulates Treg in tumor-bearing humanized mice.

No conflict of interest.
Mice were treated with RXC004 +/- mouse anti-CTLA4 antibody. CT26 cells were subcutaneously implanted into BALB/c mice. Mice were treated with RXC004 +/- rat anti-PD-1 antibody. Flow cytometry 10 days post-treatment started to utilise to measure key immune cell populations in the tumour microenvironment and spleen.

**Results:** RXC004 inhibited the palmiotyrosination of Wnt ligand in vitro in a dose dependant manner. RXC004 potently inhibits the Wnt pathway in a reporter cell assay and exhibits nM inhibition of cell growth in Wnt-dependent human pancreatic cancer cell lines. RXC004 significantly inhibited tumour growth in a CAPAN-2 xenograft when dosed orally at 1.5 mg/kg BID and 5 mg/kg QD. These treatments were well tolerated. RXC004 had no effect on B16F10 cell proliferation in vitro. However, in a syngeneic murine melanoma B16F10 model, RXC004 monotherapy significantly inhibited tumour growth at a dose of 5 mg/kg QD orally, potentially via an immunomodulatory mechanism. In the murine CT26 model, RXC004 treatment reduced tumour size when dosed in combination with anti-PD-1 antibody. Flow cytometry showed RXC004 in combination with anti-PD-1 antibody increased numbers of CD8+ cytotoxic T cells as well as decreasing FoxP3+ regulatory T cells when compared to the anti-PD-1 monotherapy.

**Conclusion:** These data indicate that RXC004 is a potent inhibitor of Wnt pathway driven cancers models. Data from syngeneic mouse models corroborated in vivo results from clinical trials targeting the Wnt/β-catenin pathway to promote immune response against human cancers [3]. RXC004 is currently undergoing IND enabling studies.

**References**


**No conflict of interest.**

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**307**

**Expression subtypes of lung adenocarcinoma and squamous cell carcinomas reveal a variable landscape under somatic genetic features suggesting differential response to multiple drug targets**

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**Background:** Gene expression-based subtyping in lung Adenocarcinoma (AD) and lung Squamous Cell Carcinoma (SQ) classifies AD and SQ tumors into distinct molecular subtypes with variable outcomes and potential response to therapy.

**Methods:** Using the TCGA lung cancer gene expression datasets (AD n=315, and SQ n=501), immune cell expression and tumor somatic mutation gene expression was investigated. Previously published AD subtypes [Terminal Respiratory Unit (TRU), Proximal Proliferative (PP), and Proximal Inflammatory (PI)] and SQ subtypes (Primitive, Classical, Secretory, Basal) were defined using gene expression patterns. Variable expression of immune cell gene signatures (Bindea et al. 24 immune cell types), expression of single immune gene biomarkers (CTLA4, PDDC1, and CD274), expression of genes from a clinical oncology solid tumor mutation panel (322 genes), and non-silent mutation burden was examined in relation to AD and SQ subtypes. Differential gene expression was assessed using the Kruskal-Wallis (KW) test with Bonferroni correction. Linear regression and Spearman correlations were used to evaluate association of non-silent mutation burden, tumor subtype, and CD274 expression with immune cell expression. Variable expression of genes was observed in the metastatic lung tissue compared to the primary tumors.

**Results:** Variable expression of 208/322 tumor panel genes (65%) in AD subtypes and 244/322 (76%) in SQ subtypes were observed (KW Bonferroni threshold p<0.000155). Most drug target genes, including but not limited to AUJKA, CHEK1, ROS1, CD274(PD-L1), CSF1R and ERBB4 in AD and SOX2, TGFBR2, SMO, CSF1R, Ptk3CA, and HGF in SQ, exhibited strong differential expression across the subtypes (p<10^-28). Immune cell expression was also highly variable across subtypes. The SQCC secretary subtype and the PI subtype of AD demonstrated the highest immune cell expression while the Classical subtype of SQ and the PP subtype of AD demonstrated low expression of immune cells. In SQ tumors, subtype was a better predictor of adaptive immune cell expression than CD274 (PD-L1) (median F-test p-value and adjusted R-squared were 2.16 ×10^-24 and 0.20 for subtype versus 1.86 ×10^-28 and 0.07 for CD274). Non-silent mutation burden was not strongly correlated with immune cell expression (Spearman correlation = -0.07 in AD and 0.08 in SQ) however, the PI subtype of AD, which is enriched for TP53 mutations, was associated with elevated immune cell expression and a high mutation burden. SQ subtypes demonstrated significant differences in many drug target tumor panel genes and in immune cell expression but did not demonstrate differences in mutation burden.

**Conclusions:** Molecular subtypes of lung AD and SQ revealed differential expression of immune cells and many key somatic mutation drug targets. Evaluation of subtypes as potential biomarkers for drug sensitivity should be investigated alone, and in combination with immune cell features and key mutation targets.

**Conflict of interest:** Board of Directors: Chuck Perou and Neil Hayes are Board Director members, equity stock holders, and consultants for GeneCentric Diagnostics. They each are also named as inventors on a US patent for lung cancer subtyping. Corporate-sponsored Research: Research was funded by GeneCentric Diagnostics. Other Substantive Relationships: Jonathan Serody is a consultant for GeneCentric Diagnostics, Hawazin Faruki, Greg Mayhew, and Myla Lai-Goldman are employees by GeneCentric Diagnostics, a biotechnology company developing molecular subtyping diagnostics for lung and other tumors.
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GITR is a type I transmembrane protein of the tumor necrosis factor receptor superfamily which is expressed primarily on T lymphocytes and natural killer cells. Ligation of GITR on activated T cells provides a costimulatory signal that positively modulates antigen-specific T cell responses, leading to enhanced cellular and humoral immunity. The anti-GITR antibody is a humanized agonistic monoclonal antibody that targets human GITR.

Here, we described an integrated pharmacology approach employed for early preclinical development of MK-4166. Due to its immune-stimulatory properties and the intended patient population (patients with cancer), the use of "Minimal Anticipated Biological Effect Level, MABEL" approach along with considerations for pharmacologically active dose with reasonable safety (ICH S9) were utilized for estimation of first-in-human (FIH) doses for MK-4166. Accordingly, development of the FIH starting dose rationale for MK-4166 was based on comparison of the data obtained from various in vitro and in vivo experiments, regulatory guidelines (ICH S9 and use of MABEL), pharmacokinetic/pharmacodynamics (PK/PD) studies in cynomolgus monkeys (pharmacologically relevant species), and nonclinical safety studies.

A mechanistic PK/PD model for implementation of a safe First-In-Human (FIH) dosing strategy was used. Studies were conducted to examine the potential relationships between MK-4166 serum exposure (PK) and receptor availability (PD) on the relevant T-cell subsets. The concentrations of the antibody in serum were determined using an electrochemiluminescence (ECL) assay, and the availability of receptor (GITR) was determined using flow cytometry. Non-linear PK properties were observed over the dose range examined. In line with changes in serum concentration-time profiles for the antibody, a dose-dependent effect in receptor availability was also observed. The relationships between receptor availability, serum concentrations of the surrogate antibody mDTA-1 and tumor suppression were described by a mechanistic PK/PD model as well.

No conflict of interest.

Identification and characterization of TSR-042, a novel anti-human PD-1 therapeutic antibody

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Background: TIM-3 is an immune checkpoint receptor expressed by T-cells that, through its interaction with PD-1, may prevent effective anti-tumor immunity. T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) is a novel immune checkpoint initially identified on interferon-gamma producing CD4+ T-helper 1 and CD8+ cytotoxic T-cells and has been implicated in the exhaustion of T-cells.

Results: TIM-3 expression has also been identified on other immune cell types, although its functional role in this context remains to be fully elucidated. Here, we describe the identification of TSR-022, a novel IgG4 anti-human TIM-3 therapeutic antibody.

Materials and Methods: TSR-022 is a potent and selective humanized monoclonal antibody, generated through the use of SHM-XEL™ technology. It was characterized in a number of in vitro and in vivo studies and completed IND-enabling preclinical activities.

Results: TSR-022 binds to recombinant human and cynomolgus monkey TIM-3 with pM affinity and does not bind appreciably to mouse TIM-3. Binding of TSR-022 to TIM-3 enhances T-cell activation, for example, increasing cytokine generation from activated CD4+ T-cells and by increasing IL-2 production in a CD4+ T-cell dendritic cell mixed lymphocyte reaction assay. Importantly, TSR-022 also increased IL-2 secretion not only alone but also in combination with anti-PD-1 antibodies, including the novel anti-PD-1 antagonist, TSR-042. As a single agent, TSR-022 did not induce cytokine release from human peripheral blood mononuclear cells and when evaluated in combination with C1q and CD16a, TSR-022 displayed properties typical for a human IgG4 antibody, suggesting it is unlikely to mediate appreciable complement-dependent cytotoxicity or antibody-dependent cell cytotoxicity. Owing to its lack of cross-reactivity to rodent TIM-3, a comprehensive safety program in cynomolgus monkey was performed. Results from single dose and repeat-dose intravenous toxicology studies indicated that TSR-022 was well-tolerated and displayed a profile that supported progressing the molecule into clinical studies.

Conclusion: Taken together, these data demonstrate that TSR-022 is a potent anti-TIM-3 receptor antibody with pre-clinical properties that support its clinical investigation in cancer patients.

Conflict of Interest: Ownership: Employees of interest in TESARO or AnaptysBio.

Development of patient derived xenograft (PDx) models in human immune-reconstituted mice to evaluate efficacy of immunotherapeutic agents in lung and breast cancer

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Background: Human immune-reconstituted mice, generated by transplanting human CD34+ hematopoietic stem cell (HSC) are extremely useful in basic and applied human disease research. Many cancer therapies, including immune checkpoint inhibitors such as the anti-PD-1 monoclonal antibody Keytruda, rely on an intact immune system to release immunosuppression and eliminate cancer cells.
Material and Methods: We evaluated the effects of Keytruda in a mouse clinical trial using patient derived xenograft (PDX) models in a humanized NSG (huNSG) immune-competent mouse background from Jackson Laboratories. To simulate the tumor and immune-system diversity found in a clinical population, we performed a checkerboard study of 5 HSC donor huNSG cohorts, across 7 small cell lung cancer (SCLC) PDX models. This design allowed for population analyses of up to 35 tumors models, as defined by the unique interaction of SCLC tumor and HSC donor. In a parallel clinical trial, we also evaluated the effects of Keytruda in huNSG-immune-competent xenograft models of triple negative breast cancer (TNBC). These TNBC studies provided a controlled setting to investigate the requirement for humanization in Keytruda responsiveness, as well as the potential for T-cell memory response in the model system. Results: Here, we show that HSC derived human immune cell engraftment does not have significant effects on patient derived xenograft (PDX) tumor growth. However, humanization is required for anti-tumor response to Keytruda. In TNBC xenograft studies, tumors showed complete regression following Keytruda treatment. Re-engraftment of same animals with tumor showed minor tumor growth before complete regression again, indicative of a memory response. Aged match control naïve animals engrafted with same cells showed normal tumor growth. In SCLC PDX studies Keytruda efficacy varied between models, which underscores the high degree of tumor diversity in this model system. Tumors from these studies were then analyzed to determine target engagement of PD-1, tumor infiltrating lymphocytes (TILs) characterization and histology comparing Keytruda responders vs non-responders. Flow cytometry and immunohistochemistry revealed patterns of immune cell infiltration that correlated to responsive or non-responsive models. Based on these results, we demonstrate that huNSG immune-competent mice can serve as an effective preclinical model system for evaluating anti-PD-1 therapy, and that the population study design can be successfully used to canvass diversity of tumor and immune biology observed in the clinic.

No conflict of interest.

313 Poster (Board P139) Interim results from the phase I study of nivolumab (nivo) + nab-paclitaxel (nab-P) in pancreatic cancer (PC), non-small cell lung cancer (NSCLC), and metastatic breast cancer (mBC)

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Background: Combining a taxane with an immune checkpoint inhibitor has demonstrated improved responses across multiple tumors. Results from the Part 1 portion of the phase I safety trial of nivo + nab-P in advanced PC (± gemcitabine [G]), NSCLC (± carboplatin [C]), and mBC will be presented. Methods: The primary objective of part 1 is to evaluate dose-limiting toxicities (DLTs). Patients (pts) treated with ≥2 cycles of nivo + nab-P who remained on study for 14 additional calendar days after the last nivo dose or who discontinued due to DLT prior to completing 2 cycles of nivo were considered DLT evaluable. If deemed safe, study arms will be expanded in Part 2 to further assess safety, tolerability, and antitumor activity. In Arm A, pts with PC and 1 prior chemotherapy (CT) regimen received nab-P 125 mg/m2 Q2W. Median age was 56.5 years (range 34−78). All had ECOG PS 0 or 1, with a median of 4 prior therapies (range 2−7). 8/12 pts (67%) had drug-related treatment-emergent adverse events (TEAEs). One pt had grade 3 asymptomatic elevated bilirubin. All other drug-related TEAEs were grade 1 or 2: maculopapular rash (2 pts; 18%), pruritis (2 pts; 18%), xerosis (1 pt; 8%), diarrhea (1 pt; 8%), elevated transaminase (2 pt; 15%), elevated amylase (1 pt; 8%), lymphopenia (2 pt; 15%), leukopenia (1 pt; 8%), and anemia (1 pt; 8%). No pt had a DLT and no treatment was discontinued for drug-related TEAEs. Two pts had elevated tumor markers at study entry declining to within normal limits on treatment: 1 (cervical Ca, DL3) has ongoing SD at 4 months; 1 (pancreatic Ca, DL2) has an ongoing confirmed PR and was an ongoing SD at 4 months; 1 (cervical Ca, DL3) has ongoing SD at 4 months; 1 (cervical Ca, DL3) has ongoing SD at 4 months. The most common any-grade AEs in Arms A and C were fatigue, nausea, and alopecia. As of June 17, 2016, 6 pts (nivo-treated) have been enrolled in Arm B, 8 (4 niv0-treated) in Arm D, 10 (5 nivo-treated) in Arm E, and 11 (6 nivo-treated) in Arm F. To date, 1 case of nonimmune hepatitis (DLT) and 1 case of pneumonitis (DLT) have been reported in Arms B and D, respectively.

Conclusions: Based on the tolerability demonstrated with the combination of nivo + nab-P or nab-P/PC in Arms A and C, respectively, the study has been expanded and pts are enrolling in Part 1 of Arm B and Part 2 of Arm C. Efficacy data in Arm C, although preliminary, is encouraging. Updated data from Part 1 for all arms will be presented.

Conflict of interest: Ownership: NT, Employment Celgene; AK, Employment Celgene. Advisory Board: HS, Celgene. Corporate-sponsored Research: ZW, Research funding, Celgene; DW, Consultant and research funding, Celgene; PO, Research funding, Celgene; JWG, Research funding, Celgene, and Consultant and research funding, BMS. Other Substantive Relationships: BG, Consultant, Celgene; HSfH, Nothing to disclose; KK, Nothing to disclose.

314 Poster (Board P140) A phase I, open-label, multiple-ascending-dose trial to investigate the safety, tolerability, biologic, and clinical pharmacology of M7824 in patients with metastatic or locally advanced solid tumors

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Background: M7824 is a novel bifunctional fusion protein targeting the PD-L1 and TGF-β pathways, in patients with metastatic or locally advanced solid tumors. Methods: Four dose levels (DL 1, 3, 10, and 20 mg/kg) were escalated in a 3+3 design. Dose-limiting toxicities (DLT) were evaluated over 3 weeks. Results: As of May 4, 2016, 12 pts with advanced cancer had enrolled, with 3 pts treated with M7824 at each dose level: 1, 3, 10, and 20 mg/kg Q2W. Median age was 56.5 years (range 34−78). All had ECOG PS 0 or 1, with a median of 4 prior therapies (range 2−7). 8/12 pts (67%) had drug-related treatment-emergent adverse events (TEAEs). One pt had grade 3 asymptomatic elevated ipapase. All other drug-related TEAEs were grade 1 or 2: maculopapular rash (2 pts; 18%), pruritis (2 pts; 15%), xerostomia (1 pt; 8%), diarrhea (1 pt; 8%), elevated transaminase (2 pt; 15%), elevated amylase (1 pt; 8%), lymphopenia (2 pt; 15%), leukopenia (1 pt; 8%), and anemia (1 pt; 8%). No pt had a DLT and no treatment was discontinued for drug-related TEAEs. Two pts had elevated tumor markers at study entry declining to within normal limits on treatment: 1 (cervical Ca, DL3) has ongoing SD at 4 months; 1 (pancreatic Ca, DL2) has an ongoing confirmed PR and was an ongoing SD at 4 months; 1 (cervical Ca, DL3) has ongoing SD at 4 months; 1 (cervical Ca, DL3) has ongoing SD at 4 months. The most common any-grade AEs in Arms A and C were fatigue, nausea, and alopecia. As of June 17, 2016, 6 pts (nivo-treated) have been enrolled in Arm B, 8 (4 niv0-treated) in Arm D, 10 (5 nivo-treated) in Arm E, and 11 (6 nivo-treated) in Arm F. To date, 1 case of nonimmune hepatitis (DLT) and 1 case of pneumonitis (DLT) have been reported in Arms B and D, respectively.

Conclusions: Based on the tolerability demonstrated with the combination of nivo + nab-P or nab-P/PC in Arms A and C, respectively, the study has been expanded and pts are enrolling in Part 1 of Arm B and Part 2 of Arm C. Efficacy data in Arm C, although preliminary, is encouraging. Updated data from Part 1 for all arms will be presented.

Conflict of interest: Ownership: NT, Employment Celgene; AK, Employment Celgene. Advisory Board: HS, Celgene. Corporate-sponsored Research: ZW, Research funding, Celgene; DW, Consultant and research funding, Celgene; PO, Research funding, Celgene, and Consultant and research funding, BMS. Other Substantive Relationships: BG, Consultant, Celgene; HSfH, Nothing to disclose; KK, Nothing to disclose.
Conclusions: Preliminary data suggest that the bifunctional fusion protein M7824 was generally well-tolerated when administered to pts with heavily pretreated advanced solid tumors in doses of 1–20 mg/kg. The pharmacological modes of action, binding to PD-L1 and elimination of heavily pretreated advanced solid tumors in doses of 1−20 mg/kg. The M7824 was generally well-tolerated when administered to pts with S104 Poster abstracts Poster Session – Immunotherapy, Wednesday 29 November 2016 Poster(BoardP141)

BioMAP systems model human disease biology to enable compound testing on human primary cells to predict clinical outcomes with respect to efficacy and safety. 2. Activities shown by compounds specific to the TME include: • Pembrolizumab restores immune activity with increased Granzyme B, IFNγ, and TNFα. • Paclitaxel shows anti-tumor activities (CEACAM5 and Keratin 20) in addition to potentiated immune activity similar to pembrolizumab.


Other Substantive Relationships: All authors are employees of Merck KGaA, Darmstadt, Germany.

Cell based assays for immuno-oncology discovery research

Conclusions:

1. BioMAP systems model human disease biology to enable compound testing on human primary cells to predict clinical outcomes with respect to efficacy and safety.
2. Activities shown by compounds specific to the TME include:
   - Pembrolizumab restores immune activity with increased Granzyme B, IFNγ, and TNFα.
   - Paclitaxel shows anti-tumor activities (CEACAM5 and Keratin 20) in addition to potentiated immune activity similar to pembrolizumab.

Conflict of interest: Other Substantive Relationships: All authors are current or past employees of Merck and Co., Inc.
Development of AVID200, a novel TGF-β targeting immunotherapy: Characterization of immunomodulatory effects

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Background: Elevated TGF-β ligand promotes cancer progression primarily by suppressing the immune system in the tumor microenvironment, in particular by suppressing T-cell recruitment and/or activation. We developed AVID200, a novel decoy receptor trap that potently blocks TGF-β and induces T-cell infiltration into tumors. This promotes the “T-cell-inflamed” tumor state, which is expected to render tumors sensitive to immune checkpoint inhibitors and other immunotherapeutics. We sought to design a trap that preferentially blocks TGF-β1 and 3 and not TGF-β2, the isoform that is important for maintenance of normal cardiac function.

Methods: AVID200 is a computationally designed avidity-enhanced receptor-based trap that binds and neutralizes TGF-β1 and 3 with low pM potency. The AVID200 lead candidate was selected based on the analysis of several trap formats, with each format exhibiting varying characteristics, including differing circulating half-lives and in vitro blocking potencies. AVID200 was evaluated for single agent effects on tumor growth and T-cell infiltration in vivo studies using the syngeneic 4T1 triple negative breast cancer (TNBC) tumor model. Additionally, ex vivo studies were performed on CD4+ and CD8+ T-cells harvested from the draining lymph nodes of treated 4T1 tumor-bearing animals. Finally, AVID200 is being tested for its ability to promote sensitivity to immune checkpoint inhibitors in combination studies.

Results: In efficacy studies using the syngeneic 4T1 TNBC model, AVID200 was shown to promote significant T-cell infiltration into tumors. This infiltration resulted in reduced primary tumor growth as well as significant reductions in metastatic lesions. Additionally, ex vivo studies revealed that AVID200 treatment decreased T-cell apoptosis, promoted T-cell proliferation in response to tumor cell lysates in the presence of dendritic cells, as well as increased the capacity of T-cells to specifically lyse 4T1 tumor cells.

Conclusion: The novel computational design of AVID200 results in a trap with low pM in vitro neutralization potency for TGF-β1 and 3. Additionally, AVID200 markedly promotes the “T-cell-inflamed” tumor state in vivo. Combination studies with immune checkpoint inhibitors will be presented.


Identification of patient-specific neoepitopes for cell-based and vaccine immunotherapy within The Cancer Genome Atlas reveals rarely shared recurrent neoepitopes

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Introduction: Recent advances in immunotherapy agents against PD-1/CTLA4 for patients with melanoma have yielded substantial clinical benefits for a subset of patients. These therapies may prove useful for other difficult to treat cases, but the prevalence of sufficient neoepitope burden has not been assessed across all cancers. Neoepitopes are tumor-specific markers that arise from mutations acquired from cancer and may represent a path to targeted therapies even in cancers with few treatable targets such as triple negative breast cancers. We analyzed whole genome sequencing and RNA sequencing data from The Cancer Genome Atlas (TCGA) to identify neoepitopes among 23 different cancer classifications containing 750 patients that could be used to develop next-generation, patient-specific cancer immunotherapies.

Material and Methods: We used WGS and RNA-seq from TCGA. Using mutation calling pipeline developed in conjunction with TCGA, we identified all somatic mutations within a tumor sample and from those generated all neoepitopes. This list was refined by RNA-seq expression status and HLA-specific netMH C binding analysis.

Results: We analyzed 750 cancer patients from TCGA, containing a mixture of 23 different cancer classifications with extensive analysis on triple negative breast cancer patients. These patient samples were selected by the availability of whole genome sequencing (WGS) data, RNA-sequencing data as well as clinical outcome data. Based on WGS DNA data, we identified an average of 23000 potential neoepitopes per patient of which an average of 9000 show expression in RNA-seq data. We predicted each patient’s HLA typing from WGS DNA and RNA-seq, and used netMH C to find average of 140 presented tumor specific neoepitopes per patient. We identified few neoepitopes that were bound and expressed that are shared between different patients. In addition to TCGA data, we performed a similar analysis on clinical samples to be tested for immune response.

Conclusions: Within the TCGA dataset, the majority of neoepitopes among patients with breast cancer were unique to each patient. Within a subtype of cancer, e.g. HER2+, there are few shared neoepitopes. For cancer patients who do not respond to targeted therapies, but whose tumors harbor sufficient neoepitope burden, high-throughput identification of neoepitopes could serve as the basis for the development of next-generation, patient-specific immunotherapies.

MDSC trafficking and function in RCC by CXCR4 in the presence of a VEGF-R antagonist is dependent on HIF-2α expression

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Background: In xenograft models of human RCC 786-0 and A498 cells, we have previously demonstrated that acquired resistance to the VEGF signaling antagonist axitinib was associated with a marked increase in the infiltration of CD11b+Gr-1+ myeloid-derived suppressor cells (MDSC). MDSC express CXCR4 and its ligand, SDF-1/CXCL12, is produced in response to hypoxia induced by VEGF antagonists. We have recently reported that both the influx of MDSC and resistance to axitinib could be prevented by concurrent administration of X4P-001 (previously AMD11070), a CXCR4 antagonist.

Material and Methods: To investigate the early factors influencing MDSC trafficking with respect to CXCR4 signaling, xenografts from 786-0 cells were established and treated with axitinib, X4P-001, the combination of both agents or saline for 3 or 8 days. At sacrifice tumors were excised and flash frozen in liquid nitrogen for Western analysis, fixed in formalin for IHC and immunofluorescence, or treated with collagenase for the analysis and isolation of MDSC.

Results: As early as Day 3, the combination of X4P-001 and axitinib had additive (synergistic) effects, with 50% suppression of tumor volume compared to controls and to the modest effects of either drug alone. This result paralleled the longer term experiments previously reported. Similarly, by IHC, the tumors from mice receiving axitinib alone had extensive MDSC infiltration by day 3 and continuing to day 8, whereas the tumors from mice receiving either X4P-001 alone or the axitinib/X4P-001 combination had significantly less MDSC infiltration. Mice treated with axitinib alone had an increase in Ki-67 positive tumor cells as early as Day 3, which was not observed in mice that received both X4P-001 plus axitinib, suggesting an anti-proliferative effect of the combination. Of note, mice receiving both X4P-001 and axitinib showed significant suppression of HIF-2α by day 3 as determined by both Western blot analysis and IHC. Furthermore, at Day 8 MDSC were focused near areas of necrosis, suggesting that the hypoxia (and resulting necrosis) induced by axitinib as early as Day 3 of treatment induced SDF-1/CXCL12 that, in turn, recruits MDSC to the tumor.

Conclusions: The resistance mechanism in RCC xenografts to axitinib occurs by Day 3 after the initiation of treatment, and is dependent on HIF-2α. CXCR4/CXCL12, and the infiltration of MDSC to the tumor. The MDSC then produce proangiogenic factors that mediate VEGF resistance. Administering X4P-001, a CXCR4 antagonist, concurrently with axitinib, blocks communication between the tumor and the MDSC, suppresses HIF-2α expression, reduces MDSC tumor infiltration, and appreciably improves the anti-tumor treatment effect.

Conflict of interest: Ownership: Dr. Arbeil and Dr. Wang are employees of X4 Pharmaceuticals. Corporate-sponsored Research: The research summarized in the abstract was conducted at the Drs. Panka and Miers laboratory and is sponsored by X4 Pharmaceuticals.
Background: Significant progresses have been recently accomplished in immuno-oncology and in the development of cancer immunotherapies. However, novel solutions are necessary to the peripheral tolerance and the immunosuppressive tumor microenvironment that prevent the eradication of cancer. VAXIMM is developing first-in-kind Salmonella typhi-based oral T-cell vaccine platforms for the initiation of anti-angiogenic and anti-tumor cellular immune responses via a unique mode-of-action. This study summarizes the non-clinical safety profile as well as the preclinical anti-cancer efficacy for Salmonella typhi murine vaccine VXM01m, VXM04m and VXM06m which encode murine vascular endothelial growth factor receptor 2 (VEGFR2), mesothelin (MSLN) and Wilm’s tumor 1 (WT1) protein antigens, respectively.

Material and Methods: The preclinical safety profile of the control Salmonella typhi empty vector vaccine VXM00m, empty, as well as VXM01m, VXM04m and VXM06m and the VXM01m/VXM04m combination was assessed in C57BL/6J mice after single or repeated administrations by gavage with doses up to 10⁵ CFU in 13- and 26-week GLP-compliant toxicity studies. The anti-tumor efficacy of VXM01m and VXM04m was evaluated in the Panc02 syngeneic model of pancreatic adenocarcinoma expressing MSLN, and the anti-cancer activity of VXM06m was evaluated in the FBL-3 disseminated model of erythroleukemia expressing WT1.

Results: VXM01m, VXM06m as well as VXM01m combined with VXM04m were generally well tolerated in the repeated dose toxicity studies conducted. Treatment-related effects were limited to an increase in the number of animals with inflammation/single necrosis in the liver, which was attributed to the Salmonella typhiurium vector. Treatment of Panc02 tumor-bearing mice with VXM01m and VXM04m single agents resulted in a significant reduction in the tumor growth rate, compared to the control group, with a median T/C of 37.6% and 19.4% respectively 35 days after tumor challenge. Treatment of mice bearing FBL-3 leukemia with VXM06m generated a rapid and sustained anti-tumor effect with 100% (10 out of 10) of surviving animals 175 days after leukemia challenge, in contrast, treatment with VXM0_empty did not show any anti-cancer effect, with a median survival of 45 days and 0% (0 out of 10) of cancer regression.

Conclusions: VXM01m, VXM04m and VXM06m were tolerated at the effective doses in this study, demonstrating consistent anti-cancer activity with substantial T cell responses in different animal tumor models. This study provides further evidence that VAXIMM’s versatile oral T-cell vaccine platform can be used to stimulate anti-tumor immunity against various antigens. Further studies of VAXIMM’s vaccine cancer candidates, as monotherapy as well as in combination, are warranted.

Conflict of interest: Ownership: I. Kobli (VAXIMM), A. Meichle (VAXIMM), K. Breiner (VAXIMM). Advisory Board: P. Beckhove (Bristol-Myers Squibb). Other Substantive Relationships: S. Wieckowski, I. Kobli, A. Meichle and H. Lubenau are employees of VAXIMM.

321A Poster (Board P148)
Imprive PGG, a yeast β-galactan PAMP, induces a unique cytokine profile and enhances immune checkpoint inhibitor therapy
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Significant preclinical and clinical research has focused on the prospect of using PAMPs to spark a coordinated anti-cancer immune response in combination with checkpoint inhibitors (CPI). PAMPs are unique combination partners as they can sensitize tumors to respond to CPI in several ways, including activation of antigen presenting cells to prime tumor-specific CD8 T cells and thwarting immunosuppression to boost the effector function of T cells at the tumor site. However, many PAMPs elicit intolerable, and sometimes fatal, cytokine storms when administered systemically and PD-L1/STING agonists. Imprive PGG (Imprive PG4) is a soluble yeast β-[1,3/1,6 glucan, is a PAMP that has been successfully administered intravenously, is well-tolerated, and shows promising efficacy in a series of clinical trials. Imprive enhances the direct tumor killing function of innate effector cells, promotes re-polarization of the immunosuppressive tumor microenvironment, and drives the activation of antigen presenting cells, enabling CD8 T cell expansion and IFN-γ production. In multiple preclinical cancer models, Imprive has shown profound anti-tumor efficacy in concert with tumor-targeting and anti-angiogenic antibodies. In this study, we sought to explore the ability of Imprive to synergize with CPI in the context of the cytokine profile, and to evaluate what makes Imprive a unique PAMP, especially in the context of its cytokine profile.

In the melanoma CT26 colorectal tumor model, Imprive and an anti-PD-1 antibody given in combination repressed tumor growth more than either single agent. In the MC38 tumor model, 33% of mice receiving an anti-PD-L1 antibody were tumor free whereas >80% of those dosed with Imprive and the anti-PD-L1 antibody were tumor free. Moreover, those dosed with Imprive alone, had >80% of mice free remained tumor free upon re-challenge with MC-38 tumor cells, suggesting that Imprive based therapy enhanced immunologic memory. For comparison of cytokine profiles, a collection of different PAMPs representing ligands for a variety of Pattern Recognition Receptors (PRRs: TLR-2, -3, -4, -5, -7/8, -9, NOD-1, -2, RIG-1, STING, γ/δ-glucan receptors) were tested for their ability to produce cytokines in whole blood from healthy human subjects. Unlike the other classes of PRR agonists, Imprive consistently induced chemokines involved in leukocyte trafficking (IL-8 and MCP-1), but not the pro-inflammatory cytokines IL-6, IL-1α, and TNF-α that contribute to toxicity. Furthermore, transcriptional profiling after in vivo dosing of Imprive and an antibody to PD-L1 in the CT26 tumor model revealed a profound difference in the gene expression profile resulting in an anti-inflammatory response with substantially reduced immune cell recruitment.

Conflict of interest: Ownership: All of the authors are employed by and own stock in Biothera Pharmaceuticals Inc.
levels coincide with enhanced Imprime-induced immune activation and correspond with enhanced clinical responsiveness. These data support the use of pre-treatment ABA levels to select patients for Imprime-based therapy.

**Conflict of interest:** Ownership: All authors are employees of Biothera Pharmaceuticals, Inc. Corporate-sponsored Research: All research was supported by Biothera Pharmaceuticals, Inc.

### Animal Models

**322** Poster (Board P001)

**ADAMTS18 inhibits colitis and colitis-associated colon cancer:** Evidence from ADAMTS18 deficient mice

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ADAMTS18 (a disintegrin and metalloproteinase with thrombospondin motif 18) is a member of ADAMTS family that is known for its crucial role in development, inflammation, cancer, and vascular biology. ADAMTS18 gene was found inactivated via methylation in many tumor cell lines and carcinoma specimens, and is therefore proposed as a tumor suppressor gene (Oncogene 26: 7490, 2007). Clinically, mutation of ADAMTS18 is strongly associated with colorectal cancer (CRC). (Science 314: 258, 2006). However, a more detailed knowledge about how ADAMTS18 exerts its effect on CRC progression remains largely unclear. Here, we generate ADAMTS18 knock-out (KO) mice and investigate the role of ADAMTS18 in CRC progression.

In carcinogen azoxymethane (AOM)-induced colorectal tumor model, there were no significant differences in tumor phenotypes between wild-type (WT) and KO mice. When combined use of inflammatory agent dextran sulfate sodium (DSS) with AOM, KO mice demonstrated higher colon cancer incidence rates, more tumor numbers and severe dysplastic scores than WT mice (tumor incidence rates, WT vs. KO, 71% vs. 100%; tumor numbers, WT vs. KO, 1.5±1 vs. 6.3±1.9; P=0.026). ADAMTS18 KO tumor cells showed more BrdU incorporation than WT tumor cells. The results from in situ TUNEL assay showed ADAMTS18 KO tumor cells underwent less apoptosis than WT tumor cells. In DSS-induced colitis model, ADAMTS18 KO mice showed more body weight loss than WT mice after induction with DSS. The lengths of colons were shorter in KO mice than in WT mice. Histological analysis showed that KO mice displayed more severe inflammatory infiltration and mucosal congestion than WT mice. The results of sandwich ELISA revealed that serum levels of both LPS and TNF-α were significantly elevated in KO mice compared to those in WT mice (LPS, WT vs. KO, 8.7±1.8 vs. 11.2±2.4 μg/L, P=0.044; TNF-α, WT vs. KO, 260.4±51.8 vs. 332.1±69.4 ng/mL, P=0.049). Mechanistic studies revealed that both p38MAPK and ERK1/2 were significantly activated in colitis and CRC tissues of KO mice compared to those of WT control, which are crucial regulators of cell proliferation, apoptosis and the inflammation response. Furthermore, MAPK activity in ADAMTS18 KO mice is associated with the upstream RhoA/ROCK (Rho-associated coiled-coil containing protein kinase, monitored by MyPT1 and MLCK) signaling pathway, which breaks down cell-cell junction, increases intestinal permeability, promotes LPS entry and subsequent MAPK activation. Collectively, our findings indicated that ADAMTS18 plays a crucial protective role in the development of colitis-associated colon cancer, which holds promise to design new therapies for the treatment of colorectal cancer.

**No conflict of interest.**

**324** Poster (Board P003)

**Transcriptome-based network analysis of the human xenograft tumor–mouse stroma compartments**

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**Background:** Tumor microenvironment plays critical roles in tumor growth/progression and response to pharmaceutical. However, investigating stroma-specific components is rather challenging for the difficulty to separate stroma from tumor cells, either physically or in silico via bioinformatics. Patient derived xenograft (PDX), where human tumor and mouse stroma can readily be separated in silico [1], provides a model to study tumor microenvironment, including tumor–stroma interactions. Methods: Whole transcriptome sequencing reads on a cohort of 872 subcutaneous PDXs in athymic mice [2] were aligned to human and mouse genomes to discriminate human cancer and mouse stroma content respectively [2]. Low expressed/less variable genes were removed across the two components. The gene expression profiles of 35 cell types (including endothelia, fibroblast and leukocyte) from Immunological Genome Consortium (ImmGen) [2] were used to create lineage-specific signatures and compute relative fractions of each cell type by CIBERSORT [3] in the stroma composition. Weighted gene co-expression network analysis (WGCNA) [4] was applied on human and mouse gene expression data respectively to identify distinct gene modules associated with different biological functions. Human-mouse gene correlation analysis was performed to construct cross-species expression networks. Results: The average mouse-to-human read ratio is 11.14%, consistent with the report [2]. Deconvolution of mouse gene expression identified various fractions of stromal cells, including immune cells. The fractions vary across cancers: e.g. fibroblasts ranges from ~10% to ~25%, T/CDC4 memory lymph node consists about 20% of stroma in lymphoma. The existence of large fraction of macrophages (>20%), tissue resting lymphocytes, and low amount of tumor-associated macrophages (~3%) suggested weak non-T immune interactions across species. Distinct co-expressed gene modules from human (50x) and mouse (28x) were determined by WGCNA, revealing that human clusters were more correlated to cancer type than mouse clusters, particularly strong in pancreatic, colorectal and lung cancers. Knowledge-base functional analysis of the human modules revealed the association of diverse biological processes. In addition, cross-species correlation network identified novel relationship between human and mouse genes over all PDXs and in specific cancers. For example, human transcriptional regulator ZBED6, affecting cell cycle/CRC-growth [5],
is highly correlated with oncogene Cbl/tumor suppressor Bnap2 [6], with confirming the reported correlation between hu-MIF and mu-Dx1 [2].

Conclusions: Stromal composition seemingly varies across cancers in sub-Q-PDXs, with each cancer types associated with characteristic mesoderm gene expression modules, suggesting PDX as a useful experimental model to study tumor-stroma interactions.

No conflict of interest.

325 Poster (Board P004) Immunophenotyping, RNAseq, microbiota analysis of syngeneic mouse models treated with immune checkpoint inhibitors to support biomarker and immune-oncology drug discovery


Background: Recent clinical success of anti-CTLA4, anti-PD1, and anti-PD-L1 antibodies has attracted intense interests in immune-oncology (IO) drug discovery. The importance of immune cell phenotype and the gene signatures to patient survival and response prediction has been actively investigated and highlighted. Preclinically, syngeneic tumor models have been widely used to evaluate cancer immunotherapeutics. Previously we have generated a comprehensive data set on syngeneic models that included 24 different anti-PD-L1, and anti-CTLA4 efficacy benchmarking, baseline FACS analysis of a number of tumors, as well as baseline RNAseq analysis of the same tumor. In the current study, we set out to research a set of syngeneic models with detailed profiling against both large and small molecule IO modulators. In addition to efficacy and baseline profiling analysis, the new dataset include PD response after treatment with immune checkpoint inhibitors, as well as longitudinal microbiota analysis to track changes of animal’s gut microbiota during treatment.

Material and Methods: Syngeneic cell line models were used to evaluate efficacy upon treatment with T cell checkpoint, e.g. PD1, PD-L1, CTLA4,OX-40 and IDO inhibitors. Tumors from different syngeneic models were collected before the treatment for RNAseq analysis. Tumors were also collected after treatment for immune cell analysis with FACS for PD response. Feces from individual mouse were collected during the study to track microbiota changes using 16S sequencing.

Results: Crown has established the largest collection of syngeneic models with well characterized immunotherapy data. Those models have a diverse response to PD1, PD-L1, CTLA4,OX-40 and IDO inhibitors, ranging from close to 100% inhibition to inducing tumor growth upon treatment. We have also generated detailed maps of the expressionional and mutational profiles of those models. Mutational analysis indicated a number of syngeneic models harbor mutations that may be useful for combination studies of targeted therapies and checkpoint therapy. Immunophenotyping of the post-therapy tumor samples has identified the role of certain population of immune cells in drug response. Microbiota analysis provided additional QC step that may help explain the variability observed in many syngeneic studies.

Conclusions: Our detailed profiling data may help researchers in selecting the best models for their agents or combination strategies. The tools we provide may also help understand the mechanism of action of specific IO therapies.

Conflict of interest: Ownership: Crown Bioscience Inc.

326 Poster (Board P005) Development and validation of a patient derived xenograft based preclinical platform for immuno-oncology drug development

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Patient-derived tumor xenografts (PDX) have played a major role in the development of new cancer therapies. However, one major drawback of PDX is the lack of an immunological competent host. To overcome this hurdle the use of different humanized mouse strains is gaining more and more importance. In the current study we compared two humanizing approaches in NOD/Shi-scid/IL-2R-null (NOG) mice bearing twelve different PDX models treated with Nivolumab, Ipilimumab and the combination thereof. 48 NOG mice were humanized with CD34+ hematopoietic stem cells (HSC) from cord blood (CB) whereas 25 NOG mice received HSC from fetal liver (FTL). Following engraftment of human immune cells tumor material was implanted subcutaneously. Treatment started at tumor size of 60–150 mm³. Individual mice were treated with Nivolumab, Ipilimumab or the combination thereof. The panel of PDX models consisted of one melanoma, five adenocarcinoma, three squamous cell and three large cell carcinoma of the lung. With n=1 per treatment arm and model the study design followed the screening approach of the single mouse trial (SMT, Gredy et al, 2015). Infiltration of human immune cells was determined by flow cytometry (FC) in blood, bone marrow, spleen and tumor. Organ and tumor tissue was sampled for subsequent immunohistochemistry (IHC). All investigated PDX models showed a distinct growth behavior independent of the host immune status (CB vs FTL) and analogous to the corresponding features depicted in conventional NOG. 100% of the implanted animals could be assigned to a treatment arm qualifying this study layout for large screening approaches. All three treatment regimen displayed a discrete activity pattern throughout the PDX panel: Ipilimumab was slightly more active as Nivolumab. Nevertheless, combined therapy showed superior antitumor activity as compared to single agent administration. The sensitivity pattern of the PDX models was not influenced by the host’s immunization protocol (CB vs FTL). No clear correlation between the RNA expression of CTLA4 receptors CD80 & CD86 and PD-L1 and the sensitivity of the respective model was identified. Of note, the most sensitive model lung cancer PDX LXFA 1674 displayed a markedly higher expression of CD86 as all other models. FC analyses revealed an increase of activated CD4+ and CD8+ T cells under immunotherapy in all investigated organs. Numbers of tumor infiltrating lymphocytes were significantly increased specifically by Ipilimumab treatment. A link between extend of T cell activation and antitumor activity could not be drawn. IHC confirmed increased expression of CD86+ and PD-L1+ tumor tissue and spleen. Our investigations support the use of PDX based humanized mouse models in a SMT format, as it enables screening approaches using complex and clinically relevant mouse models.

No conflict of interest.

327 Poster (Board P006) Mouse tumor HuGEMM/hmCTLA-4 models for assessing human anti-CTLA-4 therapeutics

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Background: Blockage of immune checkpoint e.g. by anti-CTLA-4 antibody, becomes breakthrough cancer therapy [1]. Experimental model plays essential roles in developing such new immune-oncology (IO) therapy or new combination strategy. Syngeneic mouse tumor model has been widely utilized as model for testing surrogate I/O therapy by utilizing mouse immune [2], but it cannot be used for testing human biologics, due to non-cross activity between species. The replacement of mouse therapeutic target by human counterpart via knock-in while maintaining normal immunity could be a potential practical precision tool to assess human biologics in vivo as we have previously described [3].

Methods: We have engineered a chimeric human/mouse CTLA-4 gene (hm/CTLA-4) composed of human exome 238 and mouse exome 184. We knock-in this recombinant gene into C57Bl/6 mouse genome to generate HuGEMM-hm/CTLA-4+/+ mouse, which was tested for growth of MC38 syngeneic mouse cell line and MuPrime SK6605 (a mouse squamous cell carcinoma previously described [4]) derived grafts and for antitumor activity by human CTLA-4 antibodies.

Results: HuGEMM-hm/CTLA-4+/+ mice were found to express the chimeric gene in T-lymphocytes, but not wild type mouse CTLA-4 gene. Syngeneic MC38 cell derived tumor was shown to grow well in HuGEMM-h/mCTLA-4+/+ mice after subcutaneous engratment, as similarly seen in engraftment pariental wild-type C57Bl/6 mice, suggesting that the anti-human CTLA-4 immunity in chimeric mice seems not affect the growth of the cells in a significant way to impact the present experiments. MC38 tumor responded to anti-human CTLA-4 antibody including ipilimumab-like antibody, which were dosen either immediately after implantation or at staged tumors, suggesting that alteration of CTLA-4 mice is sufficient for evaluating human CTLA-4 antibody. The tumor response seems correlated with the increase in CD8+ tumor infiltrate lymphocytes (TIL). At present, several other experimental huCTLA-4 antibodies are also being evaluated in this systems, and MuPrime-SK6605, responsive mouse surrogate antibody well [4], is also tested assessed in this model. The data of both experiment will be presented at the meeting. In addition to this huCTLA-4 and previously described huPD1-HuGEMM mice, we are also engineering other HuGEMM-hu-CKPT (e.g. OX40, 4-BB, TIM3, CD3, etc.) for evaluating other checkpoint inhibitors.

Conclusions: Our data suggests HuGEMM-hm/CTLA-4+/+ mouse can be explored to evaluate new anti-human CTLA-4 antibody and also their combinations with other therapeutic agents.

No conflict of interest.
Autophagy dependence and effect of autophagy inhibition on chemosensitivity in canine osteosarcoma cell lines

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Background: The process of autophagy is being actively studied to determine its role in tumor cell death in response to anticancer drug therapy. A number of studies have shown that autophagic processes can play a role in the sensitivity of human osteosarcoma (hOSA) cells to chemotherapy. Naturally occurring osteosarcoma in dogs is 30-50 times more prevalent than that seen in human adolescents and the diseases are strikingly similar in terms of etiology, pathology and molecular signatures. Thus canine osteosarcoma (cOSA) is generally regarded as an excellent translational model for testing therapeutic modalities for potential use in hOSA. For that purpose, these studies determined the autophagy dependence of 6 cOSA cell lines as well as whether autophagy inhibition can be used in combination with cytotoxic chemotherapy as a strategy in treating cOSA.

Materials and Methods: Six cOSA cell lines that stably express nucleare RFP (Abrams, D17, Gracie, McKinley, Moresco and SAB) were screened for autophagy dependence by measuring sensitivity to hydroxychloroquine (HCQ) by Incucyte® ZOOM live cell imaging. Sensitivity to doxorubicin (DOX) and combined HCQ and DOX were also measured. Basal autophagic flux was measured using CYTO-ID® Autophagy detection kit and accumulation of LC3II following nutritional stress and autophagy inhibition by HCQ.

Results: Basal autophagic flux was detected in all cOSA cell lines. Sensitivity to DOX and the combination is shown in the table below as measured by Dm and combination index (CI) values. The data show that cOSA cells are relatively sensitive to HCQ with Dm values ranging from 8.3 to 28.4 μM. CI values show that HCQ and DOX combined effects on cOSA cells are additive.

HCQ Dm (μM) | DOX Dm (ng/ml) | CI value
--- | --- | ---
Abrams | 8.3 | 27.1 | 0.84–1.56
D17 | 19.6 | 3.9 | 0.88–1.85
Gracie | 9.0 | 6.4 | 0.93–1.26
McKinley | 9.1 | 15.5 | 0.75–1.13
Moresco | 28.4 | 17.2 | 0.70–1.38
SAB | 10.6 | 9.6 | 1.10–1.89

Conclusions: cOSA cell lines are sensitive to HCQ and DOX at concentrations that are achievable in dogs based on comparing measured tumor tissue trough levels of HCO in dogs (Autophagy 10:1415, 2014) to measured Dm values and to DOX AUC in dogs treated at 30 mg/m² (J Vet Med A 57:484, 2010). This suggests that autophagy inhibition by HCQ may be a viable strategy for enhancing chemotherapy response to DOX in cOSA and merits further study.

No conflict of interest.
a challenge to develop targeted therapies. Here we describe two genetically defined AML mouse models that are derived from patient leukemia cells isolated from leukapheresis samples. J000096994 is FLT3-ITD positive and J000096854 is FLT3-ITD positive and IDH1 positive. Both AML lines efficiently engrafted in the triple transgenic NSG\textsuperscript{TM}-SGM3 mouse model that express human IL-3, GM-CSF and SCF to support the stable engraftment of myeloid lineages. However, under the same condition J000096994 did not engraft in NSG\textsuperscript{TM} mice without expression of the cytokines and J000096854 engrafted in NSG\textsuperscript{TM} together. These observations suggest that in the characterization of both AML lines, J000096854 exhibited faster engraftment kinetics than that of J000096994. Over 1% the cells in the peripheral blood of NSG\textsuperscript{TM}-SGM3 mice were human CD33\textsuperscript{+} at 6 and 10 weeks post engraftment while it reached over 10% for J000096994 at 16 weeks. While there was a difference in the engraftment kinetics in peripheral blood between the two models, the engraftment levels of human CD33\textsuperscript{+} cells in spleen and bone marrow were comparable at endpoint. Moreover, AML cells were able to be serially transferred to recipient NSG\textsuperscript{TM}-SGM3 mice. Both models displayed positive response to cytarabine (ara-C) treatment with levels of human CD33\textsuperscript{+} cells reduced to the nadir two weeks after the 5-day treatment cycle was initiated. This presentation will demonstrate that two patient-derived AML models have been established in NSG\textsuperscript{TM}-SGM3 mice and that they have been characterized to show distinct engraftment kinetics and positive response to an AML standard of care drug ara-C. These AML models may be useful tools for testing experimental therapeutics that target FLT3 and IDH1 for treating AML.

**No conflict of interest**

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### 333 Poster (Board P012)

The Eμ-TCL-1 mouse model of chronic lymphocytic leukemia

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**Background:** Chronic lymphocytic leukemia (CLL) is a heterogeneous disease characterized by the accumulation of CD5\textsuperscript{+}CD19\textsuperscript{+} B cells in all lymphoid compartments. Apart from hematopoietic stem cell transplantation, current treatment options are not curative, and in many patients, the disease eventually relapses or becomes resistant to standard therapies. Precinical evaluation of novel therapeutic approaches for CLL has been limited by the inability to propagate patient samples in vitro culture for prolonged periods and the lack of representative cell lines or mouse models. Croce et al. developed and characterised the Eμ-TCL-1 mouse model, where the oncogene TCL-1 is overexpressed in B cells, as a putative CLL model. The aim of our studies is to further characterise the mouse model using next-gen sequencing approaches.

**Materials and Methods:** Next-gen sequencing was performed using RNA isolated from CD5\textsuperscript{+}CD19\textsuperscript{+} cells from the spleens of transgenic mice. We compared the transcriptome profiles of murine leukemic cells with normal B cell subsets and a cohort of human CLL patients (Ferreira et al., 2014).

**Results:** We show that the molecular signatures derived from CD5\textsuperscript{+}CD19\textsuperscript{+} TCL-1 leukemic cells from Eμ-TCL-1 mice are distinct from other normal B cell subsets. Strikingly, molecular signatures of these leukemias demonstrate minimal overlap with those of the most human population (Ferreira et al., 2014). Nonetheless, more stringent analysis revealed that the mouse model does bear a close resemblance to a subgroup of human CLL characterized by high TCL1 expression. TCL1 overexpression has been associated with adverse outcome in CLL patients. Interestingly, unlike prototypical CLL disease, dysregulation of BCL-2 family protein expression was not detected in leukemic cells from Eμ-TCL-1 transgenic mice, which appear refractory to the inhibition of BCL2, at least in vitro. Moreover, the leukemias isolated from sick mice showed a surprisingly wide range of sensitivity to standard-of-care agents such as fludarabine.

**Conclusions:** Together, our studies suggest that the leukemias which develop in Eμ-TCL-1 transgenic mice represent a highly aggressive, chemoresistant subtype of CLL. The goals of our ongoing studies are to further characterize this model of CLL and utilize it to test novel therapeutic approaches for treating CLL, particularly chemoresistant disease.

**References**


**No conflict of interest**

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### 334 Poster (Board P013)

Genomic characterization of immune targets in patient-derived xenograft models for translational assessment of immunotherapy

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**Background:** Although oncology therapies reactivating the immune response by targeting checkpoints such as PD1 and PD-L1 are now FDA approved, development of therapeutics regulating other immune targets remains an area of active research, and comparison between human and murine immune systems have generally limited the use of animal models in this setting. Whilst patient-derived xenograft (PDX) models are robust pre-clinical tools, whether they reflect human immune marker expression patterns remains unclear. Here we describe the genomic characterization of immunotherapy targets across a large panel of PDX models. This dataset is essential for planning downstream translational studies with PDX models in humanized mouse systems for evaluating immuno-oncology (IO) agents.

**Materials and Methods:** RNA sequencing was used on PDX models to determine expression of genes considered targets for IO agents, including PD-L1, LAG3, IDO1 and 2, MUC1, and MUC4. Given the reported correlation between mutation load and responses to IO agents, the total number of germline and somatic mutations (single nucleotide variations/small indels) in each PDX model was calculated from whole exome sequencing data. HLA locus analysis by sequence-based typing (SBT) was performed for graft-host alignment.

**Results:** A total of 327 PDX models across 8 tumor types (melanoma, lung (NSCLC/SCLC), ovarian, breast, pancreatic, head and neck, sarcoma, and colorectal) were sequenced. Overall, MUC4 was the most prevalently expressed target, showing >2-fold expression (relative to all PDX models) in 18\% of PDX models, followed by PD-L1 (15\%) and IDO1 (13\%). Across specific tumor types, PD-L1 was most prominently expressed in lung (41\% of models), IDO1 and LAG3 in ovarian (33\% of models for both genes), and MUC4 in lung and pancreatic (~33\% of models for both genes). Melanoma and lung PDX cohorts had the highest percentage of models showing >2-fold expression of at least one IO target (76\% and 67\% respectively). Melanoma, gastric, and colorectal PDX demonstrate the greatest confluence of mutations, averaging >800 mutations/model. In contrast, sarcoma had fewer, averaging <400 mutations/model. In keeping with population data, HLA-A2 (45\%) was the most commonly identified HLA antigen, followed by HLA-A1 (19\%) and HLA-A* (13\%).

**Conclusion:** PDX models are robust translational models; however, evidence of their utility for evaluating IO agents has been limited. We have now described the differential expression of various new and well-characterized immune targets in a large number of solid tumor PDX models. Such models may be reasonable surrogates for screening novel immunotherapies in mice the context of a human immune system.

**Conflict of interest:** Ownership: David Sidransky, David Cerna, Angela Davies, Daniel Ciznadjia, Neal Goodwin, Ido Sloma, Ido Ben Zvi. Board of Directors: David Sidransky.

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### 335 Poster (Board P014)

Patient-derived xenograft (PDX) models of BRCA-deficient and BRCA-like ovarian tumors reflect clinical responses to PARP inhibition

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**Background:** PARP inhibition in BRCA-deficient/BRCA-like ovarian tumors can lead to synthetic lethality. The FDA has approved a PARP inhibitor to treat BRCA-deficient ovarian carcinomas and other drugs targeting PARPs (PARPi) are in development, generating further interest for this target. PDX models could enable evaluation of PARPi resistance/response mechanisms, improving patient stratification protocols and optimizing therapeutic strategies. In this pilot study, we evaluated resistance to PARPi in 2 PDX models generated from ovarian patient-derived xenograft models and correlated responses to clinical outcomes.

**Materials and Methods:** PDX models developed from ovarian patient-derived xenograft models were subjected to next-generation sequencing to identify genetic alterations in BRCA 1/2 and regulators of alternative DNA repair processes that contribute to a BRCA-like phenotype (e.g. ATM, RAD51, FANCA, and FANC2D). Models were screened against the FDA-approved PARPi, i.e., olaparib and carboplatin/paclitaxel in BRCA-deficient/BRCA-like ovarian PDX models and correlated responses to clinical outcomes.

**Conclusion:** Patient-derived xenograft (PDX) models of BRCA-deficient and BRCA-like ovarian tumors reflect clinical responses to PARP inhibition.
Characterizing the effect of immune checkpoint inhibitors on syngeneic tumor models through gut microbiome sequencing and immune-phenotyping

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Background: Syngeneic mouse models present an important tool to understand the local and systemic effects of immune modulating therapies. Immune checkpoint inhibitors have become a mainstay of oncology treatments, yet many patients experience partial responses. Thorough characterization of pre-clinical models is paramount in order to successfully predict response to treatment in the clinical setting.

Material and Methods: We examined the efficacy of anti-PD-1, anti-PD-L1, and anti-CTLA-4 therapies on our syngeneic tumor model platform. Immune-phenotyping was conducted at various time points. Gut microbiome was examined by r16S sequencing to analyze statistically significant shifts longitudinally and between different arms and animals at cross-sectional time points.

Results: Immune checkpoint inhibitors had variable efficacy across different tumor models. We also observed shifts in immune cell populations such as T and regulatory cells and M1/M2 macrophages, as treatments continued. Gut microbial community analysis enabled analysis of enriched microbes between groups and individual animals, as well as across time points.

Conclusions: Here, we report on the establishment of syngeneic mouse models including efficacy, flow cytometry analysis of tumor-infiltrating lymphocytes, and gut microbial community analysis.

No conflict of interest.

Vaccination and immunomodulators in ovarian cancer: a phase II study of IMMU-110, the first human antibody against the mesothelin tumor antigen


Background: The mesothelin (MSLN) tumor antigen is a highly expressed, low molecular weight glycoprotein, which is elevated in both malignant and non-malignant fluid. It has been reported in a number of solid tumors, and has been identified as a potential immunotherapeutic target.

Material and Methods: A total of 51 ovarian PDX models were developed from 48 patients. Twenty-one (70%) models harbored deleterious mutations in BRCA 1/2. Of 9 BRCA negative models, 6 had mutations in molecules contributing to a BRCA-like phenotype such as PALB2 and FANCA, whilst 3 models had no identifiable mutations in these genes/pathways. We screened 14 ovarian models (3 BRCA 1/2 wild type, 8 BRCA1 mutated, and 3 BRCA2 mutated) against carboplatin/paclitaxel and olaparib. Based on tumor growth, 4 models were considered resistant, 5 models platinum sensitive (definitive TR), and the remainder of intermediate sensitivity (TR equivalent to stable disease). TR values aligning with stable disease were observed in 4/14 (29%) ovarian PDX models treated with olaparib, which corresponds with reported clinical trial RR (~33%). There was no correlation between disease. TR values aligning with stable disease were observed in 4/14 (29%) ovarian PDX models treated with olaparib, which corresponds with reported clinical trial RR (~33%). There was no correlation between disease.

Conclusion: In this pilot study, RR to olaparib in ovarian PDX models with BRCA mutations/BRCA-like phenotype was similar to clinical trial outcomes. In keeping with literature reports, platinum pretreatment did not affect model responsiveness to olaparib. Nevertheless, whilst the majority of models responding to olaparib harbored mutations in BRCA/BRCA-like genes, one was wild type with respect to these pathways. Further, other BRCA mutated/BRCA-like models failed to respond to PARP inhibition. This suggests a need for further interrogation of de novo resistance/sensitivity mechanisms, which the PDX platform is well suited to uncover.


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Characterizing the effect of immune checkpoint inhibitors on syngeneic tumor models through gut microbiome sequencing and immune-phenotyping

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Combination strategies with checkpoint immunotherapy and inducers of immunogenic cell death (ICD) in immune competent syngeneic models

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Background: A number of treatment strategies such as radiotherapy (RT), oncolytic viruses and chemotherapeutic agents such as oxaliplatin, doxorubicin, bortezomib and mitoxantrone have been highlighted as potential inducers of immunogenic cell death (ICD) through a well-defined mechanism resulting in the increased presentation of cell-associated antigens to CD4+ and CD8+ T lymphocytes by dendritic cells. Thus combination strategies of ICDs with immunotherapy (IT) could provide opportunities to harness the immune system to extend survival, even among metastatic and heavily pre-treated cancer patients and may increase the efficacy of immunotherapy in those cancer types with low immunogenic status. Here we report the application of IT and vaccination in combination with IT (anti-CTLA-4) to examine its impact on tumour growth and immunity.

Materials and Methods: Bioluminescent 4T1 mammary carcinoma cells or CT26 mouse colon cells were implanted subcutaneously or orthotopically into BALB/c mice. Subcutaneous tumour growth was monitored by caliper measurement and bioluminescent imaging (BLI) was carried out to confirm orthotopic and/or metastatic growth. Established tumours were treated with IT in combination with checkpoint inhibition (CTLA-4) or RT; both the clinical and condition of mice were monitored daily. At termination the tumours were collected and assessed for immune cell infiltration and/or ICD markers by FACS and IHC.

Results: CT26 but not 4T1 tumours exhibited a moderate response to IT, whilst treatment with RT resulted in a statistically significant tumour growth inhibition (TGI) in both models. Combination of both regimens resulted in an additive TGI over monotherapy, additionally, for CT26, tumour response...
in the IT + IGMI, but not the RT group, continued following the cessation of dosing resulting in complete regression in 80% of remaining tumours. In total, protein extracts from matched FF and FFPE tissue is well feasible and can provide biologically significant information for a large panel of proteins using minute amounts of tissue. The identified panel of markers may be useful for pharmacodynamic studies of drug effect bridging pre-clinical to clinical results. The approach may be a versatile tool in clinical research, translational medicine and pharmaceutical drug development to stratify patient tumours and to predict drug response.

No conflict of interest.

341 Poster (Board P020)

Establishment and characterization of patient-derived xenograft (PDx) models from peri toneal metastasis of colorectal carcinoma as novel platform for drug testing and biomarker evaluation

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Background: Colorectal carcinoma (CRC) is associated with high incidence and high mortality rate, particularly if metastasized to distant sites. One important site for CRC metastasis is the peritoneum. At time of first diagnosis 4 to 7% of the patients suffer from peritoneal metastasis (PM) of CRC. The PM is associated with poor prognosis and limited therapeutic options. Therefore, availability of adequate in vivo models for PM could significantly promote the evaluation of chemosensitivity of PM towards standard, targeted and novel drugs as well as analysis of novel prognostic or even predictive biomarkers. Such models could be employed for patient stratification and individualized concepts to improve the therapeutic outcome of PM patients. During the last decade patient-derived xenograft (PDx) mouse models have gained importance, since they closely resemble the molecular and biological features of the original primary tumours. So far no PM PDx models have been established from CRC. We therefore focused on the establishment of a novel CRC PM panel of PDx as useful platform for preclinical studies.

Material and Methods: For the PDx establishment colorectal surgical specimens were subcutaneously (s.c.) transplanted onto NOD scid gamma (NSG) immunocompromized mice. Engrafted tumors were transferred to NMRI nu/nu mice for further passaging. Engrafted tumors were characterized by histopathological and gene expression analyses. Real-time RT-PCR and immunohistochemistry. Cmosensitiveness of PDx models was evaluated in vivo by application of a panel of conventional chemotherapeutic and targeted drugs.

Results: For PDx establishment 58 CRC surgical specimens (taken from peritoneum and omentum) were transplanted onto NSG mice. From those, currently 19 PDx have engrafted, growing stably on NMRI nu/nu mice. Histopathological evaluation revealed maintenance of the CRC histology of
the PDX. For selected PDX, orthotopic transplantation into the peritoneum revealed their potential to form tumors in kidney, ovaries and abdominal diaphragm. The chemosensitivity testing of conventional and of targeted drugs in 10 of the PM PDX models revealed heterogeneous response of the PDX to targeted drugs in 5-FU, irinotecan, docetaxel, cetuximab, erlotinib and regorafenib. More interestingly, different responses were observed in PDX from omentum vs. peritoneum, derived from the same patient, indicating some heterogeneity within the PM.

Conclusions: This PDX mouse model panel of the CRC PM can be used for further studies to test novel therapies or combinations for PM of CRC. Our results demonstrate, that this novel panel of PDX maintains the morphology of the patient tumor in early passages, reflects heterogeneous response rates, possess potential of disseminated growth in the peritoneum and can be used as preclinical in vivo platform for translational studies of potential clinical use.

No conflict of interest.

342 Poster (Board P021)
Establishment and characterization of a hormone dependent, PSA/PSMA positive prostate PDX model
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Background: Preclinical xenograft models of cancer are routinely used to screen potentially useful therapies. While some tumor types have abundant models available for evaluation, few prostate cancer models are available, with the majority representing hormone-dependent, PSA/PSMA positive prostate cancer. To address this issue we established a prostate cancer patient-derived xenograft (PDX) designated ST1273 in athymic nude mice and characterized and compared the model against cell-based prostate cancer xenografts and a second prostate cancer PDX.

Materials and Methods: ST1273 was established from a primary biopsy collected from a sixty-five year old Hispanic male with prostate cancer. Receptor expression of the resulting model was determined using immunohistochemistry and mutations identified by NGS. Results were compared with the five cell-based xenograft (CBX) prostate models LNCaP, PC-3, DU145, E666A-hT and VCaP and a second prostate PDX designated ST2347. In vivo studies were performed evaluating ST1273 and CBX models towards approved therapies and ST1273 towards two investigational PSA/PSMA-targeting antibody-drug conjugate (ADC) therapies. In vivo study endpoints included tumor volume and time from treatment initiation with T/C values and tumor regression reported at study completion.

Results: ST1273 growth was found dependent on exogenous hormone. ST1273 and LNCaP were found to express PSA while VCaP expressed glucocorticoid receptor (GR). ST1273 and CBX models reported variable activity towards docetaxel and the targeted therapies abiraterone and enzalutamide. Interestingly treatment with one of the ADC molecules resulted in durable tumor regressions in ST1273.

Conclusion: We have established and characterized a PDX representing hormone-dependent, PSA/PSMA positive prostate cancer designated ST1273 and benchmarked it against standards of care and CBX prostate xenografts. We also demonstrated its utility towards evaluating PSA/PSMA-targeting therapies.

No conflict of interest.

343 Poster (Board P022)
NSG<sup>–/–</sup>Quad mice, a new humanized mouse model with improved human innate immune cell development
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The JAX<sup>®</sup> Onco-Hu<sup>®</sup> Platform utilizes CD34<sup>+</sup> HSC humanized NSG<sup>–/–</sup> and NSG<sup>–/–</sup>SGM3 mice engrafted with human patient-derived xenografts and human tumor cell lines to enable the in vivo investigation of the interactions between the human immune system and human cancer. We have recently illustrated the efficacy of PD1 and CTLA-4 check-point inhibitors in both humanized mouse strains engrafted with Non-Small Cell Lung Cancer and Triple-Negative Breast Cancer tumors. A major avenue of our investigation is to increase the understanding of the role of the human immune system in the control of cancer by generating murine humanized models with a more complete hematopoietic system and robust innate immune cell population. Monocytes/macrophages and NK cells are important in tumorignoresis and are potential targets for cancer immunotherapy. The HSC humanized triple transgenic NSG<sup>–/–</sup>SGM3 mice express three human cytokines SCF, GM-CSF, and IL-3 have more robust and faster engraftment levels in myeloid and lymphoid populations compared to its NSG<sup>–/–</sup> predecessor. Here we introduce a new mouse strain, the NSG<sup>–/–</sup>Quad mouse, in which the fourth cytokine human CSF-1 is expressed on the NSG<sup>–/–</sup>SGM3 background through the crossing of the NSG<sup>–/–</sup>SGM3 and NSG-CSF<sup>+</sup> mouse strains. This could further support the development and function of myeloid cells and natural killer cells derived from human CD34<sup>+</sup> HSC cells. For the first time, we present the hematopoietic engraftment protocol tailored for the new strain and provide a direct comparison of human immune system reconstitution in the peripheral blood of NSG<sup>–/–</sup>SGM3 mice vs. NSG<sup>–/–</sup>Quad recipient mice. The functions of human monocytes and NK cells in NSG<sup>–/–</sup>Quad mice will be discussed including responses to inflammatory cytokine activity, respectively. Lastly, we will show the growth curves of human tumor xenografts implanted in HLA partially matched human HSC engrafted NSG<sup>–/–</sup>Quad mice and their potential in further understanding the immune cell and tumor interactions.

No conflict of interest.

344 Poster (Board P023)
Inactivation of KLF4 promotes T-cell acute lymphoblastic leukemia and activates the MAP2K7 pathway that can be targeted for therapy
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T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive blood cancer that affects children and adults, originates from the malignant transformation of lymphoid progenitor cells and is considered to be a high-risk disease due to the poor response to treatment and increased incidence of relapse. Although relapse is the leading cause of death in children with cancer, there is no targeted therapy available for these patients to eliminate chemoresistant leukemia initiating cells (LIC). We have discovered that levels of the Krüppel-like factor 4 (KLF4), which is known to promote stemness and to reprogram somatic cells into pluripotent stem cells, are significantly low in T-ALL patients, particularly in the ETP and TLX ALL subgroups associated with poor prognosis. In contrast to normal blood cells, we found that lymphoblasts from children with T-ALL exhibit low levels of KLF4 transcripts and hypermethylation of the proximal KLF4 promoter. To study the role of KLF4 and the genetic events governing the transition from a pre-leukemic state to aggressive disease, we used a T-ALL mouse model based on the expression of the gain-of-function NOTCH1-L1601P-αβδ mutant that has weak leukemicogenic capacity and is frequently found in patients with T-ALL. Deletion of the Klf4 gene accelerated the onset and worsened disease progression by deregulating the proliferation of leukemic cells and increasing the frequency of LIC identified by limiting-dose transplantation and immunophenotypic identification. A combined analysis of global gene expression and genome-wide binding revealed that KLF4 directly repressed the Map2k7 gene encoding the dual specificity mitogen-activated protein kinase 7 (Map2k7) involved in stress MAPKK pathway. Most remarkably, T-ALL cells from both Klf4<sup>+/−</sup> leukemic mice and pediatric patients displayed elevated levels of total and phosphorylated Map2k7 and subsequent activation of the downstream targets JNK, c-Jun, and ATF2. Pharmacological inhibition of activated JNK induces cytotoxicity in T-ALL cell lines, xenograft model of T-ALL, and cells from relapse T-ALL PDX, suggesting an alternative approach to treat T-ALL. Collectively, these data uncover a novel function for KLF4 by regulating the MAP2K7 pathway in T-ALL that can be targeted to eradicate leukemia-initiating cells in T-ALL patients.

No conflict of interest.

Cytotoxics

345 Poster (Board P024)
A site-specifically conjugated anti-CD22 antibody bearing an MDR1-resistant maytansine payload yields excellent efficacy and safety in preclinical models
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Background: Hematologically-derived tumors make up ~10% of all newly-diagnosed cancer cases in the U.S. Of these, the non-Hodgkin lymphoma (NHL) designation describes a diverse group of cancers that
collectively rank among the top 10 most commonly diagnosed cancers worldwide. Although long-term survival trends are improving, there remains a significant unmet clinical need for treatments to help patients with relapsed or refractory disease, one cause of which is drug efflux through upregulation of xenobiotic pumps, such as MDR1. We describe a site-specifically-conjugated antibody–drug conjugate targeted against CD22 and bearing a noncleavable maytansine payload that is resistant to MDR1-mediated efflux. The construct is efficacious against CD22+ NHL xenografts and can be repeatedly dosed in cynomolgus monkeys at 60 mg/kg with no observed adverse effects. Together, the data suggest that this drug has the potential to be used effectively in patients with CD22+ tumors that developed MDR1-related resistance to prior therapies. CD22 is a clinically-validated target for the treatment of NHL and ALL, but no anti-CD22 agents have yet been approved. An opportunity exists for a next-generation anti-CD22 antibody–drug conjugate (ADC) to address unmet medical needs in the relapsed/refractory NHL and ALL populations.

Materials and Methods: An anti-CD22 antibody was conjugated site-specifically, using aldehyde tag technology, to a noncleavable maytansine payload. The ADC was characterized both biophysically and functionally in vitro. Then, in vivo efficacy was determined in mice using two xenograft models and toxicity studies were performed in both rat and cynomolgus monkeys. Pharmacodynamic studies were conducted in monkeys, and pharmaco- and toxicokinetic studies compared total ADC exposure in the efficacy and toxicity studies.

Results: The ADC was very potent in vivo, even against cell lines that had been constructed to overexpress the efflux pump, MDR1. The construct was administered at 10 mg/kg x 4 doses against MDR1-expressing tumor models, and in a cynomolgus toxicity study, the ADC was dosed twice at 60 mg/kg with no observed adverse effects. Exposure to total ADC at these doses (as assessed by AoUC0-t) indicated that the exposure needed to achieve efficacy was below tolerable limits. Finally, an examination of the pharmacodynamic response in the treated monkeys demonstrated that the B-cell compartment was selectively depleted, indicating that the ADC eliminated targeted cells without notable off-target toxicity.

Conclusions: The results suggest that this novel ADC has the potential to be used effectively in patients with CD22+ tumors that have developed MDR1-related resistance to prior therapies.

Conflict of interest: Corporate-sponsored Research: The authors are employees of Catalent Pharma Solutions.

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346 Poster (Board P025)

Novel liver-targeted antimitabolic pronucleotide for advanced, second-line hepatocellular carcinoma and liver metastasis

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Background: The modification of gemcitabine, 2′-deoxy-2′-difluorocytidine (dFDC) into a monophosphate prodrug allows for targeted cell delivery based on specific enzymes recognition for the cleavage of the prodrug and the subsequent release of the dFDC-monophosphate (dFDC-MP) in the target cells. Direct release of dFDC-MP by bypassing the putative resistance pathway related to sub-expression of deoxy-cytidine kinase (dCK) in tumor cells responsible for the diminished phosphorylation of dFDC.

Material and Methods: The pronucleotide (22032) was compared to its pair diastereomer (22033) and parent nucleoside (dFdC) for its pharmacokinetics properties and its hepatocyte delivery of dFDC-triphosphate (dFDC-TP) and the generation of the gemcitabine metabolite 2′-deoxy-2′-difluorouridine (dFdU)-triphosphate (dFdU-TP) in C57BL/6 male mice when administered intraperitoneally at a dFdC equivalent released dose. The maximum tolerated dose (MTD) assessed in bablb c-nude mice following once a week (QW) and twice a week (BIW) dosing with a body weight loss lower than 10% over the treatment. The efficacy measured in huh-1 model (QW and BIW) and human primary hepatocellular carcinoma (HCC) tumor model (BIW) by monitoring weekly over 21 days the alpha-fetoprotein (AFP) level and the tumors volume and weight at study termination. The aim of the study was to demonstrate the tolerability and efficacy of the pronucleotide in both cell line (CDX) and patient derived (PDX) tumor model.

Results: The administration of 22032 in C57BL/6 mice demonstrated greater level of the active dFDC-TP anabolite (x81) in the liver as compared to dFdC at equivalent released dose. The level of metabolite dFdC-TP was similar between the two groups with an AUClast = 6420 hr×μmol/g as compared to an AUClast = 5530 hr×μmol/g for gemcitabine therefore demonstrating a dFDC-TP/dFdU-TP of 85 fold in favor of the pronucleotide indicating a liver metabolism of the gemcitabine. The pronucleotide was well tolerated QD and BIW at a dFDC equivalent released dose, respectively 1.25 and 2.5 times superior to dFdC. The antitumor effect of 22032 was significantly different from control in huh-1 and HCC primary tumors and compared favorably versus dFdC group with an average tumor size 3.6 times smaller in huh-1. The Tumor weight and AFP level correlate in both QW and BIW groups in the cell derived and patient derived model making AFP an attractive biomarker.

Conclusions: 22032 demonstrate an improved tolerability and safety profile over gemcitabine with statistically significant size reduction compared to the vehicle in both CDX and PDX model. Its high delivery of active dFdc-TP observed in the liver make it an attractive candidate to be further investigated to profile its clinical potential for liver targeted therapy.

No conflict of interest.

347 Poster (Board P026)

The biological activity of clavilactones and their analogs

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Background: Clavilactones are fungal metabolites isolated from Clitocybe clavipes, and are known as EGFR-TKIs (Epidermal growth factor receptor-tyrosine kinase inhibitors). Since the biological activities of clavilactones are poorly understood, we examined the potential use of clavilactones for cancer therapy using a series of synthetic analogs.

Material and Methods: Clavilactones A, B and their analogs were already synthesized (Org. Lett., 15, 5582–5, 2013). The effect of Seco-clavilactone B (Seco-CB) on actin polymerization was assessed by filament actin (F-actin) and globulin actin (G-actin) separation assay against NHL xenograft polymerization assay using pyrene-labeled actin. In F-G-actin separation assay, cell lysate or recombinant G-actin solution polymerized in vitro was centrifuged and separated to G-actin and F-actin fraction and each actin was detected by western blotting. To identify the Seco-CB-binding site of actin, purified actin incubated with Seco-CB was digested by trypsin and/or chymotrypsin, and was analyzed by LC/MS and MS/MS.

Results: To know whether clavilactones and their analogs have anti-cancer effects, we performed MTT assay using 10 cancer cell lines derived from distinct human tissues. MTT assay showed that clavilactone A and Seco-CB, a novel synthetic analog of clavilactone B, strongly reduced cell viability comparing with other compounds even in cell lines which did not express EGFR, indicating that the compounds showed antiproliferative effect independently of their activity of EGFR-TKI. We also observed that Seco-CB uniquely changed cell morphology in some cell lines. In the view of cell morphology, the biological effect of Seco-CB on actin cytoskeleton was assessed. We observed that Seco-CB reduced the ratio of F-actin assessed by separation of G-actin and F-actin in Seco-CB-treated A549 cells. Since Seco-CB also inhibited actin polymerization in vitro, we next challenged to identify the binding site(s) of Seco-CB on actin. MS analysis demonstrated that Seco-CB binds to Thr5 and Cys285 of α-skeletal actin. Finally, we confirmed each binding site is essential for polymerization inhibition. F/G actin separation assay using a series of recombinant mutant G-actin polymerized in vitro showed that the inhibitory effect of Seco-CB on actin polymerization is depend on binding to distinct single binding site.

Conclusion: Clavilactones and their analogs showed antiproliferative effect independently of EGFR expression levels. Additionally, we identified Seco-CB as an actin polymerization inhibitor. Further assay revealed that Seco-CB inhibited actin polymerization through a novel mode of action that binds to Thr5 and Cys285 of α-actin. Finally, we confirmed each binding site is essential for polymerization inhibition. F/G actin separation assay using a series of recombinant mutant G-actin polymerized in vitro showed that the inhibitory effect of Seco-CB on actin polymerization is depend on binding to distinct single binding site.

No conflict of interest.

348 Poster (Board P027)

Efficacy of metronomic chemotherapy with oral cyclophosphamide and methotrexate in patients with non-Hodgkin lymphoma: A retrospective analysis

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Background: Metronomic chemotherapy is an emerging strategy offering a potentially less toxic yet effective treatment modality. We evaluated the efficacy and safety of low-dose metronomic (LDM) chemotherapy with cyclophosphamide and methotrexate in relapsed or refractory non-Hodgkin lymphoma (NHL).

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Methods: We retrospectively reviewed the data of 16 NHL patients who were treated with oral cyclophosphamide plus methotrexate (CM) as LDM chemotherapy. They received oral cyclophosphamide (50 mg every day) and oral methotrexate (2.5 mg twice weekly) until there was disease progression or unacceptable toxicity.

Results: A total of 15 NHL patients were evaluable for response. The overall best response rate was 53.3% (2 complete responses and 5 partial responses), with 6.7% of patients achieving stable disease. The median duration of response was 4.8 months (range, 1.45–25.3 months); the median overall and progression-free survival was 12.1 and 6.6 months, respectively; and the median response duration was 8.7 months. Only 4 patients could be treated with further salvage chemotherapy. The CM regimen was generally well tolerated, and there was no treatment-related mortality.

Conclusions: Metronomic therapy with an oral CM regimen administered for continuous, prolonged periods represents a well-tolerated treatment for recurrent NHL, especially in elderly patients.

No conflict of interest.

349 Poster (Board P028) Phase I, dose-escalation study of the investigational drug D07001-F4, an oral formulation of gemcitabine HCl, in patients (pts) with advanced solid tumors or lymphoma


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Background: This phase 1 dose-escalation trial, using a 3+3 design, and preliminary efficacy of D07001-F4, an oral formulation of gemcitabine HCl, in pts with advanced solid tumors or lymphoma. The primary objectives were to determine the MTD, characterize DLT(s) and define the recommended phase 2 dose of D07001-F4.

Methods: Patients aged ≥20 yrs with PS 0–2 were eligible. Gemcitabine 5 mg IV in cycle 0 and D07001-F4 from 2 mg PO were given on days 1, 3, 5, 8, 10, and 12 of a 21-day cycle to determine the MTD based on DLTs in cycle 1. Plasma and peripheral blood samples were obtained for PK analysis in cycle 1.

Results: 37 pts (21 M, 16 F; median 59 years [range 30–75]) were enrolled into 10 dose escalation cohorts (2–80 mg). A median of 2 cycles were given (range 0–40). There is no DLTs occurred and the MTD is defined as the highest dose cohort 80 mg. Among 34 patients received at least one dose of D07001-F4, a total of 18 patients experienced 54 possibly drug-related AEs (2 G3, others are G1 or G2). The most frequent drug related AEs are myelosuppression (n = 27), nausea (n = 10), vomiting (n = 6), blood bilirubin increased (n = 3), and anemia (n = 2). There have been 21 SAEs occurred in 18 patients including one SAR of G1 fever and one SUSAR of G3 myositis. PK analysis shows maximum concentrations of dFdC were generally achieved within 0.25 to 1 hour following dosing. Plasma dFdC was fast eliminated after dosing, the average Cmax 35.4 ng/mL and AUC0−48 h ng/mL and were rapid decreased from 2 mg to 40 mg was observed but Cmax and AUC did not increase proportionally after 40 mg. Among 24 pts evaluated for tumor response, 18 had SD and 14 of whom were on study for ≥3 months.

Conclusions: D07001-F4 appears to be well tolerated and has shown signs of anti-tumor activity in pts with advanced solid tumors. Continued clinical development is being planned.

No conflict of interest.

351 Poster (Board P030) Pharmacokinetics (PK) of alisertib (MLN8237) in adult patients (pts) with advanced solid tumors or relapsed/refractory lymphoma with varying degrees of hepatic function

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Background: Alisertib (MLN8237) is an investigational, orally available, selective Aurora A kinase inhibitor in clinical development for multiple oncology indications. This study is being conducted to evaluate the effect of moderate or severe hepatic impairment (HI) on single-dose PK of alisertib in adult cancer pts (NCT02214147).

Material and Methods: Eligible pts who met criteria for normal, moderate or severe HI as defined by National Cancer Institute Organ Dysfunction Working Group were aged ≥18 years and had ECOG PS 0–2. All pts were given a single dose of alisertib 50 mg on Day 1 for serial PK sampling over a 7-day period. Following PK assessments, pts received repeat dosing (7 days in 21-day cycles) at starting doses of 50, 30 and 20 mg twice daily in normal, moderate and severe HI groups, respectively. Protein binding was measured. Ratios of geometric mean (GM) unbound alisertib peak concentration (Cmax) and area under plasma concentration versus time curve from 0 to infinity (AUC0−inf) in pts with moderate or severe HI were calculated and the associated 90% confidence intervals (CIs) were estimated using analysis of variance. Adverse events (AEs) were graded according to NCI-CTCAE v4.03.

Results: Preliminary PK data were obtained from 36 pts: 61% male, 86% white, median age 60 years, and mean weight 90 kg. Following a single oral dose of 50 mg alisertib, median Tmax was achieved at 2.5, 3.5 and
5 hours in pts with normal hepatic function, moderate and severe HI, respectively. Alisertib terminal half-life was 23, 37 and 50 hours in pts with normal hepatic function, moderate and severe HI, respectively. There were no readily apparent differences in plasma protein binding across the hepatic function groups. The GM unbound AUC_0−inf, in pts with moderate (n = 11) and severe (n = 8) HI were 281% (90% CI: 208%, 380%) and 234% (90% CI: 158%, 346%) of that in normal (n = 12) pts, respectively. The GM unbound \( C_{\text{max}} \) in pts with moderate (n = 12) and severe (n = 8) HI was 175% (90% CI: 133%, 232%) and 114% (90% CI: 81%, 160%) of that in normal (n = 16) pts, respectively. Alisertib exposures substantially overlapped between moderate and severe HI groups. The GM ratio [90% CI] of alisertib terminal half-life for combined moderate/severe HI vs. normal was 250% [167%, 373%]. 28/36 (78%) pts had drug-related AEs. The most common drug-related grade \( \geq 3 \) AEs were neutropenia (11%).

**Results:**

Intrinsically sensitive to DM4 were variable between the CRC PDX models included testing for known mutation hotspots in CRC, Microsatellite Instability (MSI), the CpG-island Methylator Phenotype (CIMP) and miRNA expression analysis.

**Results:**

Intrinsic sensitivity to DM4 was variable between the CRC PDX models with 11/20 showing no response and 9/20 displaying different levels of sensitivity. In CEACAM5 positive tumors (16/20 models), the response to SAR408701 (9 sensitive models/20) was highly correlated with DM4 sensitivity, indicating that the activity of an antibody may be independent of the mutation status of common oncogenes in NSCLC. Further studies are warranted to explore the potential of antibody-drug conjugates (ADCs) targeting common expression changes in NSCLC.

**Conclusion:**

The pre-clinical setting, ATM-3507 enhanced the effectiveness of standard of care therapy and may prove to be an effective adjuvant therapy for the treatment of late stage NSCLC. These data are part of a translational research program that aims to progress ATM-3507 into a first-in-human safety trial pending successful completion of the requisite pre-clinical evaluations.

**Conflict of interest:**

D. Brown is an employee at Millenium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited. A. Muehler is a consultant to Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited.

**Material and Methods:**

In vivo cytotoxicity assays were performed to measure the effectiveness of ATM-3507 as a monotherapy and in combination with representative vinca alkaloids [vinorelbine (VNB) and vincristine (VCR)] and a taxane (paclitaxel) across a panel of NSCLC cell lines (A549, Calu-6, H-1299, H-1975, H-460). Synergy was assessed using a 6 × 6 point dosing matrix and the Bliss independence additivity model. In vivo tumor growth inhibition (TGI), tolerability, and survival were evaluated using two independent flank xenograft models of NSCLC (Calu6 and A549) where ATM-3507 and VNB were dosed IV (Q4D × 5).

**Results:**

ATM-3507 was cytotoxic as a monotherapy in vitro with an IC50 range of 9–13μM. Moderate to high level synergistic interactions were observed for ATM-3507 in combination with vinorelbine and vincristine (VCR) and a taxane (paclitaxel) across a panel of NSCLC cell lines. In vivo, ATM-3507 exhibited some degree of synergy with paclitaxel in vitro. The response to the MTIATM combination was independent of the mutation status of common oncogenes in NSCLC. In vitro findings were replicated in vivo with ATM-3507 showing a dose dependent TGI as a monotherapy in an A549 xenograft model. Animals dosed with ATM-3507 (50 mg/kg) + VNB (10 mg/kg) showed an improved TGI compared to either ATM-3507 or VNB alone in both A549 and Calu6 models. This translated to significant improvement in median survival for animals treated with the ATM/VNB combination (57 days) compared to VNB alone (45 days). The underlying molecular mechanism for the ATM/VNB synergy is being investigated.

**Conclusion:**

In the pre-clinical setting, ATM-3507 enhanced the effectiveness of standard of care therapy and may prove to be an effective adjuvant therapy for the treatment of late stage NSCLC. These data are part of a translational research program that aims to progress ATM-3507 into a first-in-human safety trial pending successful completion of the requisite pre-clinical evaluations.

**Conflict of interest:**

Ownership: JS, AH and DB are employees and own shares in Novogen. Advisory Board: PG and DB are on the Scientific Advisory Committee for Novogen. Board of Directors: PG serves on the Novogen board.
Material and Methods: The cytotoxicity of the compounds was assessed in the normal-like cell line MCF-10A, and in the breast cancer cell lines MCF-7, JIMT-1, and HCC1937. All cell lines were treated with the compounds in a concentration range of 0.1 to 100 μM in a dose response assay based on MTT reduction to obtain IC50 values. The effect on CSCs was evaluated by determining aldehyde dehydrogenase (ALDH) enzyme activity and cell surface expression of CD44+/CD24− by flow cytometry. Colony formation efficiency (CFE) in serum free medium containing soft agar was used as a functional assay for CSCs. A wound-healing assay was used to assess the effect of the compounds on cell migration.

Results: The IC50 values obtained from dose response curves were between 1.7 to 9.7 μM with ambrosin always being more cytotoxic than damsin in all cell lines. Based on these data we investigated the effect on CSCs in JIMT-1 cells after treatment with 1 and 5 μM concentrations of the compounds for 72 hours. Table 1 shows that treatment with 5 μM damsin or ambrosin reduced the CSC population evaluated using different assays with ambrosin being more efficient than damsin. 

Table 1. Treatment with 5 μM damsin or ambrosin reduced the CSC population

<table>
<thead>
<tr>
<th>Compound</th>
<th>CD44+/CD24−</th>
<th>ALDH</th>
<th>CFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damsin</td>
<td>94.1±4.3</td>
<td>84.5±8.3</td>
<td>61.2±9.8</td>
</tr>
<tr>
<td>Ambrosin</td>
<td>45.1±2.0</td>
<td>50.9±4.5</td>
<td>31.3±12.5</td>
</tr>
</tbody>
</table>

*Percent of control. Mean±SE, n = 3−4.

The wound-healing assay also showed that ambrosin inhibited cell migration more efficiently than damsin.

Conclusions: Our data shows that both compounds have a general inhibiting effect on cell proliferation and in addition reduce the CSC population in a low μM range. The inhibition of cell migration may reflect the decreased CSCs population. Further studies are ongoing to deduce the molecular mechanisms behind these observations.

No conflict of interest.

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Therapeutic potential of novel PLK1 inhibitor CYC140 in esophageal cancer and acute leukemia

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Background: PLK1 is overexpressed in many cancers, including esophageal cancer and acute leukemia, and elevated expression correlates with disease progression, invasiveness and poor prognosis [1−5]. CYC140 is a highly selective and potent ATP-competitive inhibitor of PLK1, selected as a clinical candidate and nearing completion of IND-enabling studies. The aims of this pre-clinical study were to inform clinical development of CYC140, by exploring its anticaner activity, therapeutic potential and mechanism of action in esophageal cancer and acute leukemia, and to identify promising drug combinations and pharmacodynamic markers for use in the clinic.

Material and Methods: CYC140 selectivity towards PLK1 was tested in vitro in a panel of over 250 kinases. Anti-tumor activity of single agent CYC140 was explored in three cell line panels derived from esophageal cancer, acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) using cytotoxicity and clonogenic assays. Detailed mechanistic studies investigating the effects of CYC140 on cell viability, proliferation and death were carried out using flow cytometry, cell death assays and western blotting. Short pulse treatments of CYC140 were optimised to quantify the maximal difference in cytotoxicity between esophageal cell lines from malignant and non-malignant tissue. In vivo therapeutic potential was explored in acute leukemia and solid tumor mouse xenograft models. Potential CYC140 drug combinations were screened and biomarkers of cellular response were characterized.

Results: CYC140 is a highly selective PLK1 inhibitor (>50 fold selective over other PLKs). Nanomolar antiproliferative activity has been observed across a broad range of tumor cell lines including esophageal cancer, AML and ALL cell lines. We demonstrate that 6 h pulse treatments of CYC140 resulted in a therapeutic window where malignant esophageal cell lines are more sensitive to CYC140 than esophageal cells of a non-malignant origin. As expected, the cellular response to CYC140 treatment includes cell accumulation in G2 and M cell cycle compartments, mitotic cells with monopolar spindles, reduced phosphorylation of PLK1 substrates such as NPM1, inhibition of proliferation and induction of cell death. CYC140 treatment causes tumor regression and tumor-free cures in solid tumor and acute leukemia mouse xenograft models.

Conclusion: Taken together these data suggest that CYC140 is a promising anti-cancer agent with potent anti-proliferative activity and therapeutic potential in a variety of tumor indications, including esophageal cancer and acute leukemia.

References

Conflict of interest: Ownership: Cyclacel stock or options. Corporate-sponsored Research: Cyclacel employees. S. Yuki3, T. Yoshino4, Y. Komatsu1, A. Ohtsu4, N. Sakamoto5, T. Katsukara1, 1Hokkaido University Hospital, Cancer Center, Sapporo, Japan; 2National Cancer Center, Division of Translational Research, Exploratory Oncology Research and Clinical Trial Center, Kashiwa, Japan; 3Graduate School of Medicine, Hokkaido University, Department of Gastroenterology and Hepatology, Sapporo, Japan; 4National Cancer Center Hospital East, Department of Gastroenterology and Gastrointestinal Oncology, Kashiwa, Japan; 5National Cancer Center, Exploratory Oncology Research & Clinical Trial Center, Kashiwa, Japan

Phase 1 study of first-in-class dUTPase inhibitor, TAS-114 in combination with S-1 in patients with advanced solid tumors

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This abstract is part of the media programme and is embargoed until the day of presentation.
Background: PET provides a spatial distribution of metabolic activity more precisely than other imaging methods. At week 8 in the phase 3 MPACT trial, there were ≈ 5 times more patients (pts) with a metabolic response (MR) by PET than by an objective response by radiographic measurement; pts with responses identified by each modality had a median overall survival (mOS) > 10 months (Ramanathan RK, et al. Ann Oncol. 2016).

Materials and Methods: [18F]-FDG PET/CT was used to measure tumor response in pts who received nab-P 100, 125, or 150 mg/m² + Gem 1000 mg/m². Lesions were evaluated at baseline and at 6 and 12 weeks post baseline. Per EORTC criteria, complete MR (CMR) was defined as complete disappearance of abnormal FDG activity, and partial MR (PMR) was defined as an incomplete reduction in standardized uptake value (SUV) ≥ 25%.

Results: Pts (n = 37) from the nab-P 125 mg/m² cohort had baseline and follow-up PET scans. The median baseline SUVmax per pt (sum of target lesions) was 21.8. All pts in the intent-to-treat population of this cohort had a MR (12 [32.4%] with a CMR and 25 [67.6%] with a PMR) as best response (baseline to nadir per pt). Thirteen pts (35%) had their best PET response at week 6. A CMR appeared to be associated with a longer mOS (23.8 months [CMR] vs 11.2 months [PMR]). For each 1% SUVmax reduction, there was a 2% reduction in risk of death (P = 0.011). Thirteen pts (35%) had their best PET response at week 6. A CMR appeared to be associated with a longer mOS (23.8 months [CMR] vs 11.2 months [PMR]).

Conclusions: In this PET analysis, all pts treated with nab-P 125 mg/m² + Gem 1000 mg/m² for MPC achieved a MR. Pts with a MR (32.4%) had a longer mOS (23.8 months) than those with a PMR (11.2 months). The median OS of 23.8 months in the CMR group deserves further evaluation in subsequent studies with molecular correlates. There was also a significant correlation between change in tumor metabolic activity and OS. These results confirm the high metabolic anti-tumor activity (100% MR rate) of the nab-P + Gem regimen for treating MPC, as well as the utility of PET for measuring treatment response. PET/CT response can be considered when evaluating activity of investigational agents in MPC, those without a significant PET/CT response in phase 1/2 studies might be unlikely to succeed in phase 3 trials.

Conflict of interest: Advisory Board: RKR and DDVH, consultant or advisory role, Celgene. Corporate-sponsored Research: RKR, DDVH, RLK, research funding, Celgene. Other Substantive Relationships: JSL, DM, and MKR, stock ownership and employment, Celgene.
361. Phase 1 Study of first-in-class dUTPase inhibitor, TAS-114 in combination with capcetabine in patients with advanced solid tumors

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Background: TAS-114 is a first-in-class oral, dual inhibitors of deoxyuridine triphosphatase (dUTPase) and dihydropyrimidine dehydrogenase (DPD), which acts as a modulator of the pyrimidine nucleotide metabolic pathway and cell-necrosis, by enhancing incorporation of uracil and fluorouracil into DNA, while inhibiting fluorouracil catabolism. A phase 1 study of combination treatment with TAS-114 and Capcetabine is underway to investigate the safety, and to determine the maximum-tolerated dose (MTD) and recommended dose (RD) in patients (pts) with advanced solid tumors.

Conclusions: The combination regimen was well-tolerated in pts with heavily treated advanced solid tumors, resulting in a stable and minor change in global health status. Overall, this data demonstrates promising activity and tolerability of NC-6004/G in heavily pretreated pts.

363. Molecular mechanisms of dianhydrogalactitol (VAL-083) in overcoming GBM chemoresistance

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Background: Dianhydrogalactitol (VAL-083) is a bi-functional alkylating agent that readily crosses the blood-brain barrier, accumulates in brain tumor tissue and has demonstrated activity against GBM in prior NCI-sponsored clinical trials. VAL-083 induces interstrand cross-links at guanine-N7 causing DNA double-strand breaks and cell-death. VAL-083 is highly expressed in GBM CSCs and can induce cell-cycle arrest, activation of the homologous recombination pathway and ensuing cell-death, through mechanisms independent of MGMT and MMR. We furthermore investigated a potentially unique uptake mechanism of VAL-083 in recurrent GBM, after temozolomide and bevacizumab failure, suggested potential of VAL-083 to offer clinically meaningful survival benefit. Here, we used biochemical and microscopic analyses of DNA repair markers gH2A.X, ATM, RPA32 and cyclin-A2 to investigate VAL-083-induced DNA damage responses in cancer-cells harbouring wild-type or mutated MMR-proteins (MLH1 and MSH2) and p53. We report a distinct mechanism-of-action of VAL-083, showing that VAL-083 treatment leads to irreversible S-phase cell-cycle arrest, activation of the homologous recombination pathway and ensuing cell-death, through mechanisms independent of MGMT and MMR.

Conclusions: We demonstrated the feasibility of molecular analysis of bone metastases using targeted proteomics and obtained protein quantities for an entire panel of therapeutically-relevant proteins from a single bone biopsy specimen. Protonic analysis of bone metastases could impact treatment choices upon diagnosis of metastasis or at relapse, and could advise whether to continue targeted therapies in patients who have disease progression only in bone lesions.

Conflict of interest: Ownership: We have equity in NantOmics (a non-publicly traded company) NantOmics is also our employer.

362. Targeted proteomic analysis of bone metastases from lung cancer and other malignancies

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Background: Targeted therapies that are successful in treating primary tumors are often ineffective against corresponding metastatic lesions. Increasing evidence suggests that tumors and their bone metastases are biologically distinct. Accordingly, biomarker status is frequently discordant between primary tumors and their bone metastases. Histopathological diagnosis of bone biopsy specimens is challenging as the tissue must be soft and easily decalcified, which destroys protein and nucleic acid integrity. Mass spectrometry-based targeted proteomics to measure the quantities of 27 therapeutically-relevant proteins in biopsies of bone metastases from patients with various cancers.

Materials and Methods: Ten biopsies of metastatic bone lesions were obtained from patients with cancers of the lung (n = 5), breast (n = 2), prostate, and urinary bladder. Proteins were extracted using the Liquid-Tissue process and subjected to selected reaction monitoring mass spectrometry to quantify the amounts of 27 target proteins in each sample. Absolute amounts of protein in amol/g were categorized by percentile groups created by testing in >500 samples.

Results: The 10 bone biopsy samples expressed 19 of the 27 protein targets tested. Of the 5 bone biopsy from lung cancer patients, 3 overexpressed EGFR protein (with levels ranging from the 50th to 90th percentiles), 1 overexpressed KRAS protein (>75th percentile) and 3 showed moderate levels of HER2 protein (10th–75th percentiles). Bone biopsies from 2 breast cancer patients overexpressed HER2, HER3, and hENT1 (relevant to gemcitabine therapy). Metastatic bone lesions from genital malignancies overexpressed AR, hENT1, EGFR, HER2, KRAS, SPARC, and TOP1.

Conclusions: We demonstrated the feasibility of molecular analysis of bone metastases using targeted proteomics and obtained protein quantities for an entire panel of therapeutically-relevant proteins from a single bone biopsy specimen.
Poly (ADP-ribose) polymerase (PARP) is a key molecule in the DNA damage response (DDR), which is a major target of both chemotherapy and radiotherapies. PARP inhibitors therefore comprise a promising class of anticancer therapeutics. In this study, we evaluated chemotherapy and radiotherapies. PARP inhibitors therefore comprise a promising class of anticancer therapeutics. In this study, we evaluated chemotherapy and radiotherapies.

**Background:**
TAS-114 is a first-in-class oral deoxyuridine triphosphatase inhibitor, which acts as a modulator of the pyrimidine nucleotide metabolic pathway. Phase 1 study is conducting a phase 1 study of combination treatment with TAS-114 and S-1 to investigate the safety, and to determine the maximum-tolerated dose (MTD) and recommended dose (RD) in patients (pts) with advanced solid tumors refractory to standard therapy.

**Material and Methods:**
TAS-114 was administered orally BID for 14 days followed by 7 days rest at the starting dosage of 5 mg/m² with the fixed dosage of S-1 (30 mg/m²) in a fasting condition. Dose-limiting toxicity (DLT) was assessed using a 3 × 3 design during the first cycle in dose escalation cohort (DEC). Expansion cohort (EC) was conducted in parallel with DEC at the dosage with confirmed tolerability and efficacy in DEC.

**Results:**
76 pts were enrolled with 48 pts in the DEC and 28 pts in the EC. In DEC, dosage of TAS-114 was escalated up to 240 mg/m². The dosages of S-1 and TAS-114 were then escalated up to 36 mg/m² and 240 mg/m², respectively. In 240 mg/m² of TAS-114, 2 patients of 5 patients enrolled at this dose level had DLTs (Grade 4 platelet count decreased and Grade 3 dermatitis bullous). Therefore 1 lower level was expanded to 6 pts and there were no more DLTs. Based on the above results, the MTD of TAS-114 in combination with S-1 at 36 mg/m² was estimated to be 200 mg/m². However, considering other safety data and treatment continuity in DEC and EC, the RD was determined to be TAS-114 at 240 mg/m² in combination with S-1 at 30 mg/m², which is the same MTD in the Western study (TAS-114–102). Pharmacokinetics of TAS-114 and S-1 components were dose proportional and consistent with the data in the Western population. The most common treatment related adverse events were anemia, white blood cell count decreased, decreased appetite and rash. Preliminary antitumor efficacy was observed in pts with non-small cell lung cancer (NSCLC), gastric cancer, pancreatic neuroendocrine tumor and gallbladder cancer. Of note, the overall response rate were 31.3% (Including unconfirmed PR) including 2 squamous cancer patients Disease control rate (DCR) was 50% and median progression free survival was 4.2 months in heavily pre-treated patients with NSCLC (Median 3.5 prior regimens).

**Conclusion:**
TAS-114 in combination with S-1 was well tolerated in pts with heavily treated advanced solid tumors, resulting in the promising antitumor efficacy. Further safety and efficacy of the global recommended dose of this combination therapy will be investigated in a phase 2 study.

**Clinical trial information:**
NCT01610479

**Conflict of interest:**

**DNA Repair Modification**

**Efficacy of poly(ADP-ribose) polymerase inhibitor olaparib against head and neck cancer cells: Predictions of drug sensitivity based on PAR-p53–NF–κB interactions**

**Background:** Poly (ADP-ribose) polymerase (PARP) is a key molecule in the DNA damage response (DDDR), which is a major target of both chemotherapy and radiotherapies. PARP inhibitors therefore comprised a promising class of anticancer therapeutics. In this study, we evaluated the efficacy of the PARP inhibitor olaparib, and also sought to identify the mechanism and predictive marker associated with olaparib sensitivity in head and neck cancer (HNC) cells.

**Materials and Methods:** A total of 15 HNC cell lines, including AMC HNC cells, were tested. Viabilities after olaparib treatment on each HNC cell lines were evaluated, and other specific molecular tests were performed for investigating antitumor mechanisms.

**Results:** AMC-HN3 and HN2 exhibited stronger responses to olaparib. Among cisplatin-resistant cell lines, only AMC HN9-cisR cells were significantly suppressed by olaparib. We found that basal poly(ADP-ribose) (PAR) levels, but not PARP-1 levels, correlated with olaparib sensitivity. AMC-HN3 and HN4 cells exhibited higher basal levels of NF–κB that decreased significantly after olaparib treatment. In contrast, apoptotic proteins were intrinsically expressed in AMC-HN9-cisR cells.

**Conclusion:** As interference with p53 expression led to NF–κB reactivation, we concluded that elevated basal PAR and NF–κB levels are predictive of olaparib responsiveness in HNC cells; in addition, olaparib inhibits HNC cells via PAR-p53–NF–κB interactions.

**No conflict of interest.**

**Exploiting synthetic lethal interactions in DNA repair deficient tumour cells using ATR inhibition**

**Background:**
Identifying genetic biomarkers of synthetic lethal drug sensitivities provides one approach to the development of precision medicine treatments for cancer. Small molecule inhibitors of the cell cycle checkpoint kinase ATR, are a novel class of cancer therapeutics. Identifying reliable determinants of sensitivity could establish novel predictive biomarkers for their clinical use.

**Material and Methods:** Using a high-throughput RNAi chemosensitisation screen, we set out to identify candidate ATR inhibitor synthetic lethal interactions in tumour cells.

**Results:** Using the RNAi screen we discovered that silencing of the ATR1A tumour suppressor gene sensitises multiple tumour lines to ATR inhibition. We validated this observation using several in vitro and in vivo model systems, demonstrating that the synthetic lethal interaction between ATR and ARID1A is robust and operates across different tumour types. Mechanistically this effect appears to be related to a TOP2A defect in ARID1A mutant tumour cells.

**Conclusions:**
ATR inhibitors are currently in Phase I clinical trials as combination therapies. ARID1A is a component of the BAF chromatin-remodelling complex, which has been shown to be mutated in up to 20% of all human tumours. However, therapeutic approaches that selectively target ARID1A/BAF defects are not yet clinically available. The data presented here provides a preclinical rationale for assessing ARID1A defects as a biomarker of single agent ATR inhibitor response and represents a novel synthetic lethal approach to targeting tumour cells.

**No conflict of interest.**

**Chromosomal instability and chromothripsis as a prognostic factor for metastatic colorectal cancer**

**Background:**
Impacts of chromosomal rearrangements and mutations on pathogenesis, prognosis and treatment resistance, are widely described in metastatic colorectal cancer (mCRC). Chromothripsis – massive chromosome fragmentation occurred in one catastrophic cell crisis – drive the development of cancer through several mechanisms including deletion of tumor suppressor genes and increased copy number of oncogenes. Chromothripsis has been associated with poor patient survival in several cancers, indicating its potential relevance as a prognostic marker. Chromothripsis incidence and impact on survival in mCRC is unclear.

**Material and Methods:**
19 mCRC patients (pts) who received FOLFOX type first line chemotherapy in Clinic of Oncology of P. Stradins CUH in 2011–2012 where selected for study. DNA was extracted from the formalin-fixed paraffin-embedded (FFPE) samples with QIAamp DNA Mini Kit (Qiagen). DNA quality was evaluated with Illumina FFPE QC Kit using RT-PCR. DNA was restored using Illumina DNA restoration Kit. Microarray analysis was performed using IlluminHuman OmniExpress-12 v1.0 FFPE BeadChip Kit. BeadChip was machine on HiScan (Illumina). Data was visualized and analyzed by GenomeStudio (Illumina) software. Analyses were performed using R version 3.1.2. Copy number variation and break points on the chromosomes were analyzed by using "DNAcopy" package. Survival rates were estimated using the Kaplan–Meier method.
Results: The highest density of breakpoints was seen in chromosomal (chr) 1 (27–365 breaks), followed by chr 2 (25–315), suggesting importance of these chromosomes in mCRC genesis. The lowest density of breakpoints occurred at chr 21 (7–99 breaks). Total number of breaks per genome – breakpoint instability index (BPI) – was 368–4009. In 10 tumor samples (52.6%) we found multiple chromosomal fragmentation (>100 breakpoints at one chromosome) – chromothripsis. The most affected chromosomes were chr 1, chr 2 and chr 6 (52.6%). The maximal count of chr affected with chromothripsis per sample was 20.

Progression free survival (PFS) and overall survival (OS) were measured for all pts. We observe positive correlation between high BPI and better PFS. mPFS for BPI < 1400 was 14 mo, mPFS for >1400 was 8 mo (HR 3.54, p = 0.03). Chromothripsis was associated with better survival. mPFS in patient with chromothripsis was 14 mo, in patient without chromothripsis – 8 mon (HR 3.92, p = 0.02). We didn’t observe statistically significant impact of any clinical or biological factor on OS due to small patient number.

Conclusions: In our study, we found a correlation between DNA massive fragmentation chromothripsis and progression free survival in metastatic colorectal cancer. As opposed recent studies suggested chromothripsis associated with worse prognosis, we find chromothripsis as positive predictive factor to chemotherapy.

No conflict of interest.

367 Poster (Board P046)

Talazoparib in combination with temozolomide enhances antitumor effects in prostate cancer
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1Medivation, Inc., Research and Development, San Francisco, USA
2owners of Sarissa, Inc. Sarissa has a commercial interest in development and commercialization of IBR2. Published data in early 2016 at AACR

Background: The poly(ADP-ribose) polymerase (PARP) family of enzymes are responsible for DNA single-strand break repair in cells. Talazoparib is a potent, orally bioavailable, small molecule PARP inhibitor. It blocks PARP enzyme activity and traps PARP on DNA, preventing DNA repair and causing cell death in BRCA1/2 deficient cells [1]. However, when talazoparib is combined with a DNA alkylation agent such as temozolomide, antitumor effects are further enhanced [2]. Men with germline BRCA1/2 mutations have an increased risk of developing prostate cancer [3] and preclinical studies suggest that PARP inhibitors may have antitumor effects in prostate cancer models. Here, we explore the potential for antitumor effects of talazoparib as a single agent, or in combination with temozolomide, in prostate cancer cell lines and in nonclinical models of castration-resistant prostate cancer.

Materials and Methods: Cell-based cytotoxicity assays were conducted in prostate cancer cell lines (LNCaP, LNCaP/AR, 22Rv1, C4-2, PC-3 and VCaP). Cells were treated with increasing concentrations of talazoparib or temozolomide, either as a single agent or in combination, and PARP-DNA trapping and gH2Axl levels were assessed. LNCaP LNCaP/AR xenograft models and patient-derived prostate cancer models were generated and effects of talazoparib monotherapy or talazoparib/temozolomide combination therapy on tumorigenesis were evaluated. Immunohistochemistry assays were performed to determine Ki-67, gH2Axl, caspase 3, and CD31 marker expression following the different treatment regimens.

Results: Cancer cells cytotoxicity was observed in 83.3% (5/6) of the cell lines at clinically-relevant IC50 values for each drug. In prostate cancer cell lines, combined talazoparib/temozolomide treatment significantly induced gH2Axl levels compared with either agent alone. In xenograft models of castration-resistant prostate cancer, talazoparib monotherapy greatly inhibited tumor growth. Notably, talazoparib antitumor effects were further potentiated when combined with non-cytotoxic doses of temozolomide.

Conclusions: Talazoparib monotherapy has antitumor effects in cell lines harboring DNA repair gene mutations and in nonclinical models of prostate cancer. Furthermore, talazoparib in combination with a non-cytotoxic dose of temozolomide more effectively kills cancer cells compared to single-agent treatment. Our data supports the therapeutic potential of talazoparib as a mono therapy and also in combination with temozolomide in prostate cancer.

References

Conflict of interest: Ownership: VP, AP, HU are all employees of Medivation, Inc.

368 Poster (Board P047)

Synergistic antitumor activity of the targeted drugs imatinib, regorafenib, and gefitinib with the RAD51 inhibitor IBR2
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Background: The inherent genomic instability of cancer has been exploited for potential tumor-selectivity of drugs targeting specific deficiencies in DNA repair, a phenomenon referred to as “synthetic lethality” (Nature 434, 433–4, 2005). Inhibitors of poly(ADP-ribose) polymerase (PARP, olaparib) are routinely used clinically against tumors deficient in BRCA1 or BRCA2, due to the poor capacity of these cells to undergo homologous recombination repair. To make use of this potential selectivity in tumors that may not inherently hypersensitive to a particular treatment, we developed synthetic lethality by down-regulating essential components of DNA repair, in particular BRCA2 and RAD51, with siRNA to sensitize cells to chemotherapy drugs such as the DNA-crosslinking agent cisplatin and olaparib (Mol Onc 8:1429, ’14; Proc AACR 57:3718, ’16). As a next step toward developing such a combination for clinical use, we used a small molecule inhibitor of RAD51 to test in combination with established chemotherapeutic drugs. 2-(benzylsulfonyl)-1-(1H-indol-3-yl)-1,2-dihydroisoquinoline (IBR2) was obtained from the laboratory of Dr. W-H Lee, Univ. California − Irvine. IBR2 has antitumor activity in vivo at doses that are not toxic to tumor-bearing mice, and it enhanced induction of apoptosis by imatinib against K562 cells (EMBO Mol Med 5:333, 2013). Materials and Methods: Inhibition of proliferation was assayed against cell lines derived from tumors of various tissue sources: AML (Kasumi-1), CML (K562), breast (MDA-MB-468), colon (HT-29, HCT15), gastric (AGS, Hs746T, N87), and lung (A549, H69, H1650, H1975). Oncogenic drivers for these lines include BCR-ABL, c-Kit, B-Raf, k-Ras, ER, and mutant p53. Four-day drug exposures were conducted in 96-well plates (total 200 μ1 per well). Relative cell density was determined using vital stains (alamarBlue, neutral red) as a percent of the fluorescence/absorbance of control cultures.

Results: IBR2, at concentrations that inhibited proliferation between 0% and 75%, enhanced the inhibition of proliferation by imatinib, regorafenib, and gefitinib by up to 90% in all cell lines studied, depending on the drug used and concentration. In some cases complete cell obliteration was achieved. Cell lines that were significantly more sensitive to a certain drug than other cell lines (e.g., K562 - with regorafenib) were not sensitized by IBR2. IBR2 was not synergistic with olaparib. Combinations with IBR2 that were particularly enhanced included: imatinib against HT-29, AGS and H69; regorafenib against MCF-7, Hs746T, K562 and H69; gefitinib against A549, H1650 and H1975.

Conclusions: IBR2, at non-toxic concentrations, enhances cytotoxicity of several signal-targeting agents. This action may be in addition to inhibition of RAD51. IBR2 has the potential to enhance tumor-selectivity of anticancer agents. In vivo studies are ongoing.

Conflict of interest: Ownership: Dr. Vincent and Dr. Koropatskii are owners of Sarissa, Inc. Sarissa has a commercial interest in development of IBR2 as a lead compound.

369 Poster (Board P048)

Akt1 is a potential target for radiosensitization of K-RAS mutated cells
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Background: Despite the significant contribution of radiotherapy in cancer treatment radiosensitivity still occurs. One of the major resistance mechanisms is the activation of PI3K/Akt survival pathways. The PI3K/Akt pathway is frequently upregulated in human tumors due to overexpression or mutation in the components of this pathway such as point-mutation in K-RAS gene. K-RAS mutation leads to constitutive K-RAS activity and activation of Akt. Akt/PKB is a serine/threonine kinase, which exists in three isoforms known as Akt1 (PKBα), Akt2 (PKBβ) and Akt3 (PKBγ). In the present study we investigated the mechanism by which Akt1 activity leads to radiosensitivity of K-RAS mutated cells.

Materials and Methods: K-RAS wild-type head and neck squamous cell carcinoma (HNSCC) cell line FaDu, K-RAS mutated non-small cell lung cancer (NSCLC) cell line A549 and K-RAS mutated colorectal cancer cell line Caco-2 were used. Function of Akt1 on radio-induced double strand breaks (DSBs) was investigated in tumor cells with K-RAS wild-type and K-RAS mutant p53. Four-day drug exposures were conducted in 96-well plates (including control cultures). IC50 values for each drug were determined using alamarBlue, neutral red) as a percent of the fluorescence/absorbance of control cultures.

Results: IBR2, at concentrations that inhibited proliferation between 0% and 75%, enhanced the inhibition of proliferation by imatinib, regorafenib, and gefitinib by up to 90% in all cell lines studied, depending on the drug used and concentration. In some cases complete cell obliteration was achieved. Cell lines that were significantly more sensitive to a certain drug than other cell lines (e.g., K562 with regorafenib) were not sensitized by IBR2. IBR2 was not synergistic with olaparib. Combinations with IBR2 that were particularly enhanced included: imatinib against HT-29, AGS and H69; regorafenib against MCF-7, Hs746T, K562 and H69; gefitinib against A549, H1650 and H1975.

Conclusions: IBR2, at non-toxic concentrations, enhances cytotoxicity of several signal-targeting agents. This action may be in addition to inhibition of RAD51. IBR2 has the potential to enhance tumor-selectivity of anticancer agents. In vivo studies are ongoing.

Conflict of interest: Ownership: Dr. Vincent and Dr. Koropatskii are owners of Sarissa, Inc. Sarissa has a commercial interest in development of IBR2 as a lead compound.
Pharmacologic inhibition of Akt1 or its knock down by siRNA led to impaired repair of radiation-induced DSBs and radiosensitization of K-RAS-mutated NSCLC cell line A549 and colorectal cancer cell line HCT-116 cells. Akt1 mediated enhanced DSB repair and radiosensitivity was demonstrated to be dependent on the expression and activation of DNA-dependent protein kinase catalytic subunit (DNA-PKcs). Biochemical studies demonstrated that Akt1 through its C-terminal domain physically interacts with DNA-PKcs. Following induction of DSB, recruitment of DNA-PKcs to damage sites and autophosphorylation is partially dependent on Akt1.

Conclusion: Indirect inhibition of DSBs repair by targeting Akt1 may be a tumor cell specific approach to achieve radiosensitization of K-RAS mutated tumor cells.

Conflict of interest: Acknowledgement: This work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG, RO 527/7).

370  Poster (Board P049)
Inhibition of Chk1 and Wee1 as an effective therapeutic approach in diffuse large B cell lymphomas: A way to target Myc?

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Background: Diffuse large B cell lymphoma (DLBCL) is the most common type of aggressive lymphoma. Approximately 40% of DLBCL patients have refractory disease or disease that will relapse after an initial response to the combinatorial chemo-immunotherapy. Thus, finding new therapeutic approaches is urgently needed for this lymphoma. Experimental evidence showed that high levels of Myc protein is very common in DLBCL and correlated with poor survival prognosis of DLBCL patients, suggesting that Myc is a crucial target for DLBCL therapy. The checkpoint kinases Chk1 and Wee1 are key cell cycle regulators, required during normal S phase to avoid deleterious DNA breakage, and to maintain cancer cell survival under replication stress. Chk1 inhibitors have recently been shown to be effective in Myc overexpressing cells and are strongly synergistic with Wee1 inhibitors in many experimental systems. We herein investigated the effects of Chk1 and Wee1 inhibition in DLBCL cell lines differently expressing Myc protein.

Material and Methods: Eleven cell lines derived from germinal center B-cell like (GCB) and activated B-cell like (ABC) DLBCL were treated with the Chk1 inhibitor PF-00477736 and the Wee1 inhibitor AZD-1775 alone and in combination. Drug combination was quantified by calculating the Combination Index (CI) (Chou Talalay method). Cell cycle analysis by FACS and detection of apoptosis by caspase-3 activity were performed after such treatments. Myc protein levels and DNA damage markers were evaluated by Western blot Analysis.

Results: Myc protein levels and the degree of endogenous constitutive DNA damage (+H2AX activation and pS317-Chk1) detected in the different DLBCL cell lines did not correlate with the extent of sensitivity to PF-00477736 and to AZD-1775 (IC50s ranging respectively from 68 to 1023 nM and from 114 to 968 nM). The combined treatment is strongly synergistic in all the DLBCL cell lines, with CI values, evaluated at the IC50 doses, ranging from 0.06 to 0.77. The drug combination induced an S phase delay and an accumulation of DNA damage measured by +H2AX activation. Enhanced caspase-3 activity observed after the combined treatment suggested that cell death by apoptosis is also occurring. Interestingly a strong decrease of Myc protein levels not associated with Myc mRNA down-regulation was observed after the dual inhibition of Chk1 and Wee1. Molecular investigation is undergoing to elucidate the mechanisms at the basis of this Myc protein expression modulation.

Conclusions: Overall these data suggest that combining Chk1 and Wee1 inhibitors could be a new and effective therapeutic strategy to test in clinical setting in DLBCL.

No conflict of interest.

371  Poster (Board P050)
Enhancing susceptibility to PARPi in homologous recombination repair dysfunction (HRD)-associated pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies with limited treatment options. Recent genomic advances have identified a subtype with homologous recombination repair (HR) deficiency. Those patients include germline BRCA PDAC (5% of sporadic and 10–15% of Jewish Ashkenazi PDAC cases) and a ‘BRCAness’ phenotype in the absence of BRCA mutation. These patients are of critical importance as they may benefit from a personalized treatment approach. Tumors with HR deficiency are highly susceptible to PARP inhibition (PARPi). However, durable anti-tumor responses are limited and accumulating resistance is observed, thus forming the scientific rationale for the development of combination therapy. PI3K/AKT signaling has an essential role for cancer initiation, progression and importantly HR repair steady state preservation.

The goal of this study was to test the hypothesis that addition of PARPi may enhance the sensitivity to PARPi.

We have developed a unique patient-derived xenograft (PDX) from metastatic partially depenses fluid or liver biopsies thus closely mimicking the relevant clinical scenario. In order to examine feasibility of BRCA-associated PDXs for PARPi therapy, we have evaluated PI3K pathway activation.

Phosphorylated AKT was detected in BRCA1-mutated PDX, which supports the addition of PI3K to PARPi in our model. Therapeutic efficacy of PARPi (Olaparib) alone and in combination with PI3Ki (BKM120) was evaluated in 3 PDX models generated from 3 germline BRCA-mutated patients (One-BRCA1, two-BRCA2). In each experiment mice were randomized into 4 groups: (a) vehicle control, (b) Olaparib (50 mg/kg, i.P.), (c) BKM120 (30 mg/kg, PO) or (d) the combination of Olaparib with BKM120, all administered once daily, d1–5. Our results demonstrate significant attenuation in tumor growth following therapy with BKM120 (alone and combined with Olaparib) in all 3 PDX PDAC models. While in two PDX models generated from cell lines with limited clinical resistance, combination didn’t confer additional benefit, in the third naïve to treatment model, the combination demonstrated significant reduction in tumor growth compared to monotherapy of each agent.

Our results demonstrate reasonable correlation between the clinical disease course in PDAC patients and the response to therapy in PDX models generated from their tissues. Surprisingly we found BKM120 to be significantly efficacious as single agent in BRCA-associated PDX model derived from patients exhibiting clinical resistance to DNA damaging agents. In the PDX model, derived from patient naïve to therapy, synergy between BKM120 and Olaparib was demonstrated.

We anticipate that our study will promote an understanding of the role of BRCA and PI3K inhibition in HRD PDAC and propose a unique platform to test therapeutic interventions targeting these pathways in a personalized manner.

No conflict of interest.

372  Poster (Board P051)
Lack of RAD51 foci formation enables the identification of PARPi inhibitor sensitive breast tumors

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Poly-(ADP-ribose)-polymerase (PARP) inhibitors (PARPi) are active anti-cancer agents in tumors deficient in DNA repair by homologous recombination (HR). Clinical studies with single-agent PARPi are therefore focused on breast and ovarian cancers with BRCA1/2 mutations and/or those identified as HR-deficient by genomic scars. Nevertheless, there is a need to improve patient selection by characterizing the actual tumor’s HR-status. Here, we sought to validate the RAD51 immunofluorescence assay as a functional readout of the tumor’s capacity to complete HR DNA repair by examining its correlation with PARPi primary response and acquired resistance using patient-derived tumor xenografts (PDX).

No conflict of interest.
We developed two independent PDX panels, totaling 56 models. Specifically, the discovery panel consisted of 25 PDXs from patients harboring \( n = 12 \) or not \( n = 13 \) germline BRCA1/2 mutations, namely from 23 primary or advanced breast cancer and 2 high-grade serous metastatic ovarian cancer (HGSOCC). The antitumor activity of the PARPi v1.1/SK/P1/2 inhibitor olaparib as single agent (50 mg/kg) was assessed in this panel. In addition, in vivo acquired resistance was modeled in the PDXs by exposing 3 PARPi-sensitive PDXs to olaparib for >80 days, until individual tumors regrew. The tumor’s HR capacity was evaluated by measuring the percentage of cells in the S/G2 phase of the cell cycle (geminin-positive) exhibiting RAD51 nuclear foci, both in PARPi- and in vehicle-treated formalin-fixed paraffin-embedded tumors. The validation set is composed of 26 triple negative breast cancer PDXs including 5 gBRCA PDXs. The percentage of RAD51-positive cells in the discovery set ranged between 1 and 83% in PARPi-treated PDXs and between 0 and 62% in vehicle-treated tumors. Five out of 25 PDXs (20%) with low levels of RAD51-positive cells (1–3%) in PARPi-treated tumors exhibited tumor regression upon olaparib treatment. The 20 PDXs that showed disease stabilization \( n = 2 \), disease progression \( n = 18 \) and those that developed acquired resistance to olaparib \( n = 3 \) exhibited significantly higher levels of RAD51-positive cells (27–83%) in PARPi-treated tumors, \( p < 0.0001 \). Similar results were obtained in vehicle-treated tumors. Of note, PARPi responsiveness correlated with low levels of RAD51 foci independent of the BRCA status. The validation set is composed of 7 regressing- and 21 stabilized/progressing-models, whose correlation with the RAD51 predictive value will be presented.

Our results suggest that primary and acquired PARPi resistance involve restoration/restitution of HR functionality and support the potential of the RAD51 assay as a predictive biomarker of PARPi response in the clinic.

Conflict of interest: Piskorz Anna: No conflicts to disclose. Lin Kevin K.: Employee, Clovis Oncology, Inc.; Stock and other ownership interest, Aprea Biotech AB; Consulting or Advisory Role, Clovis Oncology, Inc. Morris James: Stock and other ownership, Invitada; Patents, Royalties, Other Intellectual Properties, Invitada; Other, Invitada; Mann Elaina: Employee, Clovis Oncology, Inc.; Stock and Other Ownership Interest, Clovis Oncology, Inc. Oza Amit: Consulting or Advisory Role, Amgen, Verastem, Clovis Oncology, Immunovaccine; Travel Accommodations, Expenses, AstraZeneca; Honoraria, WebRx Research Funding, AstraZeneca; Roche, Merck, Celgene; Consulting or Advisory Role, Clovis Oncology, Genentech/Roche, Esperance Pharmaceuticals, NCCN, DOD-CDMRP; Travel, Accommodations, Expenses, Millennium, Merck, AstraZeneca/Medimmune, Array BioPharma, Merrimack, Gradsial, Beyor, Clovis Oncology, Honoraria, AstraZeneca, Medimmune, Esperance Pharmaceuticals, OncoMed, Array BioPharma, Clovis Oncology, Amgen, Johnson & Johnson, Merrimack. O’Malley David M.: Consulting or Advisory Role, Janssen Oncology; Research Funding, Amgen, VentRx, AstraZeneca, GentiTechniRoche, Regeneron, Immunogen, Array BioPharma, Janssen; Research & Development, Clovis Oncology, EMD Serono, Ergomed, Aljinomoto, Friedlander Michael: Consulting or Advisory Role, Roche, Clovis Oncology, AstraZeneca; Honoraria, Roche, Clovis Oncology; Research Funding, AstraZeneca, Cragan Janiel M.: Research Funding, Clovis Oncology, Inc. (to institution). Mia Ling: Consulting or Advisory Role, biotheranostics, Giordano Heidi: Employee, Clovis Oncology, Inc.; Stock and Other Ownership Interest, Clovis Oncology, Inc. Raponi Mitch: Employee, Clovis Oncology, Inc.; Stock and Other Ownership Interest, Clovis Oncology, Inc. McNeilish Iain A.: Consulting or Advisory Role, Clovis Oncology, AstraZeneca; Research Funding, Clovis Oncology, Inc. (to institution). Swisher Elizabeth: Travel Accommodations, Expenses, Clovis Oncology, Inc. (to institution). Brenton James D.: Consulting or Advisory Role, Invitada; Speakers’ Bureau, AstraZeneca; Travel, Accommodations, Expenses, Clovis Oncology, Inc., Agnea AB; Patents, Royalties, Other Intellectual Property, Tm-Seq v2 method for ctDNA estimation; Stock and Other Ownership Interest, Invitada.

Evaluating clinical-stage PARPi inhibitors in cell-based assays to correlate PARPi suppression with functional impact on DNA repair

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Background: PARP inhibition has been proven efficacious in treating tumors harboring homologous recombination deficiency (HRD). Multiple mechanisms of PARPi inhibitors have been attributed to cause cell death in HRD tumors including catalytic inhibition and trapping of PARPi onto DNA. This study was designed to quantitatively evaluate the functional suppression of DNA damage repair by PARPi inhibitors and to correlate the outcome with the levels of PARPi inhibition.

Material and Methods: The ability of PARPi inhibitors to inhibit intracellular PARPi-1-2 activity was evaluated in an commercial assay (Trevigen 4520-
096-K) that detected the amount of PAR chains formed after inducing DNA SSBs in Jurkat cells, and by immunofluorescence in non-induced BRCA-deficient tumor cells. To correlate the level of PARP suppression required for inhibiting SSBB repair in cells, single cell gel electrophoresis (Trevigen 4204-040-K) utilized to directly measure DNA SSBBs immediately after inducing DNA SSBBs with hydrogen peroxide, and one hour after washout of peroxide. Alternatively, gamma H2AX foci were quantified in BRCA-deficient tumor cells by immunofluorescence as a means to assess functional impact on DNA repair. All assays were well-validated with high sensitivity.

Results: Niraparib and olaparib caused a dose-dependent suppression of intracellular PAR levels that were accompanied by dose-dependent increase in the amount of unrepaird single-strand DNA breaks. The interpolated EC50 for suppression of DNA damage repair induced by H2O2 is 0.38±1M and 1.89±3M for niraparib and olaparib, respectively in this assay. In both cases, blockage of DNA repair was achieved when PAR levels were suppressed by about 90%. Data from other clinical PARP inhibitors will also be discussed.

Conclusions: Evidence for functional suppression of DNA repair was observed for niraparib or olaparib at dose levels that inhibited PARP by approximately 90%. For niraparib, the cellular IC90 was well correlated with doses that achieved robust anti-tumor effects in BRCA-deficient cells in vitro [1].

References

Conflict of interest: Ownership: Keith Mikule and Sarah Wang are employees of Tesaro Inc. Sandra Woodgate and James Potter are employees of Trevigen Inc. Steffen Lawo is employee of Horizon Discovery Ltd.

**Molecular targeted agents II**

**376 Poster (Board P055)**

**CB-839, a selective glutaminase inhibitor, has anti-tumor activity in renal cell carcinoma and synergizes with everolimus and receptor tyrosine kinase inhibitors**

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Many tumor cells utilize the amino acid glutamine to meet the elevated bioenergetic and biosynthetic demands of rapid cell growth. The enzyme glutaminase converts glutamine to glutamate, which fuels the TCA cycle, synthesizes amino acids, and balance cellular oxidative stress. CB-839 is a novel inhibitor of glutaminase that is currently in Phase 1 clinical trials for the treatment of cancer. To investigate the role of glutaminase in renal cancer, we tested CB-839 on a panel of 29 kidney tumor-derived cell lines. CB-839 caused tumor cell death in 18 out of 23 renal cell carcinoma (RCC) and 0 out of 6 non-RCC cell lines. Cell lines that were sensitive to CB-839 exhibited more pronounced decreases in aspartate and malate levels compared to resistant cell lines, indicating that sensitivity to glutaminase inhibition may be dependent on the intensity of TCA flux. Expression of the enzyme pyruvate carboxylase (PC), which converts pyruvate to oxaloacetate recycling the TCA cycle, reduces the need for glutamine in cells and may be a biomarker of resistance to CB-839. Consistent with the strong dependency for glutamine metabolism, RCC cells and primary human RCC tumors expressed low levels of PC suggesting that RCC may lack this mechanism of resistance to CB-839. In addition, CB-839-sensitive RCC cell lines showed stronger pharmacodynamic decreases in mTOR signaling (decreased p-S6 and p-4EBP1 proteins), suggesting that nutrient deprivation by CB-839 treatment is sensed by the mTOR pathway. These observations led us to evaluate whether inhibitors of receptor tyrosine kinase (RTK) signaling or mTOR would combine with CB-839 to increase cytoreactivity in RCC cell lines and xenograft mouse models. We found that CB-839 synergized with the RTK inhibitors pazopanib, sunitinib, and cabozantib, and the mTOR inhibitor everolimus in proliferation assays with RCC cell lines. Mechanistic studies in Caki-1 cells revealed that the CB-839/cabozantinib combination reduced signaling through AKT and ERK, increased PARP expression and activity, and reduced glycolytic and TCA cycle activity more than either single agent treatment. In the Caki-1 xenograft model, the combination of CB-839 and cabozantinib enhanced tumor growth inhibition compared to either monotherapy. Likewise, the combination of CB-839 and everolimus inhibited both glucose and glutamine consumption, leading to decreased glycolytic and TCA cycle function and enhanced anti-proliferative activity. The CB-839/everolimus combination showed enhanced anti-tumor activity in the Caki-1 xenograft model, and tumor metabolomics showed evidence of enhanced oxidative stress in tumors treated with this combination. CB-839 is currently being tested in phase 1 clinical trials of patients with solid and hematologic tumors, and is showing promising clinical activity in combination with everolimus in RCC patients.

Conflict of interest: Ownership: Calithera Biosciences.

**377 Poster (Board P056)**

**Targeting neuroendocrine prostate cancer using a novel human antibody construct**

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**Background:** Second generation anti-androgen therapeutics have shown promise in prolonging survival in men with castration-resistant prostate cancer (CRPC), however, as with every drug, resistance is guaranteed. We now know that as men become resistant to these second-generation therapeutics, their cancer trans-differentiates from androgen receptor (AR)-driven disease to non-AR driven CRPC that expresses neuroendocrine markers (CRPC-NE). New imaging agents and therapeutics for CRPC-NE are urgently needed; however, new molecular targets need to be identified first. To identify molecular targets of CRPC-NE, a large human single-chain antibody fragment (scFv) library was screened on CRPC-NE tumor tissue using a novel ex vivo selection method. One scFv fragment, termed HA10, was found to selectively bind to the cancer stem cell antigen CD135. The antibody HA10 was able to detect CD135 protein in CRPC-NE sections by immunohistochemistry (IHC) and was also a potent in vivo imaging agent for positron emission tomography (PET) imaging.

**Material and Methods:** The scFv fragment HA10 was identified by direct biopanning on a CRPC-NE liver met acquired from the University of Minnesota Masonic Cancer Center. The HA10 scFv was cloned into full-length mouse and human immunoglobulin (IgG) constructs. IHC was performed on metastatic prostate cancer tissue microarrays (TMAs) from
the University of Washington and University of Minnesota using the mouse HA10 IgG. Human HA10 IgG was labeled with 89Zr for in vitro characterization studies in prostate cancer cell lines LAPC4 and R1 that were engineered to overexpress CD133 (LAPC4CD133 and R1CD133). The ability of 89Zr-HA10 to image CD133 in vivo by PET was examined in R1CD133 xenograft and LuCap patient-derived xenograft (PDX) mouse models.

Results: Only soft-tissue CRPC-NE metastases stained positive for CD133 with HA10 in the examined TMA. The in vitro characterization studies found that HA10 selectively bound to the LAPC4CD133 and R1CD133 cell lines over the parental cells. Additionally, HA10 was rapidly internalized by CD133-expressing cells. This internalization resulted in a sensitive PET imaging probe with a high tumor uptake of 48.3% injected dose per gram (%ID/g) in the CRPC-NE LuCap 145.1 PDX model. No significant tumor uptake was observed in the non-CRPC-NE LuCap 77 PDX model.

Conclusions: HA10 is a unique antibody that specifically binds to CD133 in vitro and in vivo. Our preliminary IHC data in TMA suggests that CD133 may be a hallmark of CRPC-NE. These data lay the foundation for further studies to evaluate the clinical potential of CD133 as a biomarker of CRPC-NE and the utility of HA10 derived therapeutics and imaging agents.

No conflict of interest.

Poster Session – Molecular targeted agents II. Thursday 1 December 2016

378 Neratinib induces estrogen receptor function and sensitizes HER2 mutant breast cancer to anti-endocrine therapy

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Material and Methods: We have established cell lines expressing either wild type or the most recurrent ERBB2 mutant alleles in ER+ PIK3CA-wild type or the most recurrent ERBB2 mutant alleles in ER+PIK3CA-wild type and structural datasets for small molecules with validated binding targets. GPCR antagonism was evaluated using beta-arrestin recruitment and cAMP reporter assays in Chinese hamster ovary cells. KB values were determined with Schild analyses and DRD2 binding was confirmed with radio-labeled ligand competition assays. ELISA was used to quantitate serum prolactin levels.

Results: A computational algorithm predicted that ONC201 antagonizes DRD2 and adrenergic receptors. In vitro studies revealed that ONC201 is a competitive antagonist of DRD2 and DRD3 with a KD value of ~3μM, >20-fold selectivity over other dopamine receptors, and no detected antagonism of other GPCRs with known ligands. High mRNA expression of DRD2 was observed in several ONC201-sensitive tumor cell lines. siRNA-mediated knockdown of DRD2 in colon cancer cell lines phenocopied effects of ONC201, including induction of apoptosis and reduction of pAkt and pERK levels. Conversely, overexpression of DRD2 increased pAkt and pERK levels and ablated ONC201-induced ATF4 upregulation, pAkt and pERK downregulation, and induction of apoptosis. In accordance with physiological DRD2 antagonism, a 2-fold mean induction of prolactin was detected in the serum of ONC201-treated patients. Expression of DRD2, which counteracts DRD2 signaling, was negatively correlated with ONC201 in vitro potency in the NCI60 panel of cancer cell lines. Furthermore, a missense DRD5 mutation was identified in RKO cells that acquires resistance to ONC201. Preliminary in vitro data also suggests that the specificity of D2-like (DRD2/DRD3/DRD4) versus D1-like (DRD1/DRD5) subfamily antagonism influences anti-cancer efficacy.

Conclusions: Selective and competitive antagonism of DRD2 by ONC201 occurs physiologically and contributes to its antitumor activity.

Conflict of interest: Ownership: Wolfgang Oster, Wafa S. El-Deiry and Joshua E. Allen are stockholders of Oncoceutics Inc. Other Substantive Relationship: Wolfgang Oster, Joshua E. Allen, Varun Vijay Prabhu are employees of Oncoceutics, Inc.

380 NPC-064, a novel mutant-selective EGFR inhibitor, targets NSCLC driven by EGFR exon 20 insertion mutations

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Background: Activating mutations in the EGFR gene are important targets in cancer therapy because they are key drivers of NSCLC. While almost all common EGFR mutations such as exon 19 deletions and L858R point mutation in exon 21 are eligible for EGFR-TKI therapies, there are no EGFR-TKI therapies available for the exon 20 insertion mutations. The existing EGFR-TKI therapies for exon 20 insertion mutations are associated with poor clinical outcome because of no selectivity between exon 20 insertions and wild-type (WT). Therefore, an EGFR-TKI which is able to achieve the selective inhibition for exon 20 insertion mutants may be gaining attention. NPC-064 could be a more efficacious treatment option for these patients.

Material and Methods: Biochemical assay were performed using a mobility shift assay. Cells engineered to express EGFR mutants including exon 20 insertion mutations were employed for cellular potency...
assessment. Cell viability assay were performed using CellTiter-Glo™ luminescent cell viability assay. The protein and phosphoprotein expression level were determined by Western blot analysis or Simple Western™ assay. Antitumor evaluation was conducted in athymic nude mice bearing NCI-H1975 EGFR/ErbB3 positive cell line which was genetically engineered to express EGFR D770,N771insSVD mutation instead of endogenous mutant EGFR.

**Results:** TPC-064 was discovered as a mutant EGFR selective inhibitor. Biochemical assay revealed that 100 nM TPC-064 showed more than 50% inhibition against these EGFR, subtypes and six other kinases. In cell viability assays using Ba/F3 cells engineered to express EGFR variants, TPC-064 inhibited exon 20 insertion mutations more potently than WT. The IC50 values were in the range of 37–239 nM for exon 20 insertion mutations and 544 nM for WT. Consistent with cell viability assay, the IC50 values of p-EGFR inhibition were in the range of 33–197 nM for exon 20 insertion mutations and 571 nM for WT. In addition to exon 20 insertion mutations, TPC-064 showed potent cell growth inhibitory activity to NSCLC cell lines harboring other mutant EGFR including exon 19 deletion and L858R/T790M with a range of 0.3–3.7 nM G68 values. In NCI-H1975 EGFR/SVD (genetically engineered human lung cancer) xenograft model, once-daily oral dosing of TPC-064 showed antitumor efficacy at 50 mg/kg or more and tumor regression at 200 mg/kg without severe body weight loss. Antitumor efficacy was associated with reduction in tumor size.

**Conclusions:** TPC-064 is a novel mutant-selective inhibitor targeting EGFR exon 20 insertion mutations while sparing WT. This mutation-selective characteristic led to significant antitumor efficacy in a mouse xenograft model, suggesting a promising therapeutic option for patients with NSCLC harboring EGFR exon 20 insertion mutations.

**No conflict of interest.**

381 Poster (Board P060) KO-947, a potent and selective ERK inhibitor with slow dissociation kinetics

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The RAS/RAF/MEK pathway is activated in more than 30% of human cancers, including tumors arising from mutations in KRAS, NRAS and BRAF. Although inhibitors of both BRAF and MEK have been approved for treatment of melanoma, acquired resistance to these inhibitors has been documented both in preclinical and clinical samples due to reactivation of ERK1/2 kinases. Here we describe the characterization of KO-947, a potent and selective inhibitor of ERK1/2 kinases, in biochemical, cellular and in vivo activity assays.

KO-947 is a low nanomolar inhibitor of ERK1/2 with limited off-target activity across a broad range of protein kinases as measured using biochemical activity assays, competition binding assays, and a probe-based competition binding assay in cell lysates. KO-947 potently inhibits ERK signaling pathways and proliferation of tumor cells exhibiting dysregulation of MAPK pathway signaling, including mutations in BRAF, NRAS or KRAS. KO-947 also inhibits MAPK signaling and cell proliferation in clinically relevant models that are resistant to BRAF and MEK inhibitors. Results from screening a large panel of PDX models demonstrate that KO-947 induces tumor regressions in BRAF or RAS mutated tumor models as well as in tumor models lacking BRAF/RAS mutations but with other dysregulation of the MAPK pathway. KO-947 is differentiated from other published ERK inhibitors by an extended residence time and high potency in cell engagement that translate into prolonged pathway inhibition in vitro and in vivo. Drug properties of KO-947 enable the achievement of optimal antitumor activity with intermittent dosing, which may provide an opportunity to maximize the therapeutic window with flexible administration routes and schedules.

These results demonstrate the potential clinical utility of KO-947 in MAPK pathway dysregulated tumors. KO-947 is anticipated to enter clinical development in the second half of 2016.

**Conflict of interest:** Ownership: Francis Burnows, Pingda Ren and Yi Liu have ownership of and are employed by Kura Oncology.

383 Poster (Board P062) mTOR Ser2481 phosphorylation may be a key target limiting the therapeutic efficacy of mTORC1 inhibitors in advanced hepatocellular carcinoma

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**Background:** Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. The multikinase inhibitor sorafenib increases the overall survival (OS) of advanced HCC patients. However, the therapeutic efficacy of sorafenib is limited to a poorly differentiated (HAK-1A) and poorly differentiated (HAK-1B) cells established from a single HCC tumor.

**Material and Methods:** Establishment of HAK-1A and HAK-1B cells: These cell lines have been established from a single nodule showing a three-layered structure with a different histological grade in each layer from a 55-year-old Japanese male patient with HCC (Yano et al., Hepatology, 1993). Xenograft mouse experiment: Cells were injected subcutaneously into the right flank of male BALB/c athymic nude mice. These mice with tumors larger than 100 mm3 were treated with everolimus (2.0 mg/kg/day) orally daily. All animal experiments were approved by the Ethics of Animal Experiments Committee at Kyushu University Graduate School of Medical Sciences.

**Results:** We first compared the cytotoxic effects of various anti-cancer drugs between HAK-1A and HAK-1B cells, and observed that rapalogs, a component of mTORC1, suppressed the cell growth and p-mTOR Ser2481 only in HAK-1B cells. (4) Tumor growth of HAK-1B was markedly inhibited by treatment of everolimus, accompanying by suppression of p-mTOR Ser2481. Furthermore, three other HCC cell lines established independently from the HCC tumors were also 2,000-fold times more sensitive to rapamycin, which correlated closely with the inhibition of p-mTOR Ser2481 by rapamycin.

**Conclusion:** Our present study demonstrates that mTOR Ser2481 phosphorylation was specifically inhibited in mTORC1-addicted HCC cells when treated with mTORC1 inhibitors. We therefore conclude that mTOR Ser2481 phosphorylation may provide a potential reliable marker for the therapeutic efficacy of mTORC1 inhibitors, and contribute to further development of optimized mTORC1 targeted therapeutics against HCC.

**No conflict of interest.**

384 Poster (Board P063) Circulating tumor cell analysis in a first-in-human phase I study of s EphB4-HSA, an inhibitor of EphrinB2-EphB4 signaling

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**Background:** Circulating tumor cell (CTC) analysis has been prognostic and predictive in large phase 3 trials, but CTCs may yield particular benefit in phase II/III trials, where patients with advanced disease often present prior to standard radiographic assessment and where additional "drug-on-target" information may be informative. To test this, we used a palyrene C slot microfilter developed by our group to enrich and analyze CTCs in the setting of a first-in-human phase I study of s EphB4-HSA, an inhibitor of EphrinB2-EphB4 signaling.

**Material and Methods:** 7.5 mL tubes of blood (CellSave, Jansen/J&J) were collected at study entry (day 0) and at day 3, week 1, and at 1-month cycles thereafter. Mononuclear cells were enriched by Ficol
centrifugation and then passed through the microfilter, which depletes white blood cells but captures CTCs based on size and deformability. Captured cells were immunofluorescence stained on filter for DAPI nuclear stain, cytokeratin, EphB4 and EphrinB2 receptors, and their respective downstream signaling mediators pAKT and pSrc. Enumeration and staining results were analyzed for associations with disease response by standard radiographic RECIST1.1 criteria.

Results: From 10/2012 to 8/2015, CTCs were identified in 59/62 (95.2%) of study patients (clinical endpoints for the phase I trial are presented separately). The median CTC count for the entire cohort was 3 (range: 0–73). A reduction in CTC counts from baseline (reduced on two consecutive time points) was associated with greater disease control (SD/CR/PR, p = 0.006). Greater CTC protein expression (EphB4 and EphrinB2 receptors, pAKT, and pSrc) at baseline was associated with a trend towards greater disease control but did not reach statistical significance.

Conclusions: In a phase I clinical trial comprised of small numbers of heterogeneous solid malignancies, decreases in CTC counts on a parylene-C slot microfilter were significantly associated with responses to therapy, and increased expression of the study drug’s putative targets in CTCs at baseline was associated with a trend towards higher response. These preliminary results support a strategy of CTC characterization as an informative secondary endpoint in early drug development.

Conflict of interest: Support: The V Foundation Translational Research Grant, Cancer Center Core Grant P30CA140489.

385 Poster (Board P064) TAS3681, a new type of androgen receptor antagonist, disrupts aberrant AR signaling that drives tumor resistance to AR-targeted therapies by downregulating full-length and splice variant AR

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Background: Therapies targeting androgen receptor (AR) signaling such as abiraterone and enzalutamide prolong life in castration-resistant prostate cancer (CRPC). However, resistance to these new therapies has already been reported. Induction of AR splice variants and AR overexpression are major issues of CRPC progression. Moreover, occurrence of AR mutation is often observed in tumor escaped from 2nd generation AR-targeted therapy. TAS3681 is an oral pure AR antagonist with AR downregulation activity. In the present study, we evaluated the impact of TAS3681 on several issues related to resistance to current AR-targeted therapy.

Material and Methods: VCaP cells transfected with wild-type AR expressing vector were treated with TAS3681 in the presence of androgen. The CellTiter96® assay was performed to measure cell viability. Prostate cancer cells expressing AR-v7 were treated with TAS3681, and full length (FL)-AR and AR-v7 protein levels were determined by Western blot. Real-time PCR was used to analyze mRNA levels of several AR-v7 target genes. TAS3681 was orally administered to confirm AR downregulation in tumor and engineered antitumor efficacy in AR-v7 positive CRPC tumor xenograft mice model.

Results: In prostate cancer cells which overexpress AR, in contrast to enzalutamide, TAS3681 effectively suppressed AR transactivation and cell proliferation via AR downregulation. TAS3681 effectively antagonized F876L mutant AR which confers resistance to cell growth inhibition by 2nd generation androgen. TAS3681 reduced both FL-AR and AR-v7 protein expression in prostate cancer cells in vitro and in vivo, and showed strong antitumor efficacy in AR-v7 positive xenografts.

Conclusions: TAS3681 demonstrates disruption of aberrant AR signaling that drives tumor resistance to current AR-targeted therapy through its pure AR antagonist profile and downregulation of both full-length and splice variant AR.

No conflict of interest.

386 Poster (Board P065) Potent and selective inhibition of CDK7 by novel covalent inhibitors

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Background: Cyclin-dependent kinase 7 (CDK7) is an important constituent of the cellular transcriptional machinery, where it phosphorylates the C-terminal domain (CTD) of RNAP polymerase II (RNAPII). Because many tumor types are critically dependent on transcription for maintenance of their oncogenic state, pharmacological modulation of CDK7 kinase activity is considered as an approach to treat cancer.

Material and Methods: Multiple series of CDK7 inhibitors were identified by iterative medicinal chemistry efforts and SAR based approach. These compounds were optimized towards attaining good physicochemical properties, high potency, good selectivity and desirable pharmacokinetic profile to achieve anti-tumor activity.

Results: From covalent CDK7 inhibitors that have been developed from two hit chemical series, we have now identified an orally bioavailable lead compound this is not only very well tolerated in an MTD study but is also highly efficacious in an AML derived xenograft model. This compound was associated in inhibiting CDK7 in biochemical as well as cellular assays and has excellent drug-like characteristics including solubility, permeability, metabolic stability and good oral bioavailability. When tested for its impact on tumor growth inhibition in a xenograft model, it has demonstrated more than ninety percent tumor growth inhibition at a dose that is significantly lower than the maximal tolerated dose. This was found to be accompanied by pS5RNAPII RNAPolII Ser5 phosphorylation suppression in an accompanying PK-PD study. Efficacy studies are planned in xenograft models from additional indications. Toxicity studies in rats and pharmacokinetic studies in higher species are about to be initiated for this compound.

Conclusion: We have identified novel and selective CDK7 covalent inhibitors from two series with desirable drug-like properties. Of these, an orally bioavailable lead compound that was evaluated for anti-tumor activity in a xenograft model has demonstrated significant efficacy.

Conflict of interest: Ownership: All authors are employees of Aurigen Discovery Technologies Limited.
Results: Cetuximab is ongoing in Cliniques Universitaires Saint-Luc. A clinical study phase I investigating CDK4/6 inhibitor effect combined to and anti-EGFR treatment. naïve, while these second model was resistant to chemotherapy, radiotherapy.

The in vivo efficacy of Ribociclib was investigated in 2 HPV-negative (western blot) of sensitive and resistant cell lines.

In this work, we are investigating the activity of a CDK4/6 inhibitor in clinical and molecular behaviors.

Background: Beside alcohol and tobacco, Human Papillomavirus (HPV) is a causal factor of squamous cell carcinoma of the head and neck (SCCHN), especially when located in the oropharynx. HPV-positive (HPV+) and -negative (HPV−) SCCHN are different entities based on differences in clinical and molecular behaviors.

HPV+ SCCHN tumors alterations that enable them to circumvent the mitotic checkpoints through impaired cyclin-dependent kinase (CDK) activities. Implicated mechanisms are (i) inactivation of CDKN2A (58%) and (ii) CIN1 overexpression, p16 inactivation and cyclin D1 overexpression, induce CDK4/6 activation and promote tumor proliferation. Therefore, there is a strong rationale to test CDK4/6 inhibitors in HPV− SCCHN.

In contrast, HPV+ SCCHN is mediated by expression of the viral E6 and E7 oncoproteins, which cause deregulation of the cell cycle by inactivating the p53 and Retinoblastoma proteins, respectively.

Material and Methods: First-in-human, multicenter, open-label, ongoing study of HDM201 in pts with advanced TP53 wt tumors, progressing on standard therapy or for whom no standard therapy exists (NCT02143635, sponsored by Novartis Pharmaceuticals Corporation). Four oraltreatmentstandardtherapyorforwhomnostandardtherapyexists (NCT02143635, sponsored by Novartis Pharmaceuticals Corporation).

Poster/Posterinthespotlight(BoardP070)
address thrombocytopenia; one involves dose regimen optimization and/or implementation of prophylactic support with the thrombopoietin receptor agonist eltrombopag to further the therapeutic potential of this class of drugs.

**Conflict of interest:** Ownership: Sébastien Jeay, Novartis shareholder; Ensar Halliovic, Novartis stock ownership; Jens U. Würtzner, Novartis stock ownership. Advisory Board: David Hyman, Atara Biotherapeutics; Sebastian Bauer, Pfizer, Novartis, Bayer, Blueprint Medicines. Corporate-sponsored Research: David Hyman, PUMA Biotechnology, AstraZeneca, LOXO Oncology; Philippe Cassier, Novartis, Roche, AstraZeneca, Merck MSD, Merck Serono, Celgene, Bayer, Blueprint, Amgen, Eli Lilly; Sebastian Bauer, Novartis. Other Substantial Relationships: David Hyman, Consulting – CynoVex, Philippe Cassier, Honoraria – Roche, Novartis, Amgen; Sebastian Bauer, Honoraria for CME-related presentations: Novartis, Pfizer, Bayer, Sébastien Jeay, Ensar Halliovic, Christophe Meille, Nelson Guerreiro, Luisa Mariconti and Arun Kumar are Novartis employees; Jens U. Würtzner is a former Novartis employee.

392 Poster (Board P071)

Synergistic anticancer effects through tumor vessel normalization by PI3K inhibitors

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**Background:** Tumor vessels are leaky and immature, which causes poor oxygen and nutrient supply to tumor resulting in cancer cell metastasis to distant organs, and anticancer drugs can't penetrate into deep tumor. Recently many approach has been tried to improve the drug delivery into tumor by tumor vessel normalization. In this study, we investigated the tumor vessel normalization by phosphatidylinositol 3-kinase (PI3K) inhibitors and its role in drug delivery.

**Material and Methods:** HS-173 (a novel PI3K inhibitor) and NVP-BEZ235 (a dual PI3K/mTOR inhibitor) were investigated for the vessel normalization in two cancer cell lines as well as xenograft and metastasis animal models. To determine tumor vessel structure and maturation, the tumor tissues were stained with CD34 and DAPI, and observed by scanning electron microscopy. For tumor vessel integrity associated with tumor vessel normalization, the tumor tissues were stained with NG2 (a pericyte marker), collagen IV (a basement membrane marker), and claudin-5 (a tight-junction marker). And FITC-dextran or -lectin was injected to tumor-bearing mice to measure perfused or leaky vessels to assess vessel functionality in vivo and in vitro. Besides, blood flow was measured by 3-dimensional (3D) ultrasound imaging.

**Results:** HS-173 and NVP-BEZ235 potently suppressed tumor growth and hypoxia, increasing apoptosis in B16 and Mia PaCa-2 xenograft. And the PI3K inhibitors induced a regular, flat monolayer of ECs in vessels. Interestingly, vascular maturity, pericyte coverage, basement membrane thickness and tight-junctions were normalized in the drug-treated groups. In addition, PI3K inhibitors decreased tumor vessel tortuosity and thin vessels, and improved vessel function, and enhanced vascular perfusion and blood flow, and reduced vascular leakage by normalizing tumor vessel structure and function as compared to the control groups. In metastasis model, HS-173 potentely suppressed the number of pulmonary metastatic nodules. Also, it improved the delivery of doxorubicin into tumoral region, resulting in that the combination of HS-173 with doxorubicin have exhibited synergistic anticancer effects compared to the HS-173 or doxorubicin monotherapy.

**Conclusion:** The PI3K inhibitors normalized tumor-associated vascular changes, inhibited vascular leakage and capillary, and improved the drug delivery into tumor.

**No conflict of interest.**

393 Poster (Board P072)

identifying synthetic lethal targets in colon cancer using CRISPR–Cas9 and siRNA screens

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Large scale cancer genome sequencing projects have found that tumour suppressors and un-druggable oncogenes dominate the spectrum of cancer driver mutations. There is an urgent need for new therapeutics to target these mutations, especially in cancers where a low frequency of neo-antigens means that immune checkpoint inhibitors are unlikely to be effective.

The concepts of synthetic lethality, non-oncogene addiction and genetic co-dependence provide a rationale for identifying vulnerabilities and potential therapeutic targets in these cancers. In colon cancer, for example, druggable cancer drivers, such as PIK3CA, are few, whereas mutations in proteins that are notoriously difficult to drug, namely APOC, TP53 and KRAS, are frequent. We have used arrayed siRNA and pooled CRISPR-Cas9 libraries to screen a panel of isogenic and non-isogenic colon cancer cell lines under conditions designed to increase the cells dependency on oncogenic pathways. This panel contains cell lines with mutations in TP53, APC, KRAS and/or PIK3CA to which we are aiming to identify genetic co-dependencies.

Our screens have identified a number of novel targets, the reduced expression of which imposes specific fitness defects in mutant KRAS and PIK3CA cancer cell lines. For example, siRNA experiments identified a novel gene whose depletion has an anti-proliferative phenotype only in the presence of a PIK3CA mutation. By contrast, CRISPR–Cas9 data indicate that this gene is essential in all cell lines tested. However, the increased penetrance of the CRISPR–Cas9 approach has uncovered several candidate synthetic lethal genes that were not found by RNA interference. Although our current screens are not saturating, we have confirmed several previously reported genetic interactions, such as a requirement for MDM2 expression in TP53 wild type tumour cells and increased sensitivity to loss of hexokinase 2 in KRAS mutant cell lines. Furthermore, CRISPR–Cas9 screens in Horizon’s X-MAN® isogenic PIK3CA cell lines identified several genes that are essential in cells harbouring mutant PIK3CA, including PIK3CA and the downstream kinases AKT1 and AKT2. We have also identified several novel targets that impact the genotypes of interest.

Intriguingly, despite our siRNA and sgRNA libraries having shared target genes, the concordance between the siRNA and CRISPR-Cas9 data is small. For example, EHM72 was a prominent KRAS synthetically lethal hit in our primary siRNA screen. However, analysis of 219 sgRNA guides targeting EHM72 revealed that the gene was dispensable across the same panel of colon cell lines. We are currently using both approaches and additional ones to validate our novel targets.

**Conflict of interest:** Ownership: All authors are employees and/or shareholders of Horizon Discovery Ltd.

394 Poster (Board P073)

FGFR-mediated reactivation of ERK signaling contributes to insensitivity of head and neck squamous cancer to MEK inhibition

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Treatments for recurrent or metastatic head and neck squamous cell carcinoma (HNSCC) have limited efficacy. The extracellular signal-regulated kinase (ERK) expression and activation exhibits aberrant overexpression in human head and neck squamous cell carcinomas (HNSCC). Here, we aimed to investigate the cause of the limited therapeutic effect of MEK inhibitor in head and neck squamous cell carcinoma (HNSCC) because MEK/ERK reactivation has been shown invitably occurs. We assessed the effects combining selumetinib with FGFR3 inhibitor (PD173074) on tumor growth. In addition, we analyzed the level of EMT-related genes and protease to determine the pathological events such as tumor invasion function and metastasis. We determined that a rationale for combining MEK inhibitor with inhibitors of FGFR3-mediated reactivation of ERK signaling transiently in head and neck cancer cells. The rebound in ERK and AKT pathway in head and neck cancer cells is accompanied by increased phospho-FGFR3 signaling after treatment of selumetinib. The feedback activation of FGFR3 is due to an autocrine secretion of FGF2 ligand. The FGFR3 inhibitor PD173074 prevents the MAPK rebound and sensitizes a responding of head and neck cancer cells to selumetinib. In addition, we investigated that rapid feedback activation of FGFR3 regulates the expression of epithelial to mesenchymal transition (EMT) markers and uPA serine protease, which resulted in high migratory potentials in tumor invasion. These results provide rational therapeutic strategies for clinical studies in this poor prognosis subtype of selumetinib. Our data determined that a rationale for combining MEK inhibitor with inhibitors of feedback activation of FGFR3 signaling in head and neck cancer cells. ERK rebound due to up regulation of FGFR3 and the ligand FGFR2 diminished the antitumor effect of selumetinib, which is overcome by combination with FGFR3 inhibitor.

**No conflict of interest.**
Assessment of EGFR-dependent signaling pathway targeting in EGFR-mutated and wild type non-small cell lung cancer patient-derived xenografts

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Background: EGFR mutations are found in 10% of Caucasian and 30% of Asian non-small cell lung cancer (NSCLC) patients. Hence, EGFR-targeted therapies have been developed for EGFR inhibition with significant initial responses. However, acquisition of drug resistance is common when using an EGFR mono-targeting strategy, with half of the patients developing resistance within 12 months after start treatment. While various mechanisms of acquired resistance have been identified, the best strategy against these resistances is not well defined. The aim of this study was to evaluate the efficacy of single or dual targeting of both Pi3K/mTOR and EGFR signaling pathways in a panel of NSCLC Patient-Derived Xenografts (PDXs) bearing various mutations.

Material and Methods: Four well caracterized PDXs obtained from NSCLC patient’s were treated with the EGFR (HER1) TKI erlotinib (30mg/kg/day, 5 days/week), the HER2/4 TKI afatinib (20mg/kg/day, 5 days/week), the anti-EGFR monoclonal antibody cetuximab (25mg/kg, twice a week), the anti-Pi3K/mTOR mTORC1 inhibitor RAD001 (2mg/kg/day, 5 days/week), and the MEK1/2 inhibitor selumetinib (25mg/kg twice a day, 5 days/week), and/or the PI3KCA-mutated LCF12 (25mg/kg/day, 5 days/week) or BKM120 (20mg/kg/day, 5 days/week). The two EGFR-TKI erlotinib and afatinib induced non-significant Tumor Growth Inhibition (TGI) of 0% to 40% in the two PI3KCA-mutated PDXs, intermediate TGI of 38% and 54% in the two KRAS-mutated models, and significant TGI of 59% and 65% in the two PI3KCA and KRAS wild-type tumors. RAD001 induced intermediate TGI of 49% and 52% in the two PI3KCA-mutated xenografts, and high significant TGI of 70% and 84% in the two KRAS-mutated models. RAD001 was not tested in the two wild-type PDXs. Selumetinib induced contrasted TGI of 9% and 63% in the two PI3KCA-mutated models and in the PIK3CA-mutated ML5 model and in the two KRAS-mutated LCF15 and LCF25 PDXs, showing in only one model (LCF15) an increased TGI in comparison to the best monotherapy (92% versus 84% after RAD001 and 69% after selumetinib).

Conclusions: In our study, PI3K/mTOR pathway targeting with BKM120 or RAD001, as well as MAPK pathway targeting with selumetinib, induced better TGI in KRAS-mutated PDXs than PI3KCA-mutated models, but without complete tumor regressions. This pharmacological study demonstrates that our panel of NSCLC PDXs constitutes a relevant preclinical tool to evaluate new therapeutic approaches, alone or in combination, using PI3K- or MAPK-directed therapies.

No conflict of interest.

Assessment of PI3K and/or MAPK signaling pathway targeting in KRAS- or PIK3CA-mutated and wild type non-small cell lung cancer patient-derived xenografts

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Background: KRAS mutations are the most frequent molecular alterations in non-small cell lung cancers (NSCLC) and are associated with poor responses to therapies. KRAS leads to the activation of the PI3K/AKT/mTOR and MAPK/MEK/ERK pathways, whose inhibition remains clinically challenging. On the other hand, PI3KCA mutations are found in about 4% of NSCLC, often together with EGFR or KRAS mutations. Moreover, PI3KCA mutations are associated with poor survival in EGFR or KRAS Wild-type NSCLC patients. Antitumor efficacy of both MAPK/ERK and PI3K pathway inhibition is currently being evaluated in various clinical trials. The aim of this study was to evaluate the efficacy of single or dual targeting of both PI3K/mTOR and MAPK signaling pathways in a panel of NSCLC Patient-Derived Xenografts (PDX) bearing various mutations.

Material and Methods: Six well-characterized PDX obtained from NSCLC patients were treated with the PI3K/AKT/mTOR PI3KCA inhibitor BKM120 (20mg/kg/day, 5 days/week), the mTORC1 inhibitor RAD001 (2mg/kg/day, 5 days/week), and/or the MEK1/2 inhibitor selumetinib (25mg/kg twice a day, 5 days/week). Five PDX were adenosarcomas (LCF4, LCF9, LCF15, LCF25, and ML5) and one was a large cell carcinoma (ML1). ML1 and ML5 harbored a KRAS mutation (G12C), LCF15 and LCF25 a PI3KCA mutation (exon 9 E545K), whereas LCF4 and LCF9 were KRAS/PI3KCA wild-type tumors. These models did not display any EGFR, nor ALK translocation.

Results: BKM120 induced non-significant Tumor Growth Inhibition (TGI) of 0% and 42% in the two PI3KCA-mutated PDX, intermediate TGI of 38% and 54% in the two KRAS-mutated models, and significant TGI of 59% and 65% in the two PI3KCA and KRAS wild-type tumors. RAD001 induced intermediate TGI of 49% and 52% in the two PI3KCA-mutated xenografts, and high significant TGI of 70% and 84% in the two KRAS-mutated models. RAD001 was not tested in the two wild-type PDXs. Selumetinib induced contrasted TGI of 9% and 63% in the two PI3KCA-mutated models and in the PIK3CA-mutated ML5 model and in the two KRAS-mutated LCF15 and LCF25 PDXs, showing in only one model (LCF15) an increased TGI in comparison to the best monotherapy (92% versus 84% after RAD001 and 69% after selumetinib).

Conclusions: In our study, PI3K/mTOR pathway targeting with BKM120 or RAD001, as well as MAPK pathway targeting with selumetinib, induced better TGI in KRAS-mutated PDXs than PI3KCA-mutated models, but without complete tumor regressions. This pharmacological study demonstrates that our panel of NSCLC PDXs constitutes a relevant preclinical tool to evaluate new therapeutic approaches, alone or in combination, using PI3K- or MAPK-directed therapies.

No conflict of interest.
Background: EGFR is a key driver of tumor growth in colorectal cancer (CRC). Many CRC patients initially derive benefit from anti-EGFR-based treatment regimens, but eventually develop resistance. Resistance can arise through acquisition or selection of mutations in KRAS, NRAS, or BRAF. We hypothesized that the mechanism could be overcome by combining MM-151, a potent oligoclonal EGFR inhibitor, with MEK inhibitor trametinib. For tumors that do not develop mutations in RAS or RAF, resistance may emerge through upregulation of ligands like heregulin (HRG) and insulin-like growth factor-1. These ligands activate prosurvival signaling through PI3K/Akt. We postulated that a biomarker-driven approach could determine which investigational agent to pair with MM-151 to overcome resistance in CRC models. In parallel, we have initiated a Phase 1, biomarker-directed open-label study evaluating the safety, pharmacology and preliminary activity of MM-151 in combination with MM-121, trametinib, or MM-141 in CRC, squamous cell carcinoma of the head and neck, and non-small cell lung cancer (NCT02538627).

Materials and Methods: We evaluated combinations of MM-151 with trametinib or MM-121 in a panel of KRAS mutant, BRAF mutant, or KRAS/RAF-wildtype CRC cell lines using an optimized in vitro culture system to measure cell viability and treatment effect on cell signaling. The screen was performed in the presence or absence of exogenous ligands. Cetuximab was used as a monoclonal anti-EGFR antibody comparator in the same treatment settings. Using these data, we selected xenograft models for in vivo analysis of combination efficacy. We also used engineered CRC xenograft models with altered biomarker profiles, including varying levels of HRG, to investigate the treatment effects of MM-151, MM-121, cetuximab, trametinib and their combinations. Animal experiments were approved by the IACUC.

Results: We found that MM-151 and trametinib exhibit additive effects on decreasing cell viability in RAS mutant, RAF mutant, and RAS/RAF wild-type CRC cell lines using an optimized in vitro culture system to measure cell viability and treatment effect on cell signaling. The screen was performed in the presence or absence of exogenous ligands. Cetuximab was used as a monoclonal anti-EGFR antibody comparator in the same treatment settings. Using these data, we selected xenograft models for in vivo analysis of combination efficacy. We also used engineered CRC xenograft models with altered biomarker profiles, including varying levels of HRG, to investigate the treatment effects of MM-151, MM-121, cetuximab, trametinib and their combinations. Animal experiments were approved by the IACUC.

Conclusion: We found that MM-151 and trametinib exhibit additive effects on decreasing cell viability in RAS mutant, RAF mutant, and RAS/RAF wild-type CRC cell lines using an optimized in vitro culture system to measure cell viability and treatment effect on cell signaling. The screen was performed in the presence or absence of exogenous ligands. Cetuximab was used as a monoclonal anti-EGFR antibody comparator in the same treatment settings. Using these data, we selected xenograft models for in vivo analysis of combination efficacy. We also used engineered CRC xenograft models with altered biomarker profiles, including varying levels of HRG, to investigate the treatment effects of MM-151, MM-121, cetuximab, trametinib and their combinations. Animal experiments were approved by the IACUC.
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400 Poster (Board P079)

The MEK1/2 inhibitor selumetinib (AZD6244; ARRY-142886) appears, as an efficient targeted therapy when used in an adjuvant setting in patient-derived xenografts of uveal melanoma


Background: Uveal melanomas (UM) constitute the most common primary intraocular tumors in adults and are characterized by a constitutive activation of the MAPK pathway due to mutations of the GTPase genes GNAQ or GNA11 in almost 80% of cases. The most commonly used treatments for UM are alkyating agents such as dacarbazine (DTIC) and temozolomide (TMZ). The MEK1/2 inhibitor selumetinib (AZD6244; ARRY-142886) has shown clinical activity compared to DTIC/TMZ in a recent Phase II clinical trial and has recently completed a Phase III clinical trial in combination with DTIC (NCT01974752). In parallel with this trial we sought to evaluate the efficacy of DTIC + selumetinib in UM patient-derived xenografts (PDXs).

Material and Methods: Three models were included in the study (MP34, MP55, and MM26), all bearing a GNAQ or GNA11 mutation. selumetinib was administered orally at 25mg/kg/day, 5 days a week, twice daily, and DTIC at a dose of 40mg/kg/day on Days 1 to 5 every 4 weeks.

Results: A significant tumor growth inhibition (TGI) of 54% was observed in the MP34 model but not in the two remaining PDXs. In one model, MM26, DTIC induced a strong TGI of about 99% with 6/6 complete remissions (CRs). The combination of selumetinib + DTIC did not significantly increase efficacy compared to monotherapy in any of the models; in the MM26 PDX model, the combination induced a similar TGI (99%) and CR rate (59) as DTIC alone. In this experiment, after two courses of DTIC + selumetinib, selumetinib was continued alone, showing a significant increased growth delay (p < 0.01 at Day 113), compared with DTIC alone. Pharmacokinetics, pharmacodynamics, and molecular studies on the three UM PDXs are ongoing. The preliminary pharmacodynamics study for 3 models showed a significant difference in ratio of p-MEK1/2 between selumetinib alone or selumetinib + DTIC for both of them, MP55 and MM26 and p-S6/S6 in the DTIC-sensitive PDX and published clinical studies demonstrating MEK inhibitor monotherapy activity indicate that MEK inhibition may have value as a treatment for UM; perhaps in the adjuvant setting in two specific clinical situations, i.e. patients with irradiated or enucleated high-risk primary intraocular or surgically resected metastatic UM.

Conflict of interest: Other Substantive Relationships: Drs Paul Smith, Emma Davies, Colin Howes, Joanne Wilson and Aaron Smith work for AstraZeneca Laboratories.

402 Poster (Board P081)

SEL120−34A, a specific, potent and orally bioavailable inhibitor of CDK8, targets STAT-dependent gene transcription in leukemia and lymphoma models

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Background: CDK8 kinase associates with the Mediator complex to regulate transcriptional programs for sustained proliferation of cancer models, especially in colorectal and hematological malignancies. Material and Methods: SEL120−34A, a type I inhibitor of CDK8, was developed by rational drug design. Binding mode of SEL120−34A was confirmed by X-ray crystallography. Kinase assays and TR-FRET were used to determine the inhibitory effect towards purified protein. Efficacy of the compound was tested in viability assays in a broad panel of cancer cell lines and in extended viability assays in leukemia and lymphoma models. In vivo efficacy of SEL120−34A was tested in leukemia models. STAT protein phosphorylation was used as a specific biomarker of CDK8 function in vitro and in vivo. Transcriptomic data were analyzed to establish global gene expression profiles after SEL120−34A treatment.

Results: SEL120−34A shows nanomolar activity and high specificity towards CDK8 in biochemical and biophysical assays. In a broad panel of cancer cell lines, the compound showed the strongest activity in hematological malignancies, especially in selected acute myeloid leukemia (AML) (GSI0 ≤ 12 nM), acute lymphoblastic leukemia (ALL) and mantle cell lymphoma (MCL) models. These findings were consistent with in vivo data, where SEL120−34A effectively inhibited tumor growth in subcutaneous responder AML and MCL models when administered orally. Both in vitro and in vivo, we observed robust and dose-dependent inhibition of STAT5 phosphorylation on serine 726. To understand the mechanism of action (MoA) of SEL120−34A, we performed RNA sequencing and microarray studies which revealed an immediate deregulation of Super-Enhancer associated genes and a prolonged effect on STAT-dependent gene transcription linked to inflammation and differentiation.

Conclusions: – SEL120−34A shows high efficacy in leukemia models in vitro and in vivo. – Novel MoA of the inhibitor involves changes in STAT-dependent gene transcription linked to inflammation and differentiation. – SEL120−34A shows favorable pharmacokinetic profile and safety indicating high therapeutic potential.

No conflict of interest.

404 Poster (Board P083)

First-in-class cell penetrating proteins targeting Mcl-1 induce tumor apoptosis and inhibition of tumor growth in vivo

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Background: Myeloid Cell Leukaemia-1 (Mcl-1) is an anti-apoptotic Bcl-2 family member which is essential for the survival of numerous cancers. Its overexpression is often associated with chemotherapeutic resistance and disease relapse. Mcl-1, however, has proven difficult to target using small molecules. Complix has developed Cell Penetrating Alphabodies (CPABs), a novel class of proteins engineered to efficiently enter cells and interfere with disease associated protein-protein interactions (PPIs). Half-life extension of these otherwise rapidly cleared CPABs has been achieved by incorporating an albumin moiety in the body of the protein. The CPAB technology has been used to develop a novel therapeutic approach to Mcl-1 inhibition.

Materials and Methods: A combination of rational design and phase display was used to engineer MCL-1 binding Alphabodies (ABs) and the AB equipped for cellular uptake. Specificity and affinity were measured in the Mammalian Two Hybrid (MTH) system and ELISA; cellular uptake assessed in vitro using cell fractionation, SDS-PAGE and confocal microscopy. Potency was assessed using ATP viability and caspase release assays. Finally the CPABs were equipped with an albumin binding domain and PK, biodistribution and efficacy assessed in tumor bearing mouse models.

Results: In ELISA, Mcl-1 targeted ABs bound Mcl-1 with affinities in the low µM range whilst binding to Bcl-2 and Bcl-XL remained below the assay detection limit. In a MTH assay, ABs inhibited the Bak-Mcl-1 but not Bak-Bcl-XL interactions. In vitro, CPABs were shown to be taken up efficiently in tumor cells, reaching levels of ~1 nM and efficiently induced cell death in the Mcl-1 dependent multiple myeloma (MM) cell line NCI-H929 (IC50 = 0.5 µM). CPABs also induced cell death of other Mcl-1 dependent tumor cell lines and death correlated with caspase-3/7 activation. In vivo, the half-life of the albumin binding CPAB was >1 hour, with associated tumor concentrations >1 µM. Immunohistochemistry confirmed the intracellular presence of the CPAB in the tumor cells. When given daily IV at 20 mg/kg, anti-MCL-1 CPABs resulted in 50% versus control in H929 xenograft model. A panel of MM cell lines was screened for sensitivity to anti-MCL-1 CPABs and potency correlated with MCL-1 gene expression. In vivo studies with the most sensitive MM cell lines are ongoing to assess the anti-tumor effect of the CPABs.

Conclusions: CPABs specifically inhibiting the Bak-MCL-1 interaction through the binding to MCl-1 were engineered. These CPABs were characterized by high binding affinity, strong in vitro potency and induced
a robust reduction in tumor growth in a mouse models. These CPABs represent the best-in-class cell penetrating protein therapeutics opening unprecedented opportunities to tackle intracellular PPI critical to diseases with unmet medical need.

No conflict of interest.

405 Poster (Board P084)

HTL001, a novel inhibitor of HOX/PBX binding, is highly cytotoxic to prostate and breast cancer cells

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Background: The HOX genes are a family of homeodomain-containing transcription factors that determine cellular identity during development and which are dys-regulated in most cancers. In this study we examined the efficacy of a novel inhibitor of HOX function, HTL001, which blocks the interaction between HOX and PBX proteins, in breast and prostate cancer.

Methods: We tested the sensitivity of prostate and breast cancer-derived lines to HTL001, a novel peptide antagonist of HOX protein binding to its PBX co-factor. Apoptosis was measured using a FACS-based assay with Annexin, and changes in the expression of previously identified HOX/PBX target genes were measured using RT-qPCR on RNA extracted from cell lines. The in vivo efficacy of HTL001 was tested in a mouse PC3 flank tumor xenograft model.

Results: Targeting HOX genes with HTL001 caused apoptotic cell death in all of the cell lines, and prevented the growth of prostate tumors in a mouse xenograft model. Furthermore, HTL001 was significantly more effective than the previously described inhibitor of HOX/PBX interactions, HXR9, both in vivo and in vitro. We show that HTL001 causes a very rapid increase in the expression of 2 genes previously identified as targets of HXR9, cFos and DUSP1, and that the latter could act as a marker of tumor response.

Conclusion: HTL001 is a significantly more effective inhibitor of the interaction between HOX and PBX proteins than the previously described antagonist, HXR9, and is highly cytotoxic to both prostate and breast cancer cells in vitro and in vivo.

No conflict of interest.

406 Poster (Board P085)

Preliminary biomarker and pharmacokinetic analysis from the completed dose escalation part of the first-in-human Phase I study evaluating MP0250, a multi-DARPin® blocking HGF and VEGF-A, in patients with advanced solid tumors

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Background: The VEGF/VEGFR and HGF/cMet pathways are implicated in tumor angiogenesis, invasion and metastasis. DARPin® (designed askyrin repeat proteins) are small proteins that can be engineered to bind to specific targets with high specificity and affinity. MP0250 is a first-in-class, tri-specific multi-DARPin® neutralizing VEGF-A and HGF as well as binding to human serum albumin to increase plasma half-life and potentially enhance tumor penetration. As specific dual blockade of VEGF-A and HGF is new, a broad panel of potential plasma biomarkers was analysed.

Methods: A phase I, open-label, multi-center study with completed dose escalation. Eligibility: Patients with advanced solid tumors. Design: 3+3 dose escalation study enrolling intravenous MP0250 every 2 weeks. Primary objectives: Safety, tolerability and PK. Secondary objective: ADA, explore potential biomarkers to assess target binding in circulation and changes in cytokine profiles. Plasma concentrations of MP0250, VEGF, HGF and cytokines were measured by immunoassays (ELISA/Luminex). PK parameters were derived using non-compartmental analysis.

Results: 24 patients were enrolled in 5 cohorts: 0.5 (n = 3), 1.5 (n = 5), 6 (n = 6), 8 (n = 7), or 12 mg/kg (n = 5) MP0250. The maximum tolerated dose was determined to be 8 mg/kg when MP0250 was given every two weeks. Adverse events, mainly consistent with VEGF inhibition, and signs of clinical activity, have been reported elsewhere (ESMO 2016, submitted abstract). Sustained exposure was observed over multiple dosing cycles of up to 1 year, indicating the absence of neutralizing or clearing ADAs.

Conclusion: In a phase I study, MP0250 was found to be well tolerated and show signs of clinical activity. MP0250 showed a favorable PK profile and sustained exposure over treatment periods. MP0250 had a strong impact on plasma levels of VEGF-A and HGF, but no other biomarkers were identified.

Conflict of interest: Ownership: Dawson, Feurstein, Zitt, Fiedler, Kuster, Bez, Schreiner, Turner, Tadjalli Mehr, Stumpf, Harstrick are employees of Molecular Partners and have shares or share options in the company, Corporate-sponsored Research: Baird, Omlin, Middleton, Rodon receive funding from Molecular Partners for providing clinical services to the phase 1 trial.

407 Poster (Board P088)

Galunisertib combined with sorafenib affects in vivo tumor growth and immune landscape in hepatocellular carcinoma (HCC)


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Introduction: The TGF-beta pathway that has been associated with hepatocellular carcinoma (HCC) progression, can be targeted by galunisertib, a selective ATP-mimetic TGF-beta receptor (TbR-I) inhibitor currently under clinical investigation in HCC patients. Our study aimed to investigate the anti-tumoral effects of galunisertib with or without sorafenib in a transgenic mouse model of HCC.

Methods: Transgenic mice developing stage-defined HCC were treated for 8 weeks (W) from W0 to W16 with either vehicle, sorafenib (30 mg/kg), galunisertib (100 mg/kg) or sorafenib plus galunisertib. Tumor growth was evaluated by ultrasound (liver size) and by the number of macronodules at sacrifice. Angiogenesis was evaluated by doppler (blood flow in the coeliac trunk) and by CD31 staining, and immune landscape by flow cytometry analysis.

Results: Liver size and the number of liver tumor macronodules were significantly lower in all treatment arms compared to placebo at both the W12 intermediary sacrifice and W16 final sacrifice; the combination of galunisertib and sorafenib showing potentiation effects (14.2±7.6, 26.3±7.2, 83.1±17.6 in the sorafenib, galunisertib and placebo arms respectively). Angiogenesis (CD31 staining) was decreased in all treatment arms and the combination of galunisertib and sorafenib was better than sorafenib and galunisertib monotherapies, reducing both the number of vessels and the vessel lumen area. These effects on angiogenesis were confirmed by Doppler, measuring the mean blood flow in the coeliac trunk (Tcm). TcM was decreased in all treatment arm compared to placebo. At W16, we observed a potentiation in the combination arm, compared to the monotherapy (a decrease of 29%, 18% and 39% of the Tcm compared to placebo in the sorafenib, galunisertib and placebo arms respectively).

Conclusion: The combination of galunisertib and sorafenib in animal studies showed promising anti-tumor activities that were associated with decreased angiogenesis.

Conflict of interest: Corporate-sponsored Research: Pr V. Paradis, Pr S. Faivre, Pr E. Raymond, Dr A. de Gramont, Dr A. Tijeras-Raballand. Other Substantive Relationships: Karim Benhadji is an employee of Eli Lilly, who sponsored this study.
408 Poster (Board P087)

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Background: Resistance to apoptosis is a typical hallmark of cancer. Inhibitor of Apoptosis Proteins (IAPs) block caspase activation, modulate NF-κB signaling pathways, and are involved in resistance to standard chemotherapies and radiation and to cancer. A phase I study of IAP inhibitor Debio 1143 in combination with standard of care (SOC) carboplatin and paclitaxel has recently shown that the combination is tolerable with signs of activity observed in patients with heavily pre-treated epithelial ovarian cancer (EOC). This study assessed the potential of Debio 1143-mediated sensitization of carboplatin and paclitaxel in in vitro models of human EOC and provides a basis for the identification of biomarkers for response in a combination therapy setting.

Material and Methods: High-throughput in vitro drug combination screening of 48 human EOC cell lines was used to identify Debio 1143-mediated SOC sensitization and combination synergy. Cell lines were treated with combinations of Debio 1143 with paclitaxel or with carboplatin, and cell viability was assessed in ATP light assays using a dose matrix design. For each agent, sensitivity cut-offs based on clinically relevant concentrations were selected to categorize cell line response. SOC sensitization by Debio 1143 was calculated, with response improvement of at least 25% at clinically relevant concentrations of each of the drugs considered as sensitization. Drug combination synergy using a Synergy Score cutoff of >10 was also analyzed. A signature predictive of response to Debio 1143 was derived by combining response and gene expression data from the EOC cell lines with data from patient derived xenograft models.

Results: Debio 1143 displayed single agent activity in 17/48 EOC cell lines. At clinically relevant concentrations, 14 and 13 out of 48 cell lines were resistant to paclitaxel and to carboplatin respectively. SOC sensitization by Debio 1143 was observed in 11 and 9 out of the 48 cell lines relative to paclitaxel and to carboplatin respectively. Combination synergy with carboplatin was observed in 10 cell lines, and with paclitaxel in 18 cell lines. No desensitization was observed in any cell line. A gene signature predicting response to Debio 1143 was derived. The signature was tested in EOC patient samples from a phase I study where Debio 1143 was administered in combination with SOC, and a good separation was obtained between responding patients and those with progressive disease.

Conclusions: Debio 1143 displayed single agent activity in a subset of human EOC cell lines, sensitizing multiple cell lines to SOC. These results suggest a potentially enhanced clinical response in EOC patients upon combination of SOC with Debio 1143. The identified gene expression signature will be further investigated clinically and may serve as a predictive biomarker for identification of a suitable patient sub-population.

No conflict of interest.

409 Poster (Board P088)

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Background: The fibroblast growth factor receptor (FGFR) pathway plays a major role in regulating several basic biologic processes, from organogenesis to metabolism homeostasis, and angiogenesis. Aberrations, including gene amplifications, point mutations, and chromosomal translocations, in FGFR or FGFR genes result in an oncological event which has diagnostic value in personalized medicine. Here, we report development of a highly multiplex quantitative assay to detect copy number variation (CNV) in the human fibroblast receptor (receptor) oncogenes 1, 2, and 3, and FGF (ligands) 3, 4, and 19 (Chromosome 11q) from FFPE specimen on the fully automated Modaplex platform.

Methods: Primers were designed using proprietary technology to amplify FGFR 1, 2, 3, Chromosome 11q, and three reference genes from FFPE extracted DNA. Reaction conditions were optimized using proprietary PCR chemistry. Assay development was carried out using commercially extracted nucleic acid. The CNV was simulated using synthetic targets (gBlocks) spiked-in to wild-type genomic DNA. DNA extracted from FFPE specimen was tested.

Results: The Modaplex CNV panel can detect copy number variation in FGFR1, 2, 3 and, chromosome 11q. Assays designed to include two amplicons for each of FGFR1, 2, and 3 genes, and one amplicon each for FGFR3, 4, 19 (in total three amplicons for Chromosome 11q) in order to provide reliability in CNV calculation. Reference genes were characterized for their ability to serve as normalization genes to be used in CNV calculation. The total run time including data analysis and setup is less than 4 hours. Analytical sensitivity studies demonstrated the assay is capable of detecting and quantifying 2 fold increase in copy number variation in the genes of interest. Dynamic range and PCR efficiency for each assay target were measured and a quantification algorithm was developed to measure CNV. Finally, the assay was tested on known wild type FFPE DNA to establish cut-off for CNV calculation.

Conclusion: The Modaplex FGFR-CNV assay provides a simple and sensitive method of detection of CNV of the FGFR and FGF genes. The assay can be performed on a single platform with a fast turn-around time of 4 hrs. The Modaplex FGFR CNV Panel offers an excellent tool for use in biomarker discovery and screening of prognostic markers from biological specimens.

No conflict of interest.

410 Poster (Board P089)

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Background: The PI3K/AKT/mTOR pathway is frequently activated during prostate cancer (PCa) progression through loss or mutation of the tumour suppressor PTEN. It has been implicated in the development of castration-resistant prostate cancer (CRPC) and is a major driver of PCa growth, being a promising target for therapy. However, recent clinical trials with mTOR inhibitors showed disappointing responses in CRPC. The mechanisms of resistance to mTOR inhibitors in CRPC need further elucidation and it is still not known whether targeting alternative nodes of the PI3K/AKT/mTOR pathway may improve response. The current study aimed to evaluate the response of PTEN-negative and PTEN-positive PCa cells to multiple inhibitors of the PI3K/AKT/mTOR signalling cascade.

Material and Methods: Half maximal inhibitory concentration (IC50) for different PI3K/AKT/mTOR inhibitors was determined by WST-1 viability assay, in PTEN-negative (LNCaP, PC3, PC346C and PC346DCC) versus PTEN-positive (DU145, VCAP, 22Rv1 and MDA PCa 2b) prostate cancer cell lines. The impact of the inhibitors on phosphorylation of PI3K downstream targets (AKT, PRAS40, GSK3, S6K1 and 4EBP1) was analyzed by phospho-specific western blotting.

Results: PTEN-negative PCa cells line showed strong response to PI3K(ΔN), AKT and mTOR inhibition, whereas PTEN-positive cells were resistant to PI3K(ΔN) and showed variable sensitivity to mTOR inhibitors. MTORC1 inhibitor showed low IC50 but reached plateau at about 70-80% growth inhibition (in vitro). In contrast, mTORC2 inhibitor could reach complete growth inhibition at ~1μM concentration, in sensitive cell lines. Perturbation of the PI3K/AKT/mTOR pathway using these inhibitors resulted in differential phosphorylation of downstream targets, which did not seem to correlate with in vitro growth inhibition.

Conclusions: Despite disappointing results with mTORC1 inhibitors in clinical trials for CRPC, the PI3K/AKT/mTOR pathway remains an interesting target for clinical intervention. The current study validates PI3K(ΔN) and AKT as alternative targets in PTEN-negative PCa, whereas PTEN-positive models showed preferential yet variable response towards mTOR inhibitors.

No conflict of interest.

411 Poster (Board P090)

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Background: Metformin, a biguanide derivative that is widely used for treating type 2 diabetes, has recently been shown to exert potential anti-cancer effects. Increased numbers of epidemiological and clinical studies have provided convincing evidence supporting the role of metformin in the development and progression of a variety of human tumors including...
breast, pancreatic, and colon cancer. Substantial pre-clinical evidence from in numerous laboratory studies strongly suggests that metformin has the ability of anti-cancer activity through the regulation of several cell signaling cascades including activation of AMP kinase (AMPK), liver kinase B1 (LKB1), and other direct and indirect mechanisms; however, the detailed mechanism has not yet been fully understood.

In vitro studies using immortalized cervical cancer cells suggest the idea that metformin has anti-cancer activity, while recently published clinical trials showed significant beneficial effects on some cancer incidence or mortality. Patient-derived xenograft (PDX) models in which patient tumors are directly engrafted into immunocompromised mice and patient-derived primary cells (PDCs) have been shown to be excellent preclinical models for biomarker discovery and therapeutic development.

**Material and Methods:** The immunohistochemistry was conducted in tissues of 12 cervical cancer patients and two PDX models. The proliferation, apoptosis, colony formation, invasion, and western blot were conducted in PDCs. All other chemicals and antibodies were commercially obtained.

**Results:** We evaluated the response of 12 PDCs to metformin treatment and found that 10 of our PDCs were resistant to metformin. We found that the mechanisms of resistance may occur through lack of sustained activation of AMPK by low LKB1 expression or amplify, high expression and activity of the phospholipase D1 (PLD1). PLD1 status may be used as a biomarker of metformin action in the cervical cancer. Dysregulation of PLD1 has been reported to be involved in tumorigenesis, but the role of PLD1 in cervical cancer is unclear. The combination of metformin with pharmacologic targeting of PLD1 induced strong antiproliferative, apoptotic, and anti-invasive effects in various PDCs dependently of the LKB1 status. Additionally, we evaluated the response in two PDX which were resistant to metformin. The combination of two drugs remarkably suppressed cervical tumorigenesis in all two cases of our PDX models compared to metformin. The combination of two drugs remarkably suppressed and anti-invasive effect in various PDCs dependently of the LKB1 status.

**Conclusions:** PLD1 has a previously unrecognized role in modulating metformin-mediated anti-cancer program and emerges as a potential therapeutic target in cervical cancer in concert with metformin.

No conflict of interest.

**412** Poster (Board P091) MONDTI: Molecular characterisation platform for identifying actionable mutations in advanced or metastatic cancers

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**Background:** In the era of molecular oncology, high-throughput genomic profiling of tumor specimens facilitates the identification of individual actionable mutations that could be used for customized targeted therapy.

**Material and Methods:** MONDTI is a precision medicine platform for molecular characterization of advanced or metastatic tumors and for identifying actionable mutations. Since 2013 to May 2016 paraffin embedded tumor samples of 249 patients were analysed using Ion AmpliSeq Cancer Hotspot Panel v2 for 50 different mutations. Furthermore, immunohistochemistry of 14 proteins and FISH of 5 targetable genomic aberrations were performed. After summarization by an experienced molecular pathologist the results were discussed in a multidisciplinary team.

**Results:** The most frequent tumors were colo-rectal (n=30, 12.24%), lymphomas (n=27, 11%), head and neck (n=19, 7.76%), pancreatic (n=18, 7.35%) and cholangiocellular (n=18, 7.35%). The mutational profile was obtained for 244 (98%) patients. Immunohistochemistry and FISH status were successfully performed on 218 (87.6%) and 229 (92%) of 249 patients, respectively. Of the 244 patients with molecular profiling results, 175 (71.72%) had one or more mutations detected. The most common mutations were TP53 (n=91, 37.3%), EGFR (n=69, 41.5%), PTEN (n=17, 7%), CDKN2A (n=10, 4.1%) and CTNNB1 (n=10, 4.1%). For immunohistochemistry the most common found aberrations were loss of PTEN (n=94, 43.1%), m-TOR upregulation (n=91, 41.5%), elevated expression levels of MET (n=82, 37.6%), EGFR (n=68, 31.2%) and PDGFR-alpha (n=42, 19.2%). Of the 249 patients in 149 (59.8%) cases respective druggable treatment according to their individual profile was suggested.

**Conclusions:** We validated the clinical feasibility of matching patients to individual therapy approaches based upon the results of a comprehensive molecular characterisation of their tumors.

No conflict of interest.

**413** Poster (Board P092) Identification of predictive response and resistance factors to targeted therapy in gastric cancer using a systems medicine approach

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**Background:** Progress in treatment of gastric cancer (GC) has been limited due to molecular and clinical heterogeneity. Novel drugs targeting the receptor tyrosine kinases (RTKs) HER2 and EGFR have shown mixed success in clinical trials. While the HER2 antibody trastuzumab has been approved for GC treatment, the EGFR antibodies cetuximab and panitumumab failed to improve patient outcomes. The SYS-Stomach consortium, supported by the Federal Ministry of Education and Research (BMBF), aims to investigate differences in the mode-of-action of both treatments and to unravel primary and secondary resistance mechanisms.

**Material and Methods:** We apply systematic molecular multi-omics and cell phenotypic measurements to GC cell lines. From these we derive mechanistic and statistical models of the signalling networks coupled to cellular phenotypes and agent-based cellular behaviour models. The models will be validated against cell culture and clinical sample derived molecular and morphological tumour characteristics based on MALDI imaging mass spectrometry, a powerful tool to investigate the distribution of molecules in tumour sample sections. Validated models will be used to predict potential response and resistance factors of EGFR- and HER2-directed treatment. These response predictors will be validated in tumour samples from GC patient cohorts treated with cetuximab or trastuzumab.

**Results:** We established a link between motility-focused phenotypic properties of GC cell lines with molecular characteristics in response to cetuximab and developed a GC specific semantic network connecting EGFR signalling to the regulation of cellular motility. This semantic model has been used to inform the development of a mechanistic mathematical model for the EGFR pathway which describes the measured kinetic and dose response data obtained for cetuximab responder and non-responder cell lines. This mechanistic model is linked to the phenotypic measurements motility and invasiveness using a regression model. Applying this model for gastric tumour growth in 3D was developed. It adapts the motility of each tumour cell according to its micro-environment, through employing the above-mentioned mechanistic model for all agents (cells).

The clinical observational VARIANZ study which aims to assess resistance mechanisms in HER2+ tumour samples from patients receiving trastuzumab, has been established; HER2 testing in a central pathology institute has been put in place. Recruitment is ongoing and we aim to sample study data in a quality, completeness and consistency beyond the state of the art. An analytical approach for the in-situ imaging of metabolites from human tissue samples was developed.

**Conclusions:** The SYS-Stomach consortium aims at identifying predictive factors to RTK-directed targeted therapy in GC using a systems medicine approach to improve the outcome of patients.

No conflict of interest.

**414** Poster (Board P093) Acquired resistance mechanisms to INC280, a MET inhibitor in MET-amplified lung cancer cells

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**Background:** MET is the receptor tyrosine kinase (RTK) which of the pathway is important to tissue damage repair and regeneration. MET
Acquired resistance mechanisms to INC280, a MET TKI in MET-amplified NSCLC cells.

**Materials and Methods:** EBC-1, a MET-amplified NSCLC cell line was gradually exposed to INC280 at the dose of 10nM to 25iM and INC280-resistant cells (EBC-R1, R2, and R3) were established by maintaining INC280 at 200nM. Human Phospho RTK array, Western blotting, and co-immunoprecipitation (Co-IP) were performed to evaluate phosphorylation and heterodimer of RTKs. EBC-1 and EBC-R1-3 cells were cultured in 96-well plates in the presence of drugs (INC280, afatinib, and BYL719). Cell proliferation was analyzed using EZ-cytox. Sanger sequencing, QuantSeq 3’ mRNA sequencing, and qPCR were performed to evaluate genetic alterations and gene expression including MET, EGFR, PI3KCA, and FGFR1 genes.

**Results:** Parental EBC-1 cells were toxic to INC280 (IC50, 3.70±0.10 nM) that down-regulated phosphorylated MET and EGFR. However, EBC-R1, R2, and R3 cells showed resistance to INC280 (IC50 >10nM) and these were dependent on EGFR signals rather than MET signals. EGFR-related genes were up-regulated in EBC-R1 cells that were completely dependent on EGFR signals. Therefore, EBC-R1 cells were sensitive to afatinib alone (IC50, 114.58±2.75 nM). EBC-R2 cells showed co-dependency on MET and EGFR signals which tended to form MET and EGFR heterodimer. INC280 plus afatinib synergistically inhibited the growth of EBC-R2 cells (IC50 of INC280, 3.16±2.38 nM). EBC-R3 was identified with an exposed higher dose by INC280 to EBC-R1 had the copy number gain of PI3KCA and increased FGFR1 expression, resulting in bypass signal of EGFR. Therefore, combination of afatinib plus BYL719 was active against EBC-R3 cells (IC50 of BYL719, 140.84±19.65 nM). QuantSeq 3’ mRNA sequencing revealed that mRNA expression of MAPK, PI3K, and EGFR pathway genes were up-regulated in EBC-R1-3 cells.

**Conclusions:** Acquired resistance mechanisms to INC280, a MET TKI in MET-amplified NSCLC cells were mediated by EGFR activation without METEGFR heterodimerization or PI3KCA copy number gain. EGFR inhibition or combined EGFR and MET blockade might overcome resistance to MET inhibitor in MET-amplified NSCLC.

**No conflict of interest.**

**415 Poster (Board P094)**

**Comparative biomarker profiles of pacritinib, momelotinib, pexidartinib, and ruxolitinib using BioMap Diversity PLUS panel**

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CTI BioPharma, Clinical Pharmacology & Translational Sciences, Seattle, WA, USA, 1DiscoverX Corp., Biology Research, Fremont CA, USA

**Background:** Pacritinib (PAC) is a kinase inhibitor with specificity for JAK2, FLT3, IRAK1, and CSF1R being investigated for treatment of myelofibrosis. Potential dose-dependent effects of PAC, two JAK inhibitors (mometolitin [MOM], ruxolitinib [RUX]) and a CSF1R inhibitor, pexidartinib, (PEX) on relevant biomarker profiles were evaluated at clinically relevant concentrations using a broad panel of in vitro human primary cell-based systems modeling vasculature, skin, lung, and inflammatory tissue and disease biology.

**Materials and Methods:** Phenotypic signature biomarker profiles of 4 concentrations of the 4 agents were generated using the BioMap® Diversity PLUS panel and key biomarker changes assessed. Using the BioMap® reference database (>4,000 agents), biomarker signatures most similar to those of the 4 agents were mathematically computed. Differentiating and common biomarkers were identified by overlaying the profiles for MOM, RUX, or PEX with PAC at selected concentrations.

**Results:** Biomarker activities observed for all 4 agents are shown in the Table. PAC exhibited decreased inflammation-related readouts (Eotaxin, sIgG, P-selectin, IL-2, TIMP-2, uPA, PAI-1, MMP-9), and hemostasis-related activities (decreased TF). At clinically relevant concentrations, the profile of PAC was similar to Axl and PI3K activities (decreased TF). At clinically relevant concentrations, the profile of PAC was similar to Axl and PI3K activities (decreased TF).

**Conclusion:** PAC exhibited decreased inflammation-related readouts (Eotaxin, sIgG, P-selectin, IL-2, TIMP-2, uPA, PAI-1, MMP-9), and hemostasis-related activities (decreased TF).

**416 Poster (Board P095)**

**The alkylphosphocholine erufosine induces ER and mitochondrial stress in head and neck cancer cells**

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Endoplasmic reticulum (ER) plays an essential role in cell function and survival. Accumulation of unfolded or misfolded proteins in the lumen of the ER activates the unfolded protein response (UPR), resulting in ER-stress. Prolonged ER stress or failure of UPR fails to rebuild homeostasis, results in apoptosis induction. Cell death induced by ER-stress predominantly occurs via the intrinsic mitochondrial apoptotic pathway mainly mediated through CHOP, which downregulates Bcl-2. A number of pharmacological agents that directly or indirectly induce ER stress have been shown to trigger apoptotic cell death. Erufosine is a novel chemotherapeutic agent belonging to the third generation of alkylphosphocholines. It simultaneously induces apoptosis and autophagy in oral squamous cell carcinoma. In this study, we investigated the effect of erufosine to cause ER- and mitochondrial stress and determined apoptosis induction in the two OSCC cell lines HNS and SCC61.

Initially we established the anti-proliferative effect of erufosine on HNS and SCC61 cells and the IC50 by MTT assay. To decide if erufosine induces ER stress, we carried out Western blot analysis for the ER Stress sensor proteins PERK, pPERK and CHOP. We carried out staining with acridine orange to assess ER stressed induced autophagy. In order to check for apoptosis induction, we carried out annexin V staining in the two cell lines. To assess the stress on mitochondria post treatment, immunofluorescence staining with mitotracker and DAPI was performed. The ATP levels after treatment with erufosine were determined and also the inhibitory activities in other Diversity PLUS systems such as 4H, BE3C and CASM3C. These results illustrate the divergent biological effects of ATP binding site agents in different classes specifically developed to target JAK2 and suggest clinical profiles are also likely to be divergent.
ROS levels were measured to assess the mitochondrial function. Western blot was performed to check for the protein levels of caspases, Bcl2 and cleaved PARP. The IC50 of erufosine ranged from 43μM to 37μM in HHN-5 and from 19μM to 7μM in SCC-61 cells following exposure for 24, 48 and 72h. We observed induction of the pERK and CHOP levels in both cell lines at protein levels after treatment. Increased levels of acidic vacuoles were seen indicating that erufosine causes induction of autophagy in the cell line after treatment with erufosine. Erufosine induced apoptosis in both cell lines as seen in the annexin V staining. The mitochondrial stress induction was confirmed by an increase in the ROS levels, changes in mitochondrial morphology by mitotracker dye and decrease in the ATP production after treatment. The Western blot analysis showed an increased cleavage of caspases and PARP and decreased Bcl-2 levels after treatment in both cell lines. In conclusion, we show that erufosine induces both ER and mitochondrial stress and causes apoptosis in OSCC cell lines. This data shows that ER and mitochondrial targeting by erufosine may represent a new facet of erufosine’s mechanism of action as well as a promising new framework in the treatment of Head and Neck Cancer.

No conflict of interest.

419 Poster (Board P098)

Human CYP1B1 activates uPA–uPAR–integrin pathway through suppressing p53 signaling in human breast cancer cell progression and metastasis

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Human CYP1B1 is known a major estrogen 4-hydroxylase and is significantly highly expressed in many cancer tissues including breast cancer but not in normal tissues. To explore the role of CYP1B1 on human breast cancer cell progression and metastasis, we studied the effects of CYP1B1 inhibition and activation in MCF-10A, MCF-7, and MDA-MB-231 cells. CYP1B1 significantly induced expression of urokinase-type plasminogen activator receptor (uPAR) as well as uPA. Treatment with DMBA, a well-known CYP1B1 inducer also enhanced uPAR expression. However, CYP1B1 siRNA and TMS (tetrathiomolybdate), a specific CYP1B1 inhibitor were able to block uPAR mRNA induction by CYP1B1. CYP1B1 overexpression or DMBA also activated P-AIF-1 expression which was suppressed by TMS. We also found that CYP1B1 induction caused an increased integrin α1 and α5 expression. uPAR promoter activity was also strongly decreased in CYP1B1 siRNA-treated cells. Interestingly, CYP1B1 siRNA did not cause a significant decrease of integrin mRNA levels although integrin α5 protein level was strongly suppressed, indicating protein degradation may play an important role in regulating integrin protein level. Surprisingly, CYP1B1 down-regulated p53 expression through MDM2 activation which may cause uPAR induction. Taken together, our data suggest that CYP1B1 promotes cancer cell progression and metastasis via activating uPAR pathway which is one of the target of p53 signaling.

No conflict of interest.

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Table: NCI-MATCH projected match rates (N = 5000 screened)

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<th>Arm/Target</th>
<th>Expected match rate (%)</th>
<th>Expected enrollment</th>
<th>Arm/Target</th>
<th>Expected match rate (%)</th>
<th>Expected enrollment</th>
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<tr>
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Results: Between 8/15 and 11/15, 739 of 795 pts enrolled submitted biopsies (2/3 from community and 1/3 from academic sites). Sequencing was completed on 645 specimens (87%). Median turnaround time for sequencing was 27 days but increased from 14 days to 36 days as the enrollment pace increased. The highest toxicity (Grade 3) possibly related to biopsy was <1%. The common tumor types sequenced (35%) were colorectal (13%), breast (13%), non-small cell lung (7%) and prostate (<3%). “Rare tumors” (65%) included endometrial (7%), pancreatic (5%), head/neck (5%) esophageal/gastric (4%), ovarian (11%), and others. At the time of interim analysis 10 arms were open. 56 pts (9%); predicted actionability was 10% had actionable mutations of interest (aMOIs); of these, 33 pts (51.5%, 95%CI 3.5, 7.1%) met eligibility for a treatment arm.

Conclusions: This is a high accrual trial led by ECOG-ACRIN and NCI, utilizing both the National Clinical Trials Network and the National Community Oncology Research Program. If the prevalence results in the first 545 pts are maintained, the expected match rate among the 24 arms is 23%. Additional treatment arms are planned.

No conflict of interest.

Poster (Board P099)

A 201 study of MM-141, a novel tetravalent monoclonal antibody targeting IGF-1R and ErbB3, in relapsed or refractory solid tumors

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Background: The insulin-like growth factor (IGF) pathway has been implicated in the development and progression of cancer based on its crucial role in tumor cell proliferation and survival. Multiple studies have evaluated anti-IGF-1R antibodies as a therapeutic agent and almost all reported sub-optimal results in non-stratified patient populations. Limited clinical efficacy may also be due to the fact that targets can compensate for IGF-1R blockade; therefore, co-inhibition of IGF-1R and ErbB3 may improve clinical response. MM-141 is a novel bispecific antibody that binds IGF-1R and ErbB3 and blocks ligand dependent and independent IGF-1R/ErbB3/PI3K/AKT/mTOR signaling. Here we report the results from a multi-arm, Phase 1, dose-escalation study using MM-141 alone, or in combination with everolimus or nab-paclitaxel and gemcitabine for patients with relapsed/refractory solid tumors.

Methods: This was a Phase 1 dose-escalation study evaluating safety, tolerability, and pharmacokinetic (PK) properties of MM-141 monoclonal antibody (Arm A) or with nab-paclitaxel/gemcitabine (Arm C) for patients with advanced solid tumors, or in combination with everolimus (Arm B) for patients with ER/PR+ breast cancer. Pre-treatment and post-treatment biopsies were obtained from a subset of patients to assess pharmacodynamic and exploratory markers. Time on study was calculated using the date from enrollment until the date of study termination.

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Results: 42 patients with advanced solid tumors were treated (18 in Arm A, 13 in Arm B, and 11 in Arm C). The most common adverse events in >20% of patients were nausea, vomiting, decreased appetite and headache. While decreased neutrophil count and an infusion-related reaction were the only limiting toxicities in Arm B, no dose-limiting toxicities were observed in Arms A or C. Only 1 patient (2.3%) had >Grade 3 hyperglycemia. Decreased levels of IGFB1 and ErbB3 were identified on post-treatment tumor biopsy samples suggesting target engagement and receptor internalization after MM-141 exposure. PK analysis further supports either weekly or bi-weekly MM-141 dosing. The mean time on study was 12.6 weeks (range 1.1 to 81.3 weeks) for the entire cohort. A retrospective analysis identified that patients with detectable serum levels of free IGF-1 prior to the start of therapy remained on study 1.8 times longer than those with undetectable levels (9 vs 15.7 weeks, respectively).

Conclusions: MM-141 was well tolerated when used alone or in combination with chemotherapy in patients with relapsed/refractory solid tumors. Clinical results suggest that stratification based on pre-treatment serum levels of free IGF-1 may be associated with longer anti-tumor activity. This hypothesis is being tested in a randomized Phase 2 study comparing MM-141 with nab-paclitaxel/gemcitabine in patients with elevated serum levels of free IGF-1.


421 Poster (Board P100)

Molecular mechanisms of resistance to first- and second-generation ALK inhibitors in ALK-rearranged lung cancer

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Advanced, anaplastic lymphoma kinase (ALK)-positive lung cancer is currently treated with the first-generation ALK inhibitor crizotinib followed by more potent, second-generation ALK inhibitors (e.g., ceritinib, alectinib) upon progression. Second-generation inhibitors are generally effective even in the absence of crizotinib-resistant ALK mutations, likely reflecting incomplete inhibition of ALK by crizotinib in many cases. Herein, we analyzed 100 repeat biopsies from ALK-positive patients progressing on current cancer therapy. We will need two or three drug combinations to overcome resistance to ALK inhibitors; however, as recently reported (PMID 26884591, 26546295), clinical trials demonstrate robust anti-tumor activity of targeted Trk kinase fusions driven cell lines was evaluated with cell-based dose-response viability and apoptosis assays. Trk auto-phosphorylation and downstream signaling pathway activation with or without inhibitor treatments was assessed using immunoblotting.

Results: Altiratinib is a potent Trk inhibitor and suppresses the proliferation of TPML3-TRK1 and ETV6-TRK3 driven Ba/F3 cells with cell based IC50 of 11.9 and 7.8nM respectively. Notably, altiratinib inhibits growth of NTRK1 V573M (2.8nM), F589L (18.3nM), G667C (1.8nM) and G667S (93.5nM) at concentrations <100nM in cell based assays. While substitutions at the G959 (R/L) residue are resistant to altiratinib in the TrkA (NTRK1) kinase domain, the paralogous mutation in NTRK3 G623R, retains sensitivity to altiratinib (IC50 < 240nM). Immunoblotting demonstrates dose-dependent suppression of Trk autophosphorylation that correlated with cell viability experiments. Altiratinib robustly induces commitment to apoptosis in wildtype and mutant NTRK fusions driven cells where sensitivity is noted in cell growth assays.

Conclusions: These pre-clinical data demonstrate that altiratinib effectively inhibits the majority of resistant mutations within the Trk kinase domain in NTRK-fusion proteins. Given its unique properties to interact with and stabilize the inactive conformer of Trk kinase domains, altiratinib may have clinical utility both as a front-line agent or secondary agent to prolong the durability of clinical response to targeted kinase inhibitors in NTRK-rearranged cancer patients.

No conflict of interest.

422 Poster (Board P101)

The type II switch control kinase inhibitor, DCC-2701 (altiratinib) effectively inhibits resistant NTRK kinase domain mutants

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Background: Oncoproteins resulting from NTRK family gene rearrangements function as dominant drivers in multiple malignancies. Ongoing clinical trials demonstrate robust anti-tumor activity of targeted Trk kinase inhibitors; however, as recently reported (PMID 26884591, 26546295), emergence of clinical resistance due to acquisition of Trk kinase domain mutations were predominant therapeutic limitations and significant clinical challenges. Altiratinib is an optimized Type II switch control spectrum selective kinase inhibitor with high affinity for Trk kinase domains. Given its unique difference in inhibitor binding mode, we investigated if altiratinib retains affinity and inhibitory efficacy for mutant Trk kinase fusions and pre-clinically determined its ability to circumvent emergent drug resistance.

Material and Methods: We generated engineered Ba/F3 cell lines harboring native and mutant TPML3-TRK1 and ETV6-TRK3 cell lines. Mutations include the previously reported entrectinib and/or LOXO-101 resistant substitutions: NTRK1 V573M, F589L, G595R, G667C, G667S and NTRK3 G623R. Inhibitor sensitivity of wildtype and mutant Trk kinase fusion driven cell lines was evaluated with cell-based dose-response viability and apoptosis assays. Trk auto-phosphorylation and downstream signaling pathway activation with or without inhibitor treatments was assessed using immunoblotting.

Results: Altiratinib is a potent Trk inhibitor and suppresses the proliferation of TPML3-TRK1 and ETV6-TRK3 driven Ba/F3 cells with cell based IC50 of 11.9 and 7.8nM respectively. Notably, altiratinib inhibits growth of NTRK1 V573M (2.8nM), F589L (18.3nM), G667C (1.8nM) and G667S (93.5nM) at concentrations <100nM in cell based assays. While substitutions at the G959 (R/L) residue are resistant to altiratinib in the TrkA (NTRK1) kinase domain, the paralogous mutation in NTRK3 G623R, retains sensitivity to altiratinib (IC50 < 240nM). Immunoblotting demonstrates dose-dependent suppression of Trk autophosphorylation that correlated with cell viability experiments. Altiratinib robustly induces commitment to apoptosis in wildtype and mutant NTRK fusions driven cells where sensitivity is noted in cell growth assays.

Conclusions: These pre-clinical data demonstrate that altiratinib effectively inhibits the majority of resistant mutations within the Trk kinase domain in NTRK-fusion proteins. Given its unique properties to interact with and stabilize the inactive conformer of Trk kinase domains, altiratinib may have clinical utility both as a front-line agent or secondary agent to prolong the durability of clinical response to targeted kinase inhibitors in NTRK-rearranged cancer patients.

No conflict of interest.
there are more than 25 other different mechanisms of actions and over 100 pharmacy stakeholders/sponsors. The prevailing view is that we will need effective combinations of targeted drugs rather than single agents. Based on our analysis, 378 various two drug combinations were theoretically possible. Taken together, roughly 695,000 patient enrollments would be required to explore all possibilities in rare cancers. Clearly, we need better predictive markers and or new inclusive clinical trials models to enroll thousands of patients, acquire the millions of data points, and the billions of dollars required to make precision oncology a reality for a meaningful number of diseases.

No conflict of interest.

424 Poster (Board P103)
The dual Rac/Cdc42 inhibitor EHop-167 as a breast cancer therapeutic

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Background: The Rho GTPases Rac and Cdc42 regulate cell functions governing cancer malignancy such as cell polarity, migration, and cell cycle progression. Accordingly, our recently developed Rac inhibitor EHop-016 inhibits breast cancer cell migration and proliferation, and reduces tumor growth, metastasis and angiogenesis in mouse models. However, the efficacy (IC50, 1100 nM for Rac inhibition), pharmacokinetics (t1/2, 4.5 h) and bioavailability (>30%) of Ehop-016 needed improvement.

Methods and Methods: The potential of the Ehop-016 structural derivative EHop-167 to inhibit Rac and Cdc42 activation was investigated in metastatic breast cancer cells, using a G-LISA assay that identifies GTP bound active Rac or Cdc42. The ability of EHop-167 to inhibit downstream signaling was assessed by western blotting with total or phospho-antibodies. Immunofluorescence microscopy and Transwell assays were used to determine the effect of EHop-167 on the actin cytoskeleton. MTT assays, flow cytometry, and caspase assays were used to measure cell viability, cell cycle progression, and apoptosis in response to EHop-167. Experimental metastasis assays were conducted in nude mice with mammary fat pad tumors from human metastatic breast cancer cells to determine the in vivo effect EHop-167 on tumor growth and metastasis.

Results: EHop-167 inhibits Rac1 with an IC50 of 103 nM and Cdc42 with an IC50 of 78 nM in metastatic breast cancer cells. Consequently, EHop-167 significantly decreases the activities of Rac and Cdc42 downstream effectors p21-activated kinase (PAK) and signal transducer and activator of transcription (STAT3), and inhibits breast cancer cell migration and proliferation. Moreover, EHop-167 affects metastatic cancer cells by inducing a loss of cell polarity, and inhibiting cell surface actin-based extensions, to ultimately result in detachment from the substrate. EHop-167 decreases metastatic cancer cell viability with a G50 of ~130 nM, without affecting non-cancer mammary epithelial cells. EHop-167-mediated inhibition of cell viability is due to G2/M cycle arrest, and subsequent apoptosis, as shown by increased caspase 3/7 activity. In vivo, EHop-167 inhibits mammary tumor growth and metastasis in immunocompromised mice by >90%.

Conclusion: EHop-167 is 10X more potent than the parent compound EHop-016 and has potential as an anticancer drug, and as a novel probe for Rac and Cdc42 function.

425 Poster (Board P104)
Disruption of the linear ubiquitin chain assembly complex (LUBAC) with hydrocarbon stapled alpha helices

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Background: The linear ubiquitin chain assembly complex (LUBAC) is an E3 ubiquitin ligase complex responsible for forming linear polyubiquitin chains in vitro by linking ubiquitin units head-to-tail using the C-terminal Gly76 and the epsilon-amino group of Met1. One known substrate of LUBAC is the regulatory subunit NEMO (NF-κB essential modulator), part of the IKK complex involved in activation of NF-κB. Constitutive activation of NF-κB is a hallmark of the activated B cell-like (ABC) subtype of diffuse large B cell lymphoma (DLBCL). LUBAC itself is composed of three proteins - HOIL-1L, HOIP, and SHARPIN - which the interaction between HOIL-1L and HOIP has been shown to be critical for LUBAC assembly and function. We hypothesized that disruption of the HOIL-1L–HOIP interaction would prevent LUBAC ubiquitylation activity and result in down-regulated activation of NF-κB.

Study design and Methods: The key protein-protein interaction occurs between the ubiquitin-like (UBL) domain of HOIL-1L and the ubiquitin-associated (UBA) domain of HOIP. We designed a family of small inhibitor peptides that mimic the alpha-helical interface of HOIP, and employed a hydrocarbon stapling methodology to increase the rigidity and stability of this alpha-helical structure. A number of second generation families of HOIP peptides have also been synthesized and characterized for their alpha-helical content in solution. The synthesized peptides were characterized for their ability to disrupt the interaction between HOIL-1L and HOIP in vitro by first analyzing their secondary structure in solution by circular dichroism spectroscopy followed by binding studies using fluorescence polarization and microscale thermophoresis. Selected compounds were then taken into cell-based studies to determine their impact on the intracellular disruption of the LUBAC complex. Intracellular interactions were studied using co-immunoprecipitation, and the effects of the compounds on the NF-κB signaling cascade and ubiquitylation of substrates were analyzed by western blotting and cell viability assays.

Results: We have found that the first generation of HOIP peptides partially prevent LUBAC formation and decrease NF-κB activity in cells. The second generation of compounds in which the sequences were modified for optimal binding and cell permeability show improved efficacy and provide a springboard for the development of more effective analogues for use in animal studies.

Conclusions: These findings continue to validate inhibition of LUBAC via disruption of the HOIL-1L–HOIP interaction as a potential target to affect NF-κB signaling. Compounds which inhibit NF-κB activity have potential use in LUBAC-dependent NF-κB activation in the ABC subtype of DLBCL, the DLBCL subtype that is most resistant to current therapy.

No conflict of interest.

426 Poster (Board P105)
Phase Ib study of afatinib plus standard-dose cetuximab in patients with advanced solid tumours

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Background: Afatinib in combination with cetuximab has demonstrated activity in patients (pts) with EGFR mutation-positive NSCLC and acquired resistance to the EGFR tyrosine kinase inhibitors erlotinib and gefitinib (Janigian, Cancer Discov 2014). Targeting the ErbB pathway may also be of benefit in the treatment of other tumour types; for example, in squamous cell cancers, which have high levels of EGFR overexpression. This multicentre, open-label, Phase Ib study (NCT02020577) assessed afatinib plus standard-dose cetuximab in pts with advanced solid tumours including squamous NSCLC and squamous cell carcinoma of head and neck (HNSCC).

Material and Methods: In Part A of the study, a 3+3 dose-escalation design was used to determine the maximum tolerated dose (MTD) of afatinib (from 30 to 40 mg daily) plus standard-dose cetuximab (400 mg/m2 loading dose followed by 250 mg/m2 weekly). Treatment was administered in 21-day cycles until disease progression or unacceptable toxicity. MTD was defined as the highest dose at which fewer than 2 of 6 pts experienced a dose-limiting toxicity (DLT) during Cycle 1. In Part B, safety, tolerability and preliminary anti-tumour activity was assessed in 3 expansion cohorts (squamous NSCLC, HNSCC and other tumours) at the MTD determined in Part A.

Results: In Part A, 3 pts received afatinib 30 mg, and 6 received afatinib 40 mg. No DLTs were observed in either dose cohort; the MTD was defined as afatinib 40 mg once daily plus standard-dose cetuximab. In Part B, 49 pts have been treated at the MTD (12 with squamous NSCLC, 15 with HNSCC and 22 with other tumours); these pts were heavily pre-treated (median 3 lines of prior therapy). Among all 58 treated pts, the most common drug-related adverse events (AEs; all grades [G], n [%]) were classified as skin and cutaneous disorders (48 [83%]) and gastrointestinal disorders (45 [78%]). The most frequently reported drug-related AEs were diarrhoea (37 pts [64%]) and dermatitis acneiform (25 pts [43%]). 18 pts (31%) had drug-related G3 AEs, most commonly dermatitis acneiform and rash (each 3 pts). There were 2 drug-related G4 AEs (hyperlipasaemia and hypersensitivity, each 1 pt) and no drug-related G5 AEs. Efficacy results showed that 27 pts (55%) in the expansion cohort (Part B) had stable disease (SD); 9/12 pts (75%) with squamous NSCLC, 10/15 (67%) with HNSCC and 8/22 (36%) with other tumours. No confirmed objective responses have been reported. Mean duration of disease control in the expansion cohort was 19 weeks.
Conclusions: MTD was defined as afatinib 40mg once daily plus cetuximab 250 mg/m² weekly (after 400 mg/m² loading dose). AEs were generally mild-to-moderate with no unexpected AEs. SD was observed in 55% of heavily pre-treated pts with squamous NSCLC, HNSCC, and other tumor types.

Conflict of interest: Other Substantive Relationships: Soria, Compensated consultancy for BIB; Ould-Kaci, BI Employee; Esler, Serves as a consultant to Boehringer Ingelheim and receives compensation for these services; Nazabadokio, BI Employee.

427 TGF-βi receptor I/II signaling at primary cilia membrane is regulated by ceramide to modulate cell migration

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Mechanisms that regulate TGF-βi receptor I/II (Tj)I/II trafficking to primary cilia membrane for mediating signal transduction remain unknown. Here, we show that ceramide synthase 4 (CerS4) generated ceramide, bioactive sphingolipid, stabilized Smad7-TjI/II association, which then inhibited the trafficking of TjI/II to primary cilia membrane. Expression of a mutant TjI/II, which is resistant to Smad7 binding/inhibition, restored receptor signaling to increase migration in response to CerS4/ceramide induction. Genetic or molecular alterations of CerS4 abundance prevented Smad7-TjI/II inhibitory complex, and increased association between Arl6 transporter and TjI/II via novel cilia targeting signal (31-ATALQ-35). Mutation of the cilia targeting signal abolished the trafficking of the receptor to the cilia membrane in response to CerS4 knockdown in various cell types. Localization of TjI/II in response to primary cilia activated sonic hedgehog (Shh) receptor smoothened (Smo), inducing migration/invasion and liver metastasis both in wild type and CerS4−/− knockout mice in response to endogenous CerS4/ceramide knockdown in 4T1 mammary cancer cells, injected in the mammary pads. Smad7 overexpression or primary cilia inhibition by shRNA-mediated knockdown of intraflagella transport protein 88 (IFT88) prevented TjI/II-Smo crosstalk and attenuated liver metastasis of mammary cancer cells stably transfected with shRNA against CerS4/ceramide. Overall, these data define a key mechanism for the regulation of TjI/II targeting selectively at the primary cilia membrane by CerS4/ceramide-Smad7 inhibitory complex to control Shh-mediated cell migration and invasion without affecting canonical TGF-βi signaling.

No conflict of interest.

428 p65BTK targeting restores the apoptotic response to chemotherapy of p53-null drug-resistant colon cancer cells

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We recently identified p65BTK, a novel oncogenic isoform of Bruton tyrosine kinase abundantly expressed in colon cancer cell lines and tissues (Grassilli et al, Oncogene 2016) and showed that its inhibition affects growth and survival of colon cancer cells. The purpose of this study was to investigate whether its expression: (1) varies in colon cancers at different stage and grade; (2) plays a role in the response to chemotherapy and targeted therapy.

immunohistochemistry and ELISA assay using anti-p65BTK specific polyclonal antibodies raised in the lab were employed to study p65BTK expression in colon cancer patient specimens. Silencing experiments in drug-resistant cells and overexpression experiments in drug-sensitive cells were performed to study the role of p65BTK in the response to therapy. Cell viability/cell death assays were performed to study the effects of p65BTK silencing or inhibition (by different specific inhibitors) on the response to therapy in vitro. Xenograft experiments were carried out to assess the effect of p65BTK inhibition on the response to chemotherapy in vivo. Caspase assay, protein arrays and transcriptional profiling were used to define the molecular mechanisms in cells re-sensitized to chemotherapy upon p65BTK inhibition.

We found that p65BTK expression significantly increases with the stage and the grade of colon carcino and correlates with cancer progression; in addition, p65BTK is strongly expressed in organoids and in cancer stem cells derived from colon cancer specimens. In vitro, p65BTK silencing and its inhibition by different specific inhibitors sensitize drug-resistant p53-null colon cells and patient-derived organoids to 5FU. At variance, blocking p65BTK does not restore the response of resistant cells to anti-EGFR receptor antibodies (panitumumab, cetuximab) and inhibitors (afatinib, poziotinib) or to bevacizumab. Conversely, p65BTK overexpression (but not overexpression of a kinase-dead mutant) prevents p53-wt colon cancer cells from 5FU-induced cytotoxicity. Accordingly, p65BTK inhibition sensitizes drug-resistant p53-null colon cancer cell to 5FU through induction of apoptosis. Finally, in xenograft experiments we confirmed that the combination of 5FU with a BTK inhibitor (ibrutinib) significantly reduced tumor volume in mice compared to the use of 5FU alone.

In conclusion, our data indicate that p65BTK targeting restores the apoptotic response to chemotherapy of p53-null drug-resistant colon cancer cells and suggest that the addition of BTK inhibitors to classic chemotherapy may represent a novel approach to bypass drug resistance.

No conflict of interest.
Pediatric low-grade gliomas with CRAF fusions respond differentially to targeted therapeutics based on their dimerization profiles

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Background: Pediatric low-grade gliomas (PLGGs) are the most commonly diagnosed brain tumors in children. PLGGs have been defined by activating BRAF gene mutations/fusions that dysregulate the mitogen-associated protein kinase (MAPK) pathway, leading to clinical testing of MAPK inhibitors for PLGGs. However, recent large-scale sequencing studies have also identified novel CRAF (or RAF1) fusion proteins, QKI-RAF1 and SRGAP3-RAF1, as potential PLGG driver mutations. As CRAF and BRAF are shared targets of MAPK therapeutics, we sought to investigate the mechanistic and/or differential response of CRAF fusions to clinically relevant RAF inhibitors and downstream pathway inhibitors. We focused on comparing the effects and dependency on RAF dimerization for successful targeting.

Materials and Methods: Heterologous cell model systems with stable expression of CRAF fusions were generated and used for testing downstream signaling pathways via immunoblotting. Soft agar assays and mouse flank xenografts were used to characterize oncogenic properties. We tested responsiveness to first- and second-generation RAF inhibitors, PLX4720 and PLX8394 respectively, novel RAF dimer inhibitors, MEK1, and mTORC1 as single agents or in combination. Myc- and Flag-tagged constructs of CRAF fusions were used in co-immunoprecipitation assays to assess dimerization profiles of CRAF fusions with or without inhibitors.

Results: We found that CRAF fusions respond differentially than BRAF-fusions and do not respond to RAF inhibitors, show partial response to single-agent MEK inhibitors, but robustly respond to combinatorial targeting of both MAPK and PI3K pathways and novel RAF dimer inhibitors. Upon comparing the homo- and hetero-dimerization profiles of QKI-RAF1 and BRAF fusions in the presence of RAF inhibitors, we found that QKI-RAF1 retains robust homo- and hetero-dimerization that, in contrast, are disrupted in BRAF fusions that respond to RAF inhibitors. This suggests that dimerization is essential for MAPK pathway activation and determines responsiveness to RAF inhibitors. Furthermore, we tested the novel RAF dimer inhibitor, LY3009120, and found that LY3009120 stabilized CRAF fusions in an inactive dimer conformation and suppressed oncogenic potential.

Conclusions: In summary, our work demonstrates that CRAF fusions are distinct from BRAF fusions in responsiveness to targeted therapies. Our study suggests that molecular classification of PLGGs should inform therapeutic intervention of RAF-altered PLGGs even within RAF-mutant subtypes.

No conflict of interest.

Detection of IDH1 mutations in circulating free DNA in patients with cholangiocarcinoma

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Background: To date there are still no effective therapies for patients with oncogenic KRAS, driving the impetus to develop novel targeted therapies against its effectors. The impact of oncogenic KRAS on the intracellular redox balance is still controversial. A number of papers reported that oncogenic RAS enhances intracellular ROS levels to support tumorigenicity, while recent major work by several groups described that RAS drives antioxidant programs necessary for oncogenesis. It is therefore critical to further explore the role of oncogenic KRAS on redox balance and its impact on tumorigenicity. To this end, we investigated the effect of oncogenic KRAS on the cellular response to oxidative stress and how this process contributes to KRAS-mediated tumorigenicity.

Materials and Methods: NIH3T3 fibroblasts stably transduced with KRASG12V or empty vector (MSCV) were treated with H2O2 and analyzed by whole transcriptome microarray. Cellular transformation was analyzed using soft agar colony formation assays. To assess the tumorigenic potential of the studied tumor cell lines in vivo, we generated xenografts in nude mice.

Results: Transcriptome analysis revealed that xCT had the highest positive fold change in KRASG12V-transfected cells in response to exogenous H2O2 as compared to control MSCV cells. In addition, gene expression analysis in colorectal and lung cancer patient samples show that xCT expression is upregulated in subgroups with KRAS mutations. xCT is responsible for the cellular uptake of cysteine, the rate-limiting precursor in the synthesis of glutathione. As such, we investigated the contribution of xCT in KRAS-mediated tumorigenesis using xCT−/− mouse embryonic fibroblasts (MEFs). We found that while KRASG12V overexpression in xCT+/+ MEFs allows growth in soft agar, KRASG12V was not able to support growth of xCT−/− MEFs in these conditions, indicating that xCT is required for KRASG12V transformation. Further, treatment of KRAS-transformed cells with Erastin, a small molecule inhibitor of xCT, also reduced colony formation. Finally, we observed that KRASG12V xCT−/− tumor xenografts exhibit a three-fold reduction in doubling time as compared to KRASG12V xCT+/+ tumors, pointing to the requirement of xCT for KRASG12V-mediated tumorigenicity in vivo.

Conclusion: Our work suggests that oncogenic KRAS signaling supports tumorigenicity through induction of the antioxidant function of xCT. We also highlight the potential utility of Erastin as a therapy for KRAS-driven tumors due to its inhibitory effect on xCT and intracellular redox balance.
patients with measurable disease (n = 14) but was not detected in 1 patient who was disease-free following surgical resection 2 years prior. Changes in the mutant AF from baseline correlated with radiographic response to chemotherapy.

Conclusions: Serial analysis of plasma-derived cfDNA for the detection of IDH1 mutations in patients with metastatic cholangiocarcinoma by ddPCR is feasible. Further evaluation as a potential predictive biomarker of response to chemotherapy and targeted therapy directed towards IDH1 mutations in cholangiocarcinoma is warranted. We gratefully acknowledge the support of the Integrated Genomics Operation and Bioinformatics Core (P30 CA008748), and the Marie-Josée and Henry R. Kravis Center for Molecular Oncology, MSKCC.

Conflict of interest: Advisory Board: Agios.

445 Poster (Board P114)
Landscape of RAF1 fusions in solid tumors and therapeutic utility of sorafenib
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Background: Fusions involving the RAF1 (cRAF) kinase have been reported in several solid tumors as likely driver mutations. We now present the landscape of RAF1 fusions identified through clinical grade comprehensive genomic profiling of 79,209 solid tumors. A clinical response to sorafenib in a patient with pancreatic acinar carcinoma harboring a RAF1 fusion demonstrates the potential clinical utility of the RAF inhibitor sorafenib.

Methods: Data from clinical grade comprehensive genomic profiling performed on 79,209 solid tumor samples using the FoundationOne assay was reviewed to identify cases harboring RAF1 fusions. A patient with refractory pancreatic acinar cancer was referred for treatment as part of a phase I trial of sorafenib combined with a glutamate antagonist, based on the presence of a KANK4-RAF1 fusion identified by FoundationOne assay. A cDNA encoding KANK4-RAF1 fusion was synthesized and used to express fusion gene in NIH3T3 cells; cell growth and survival was measured using colony formation.

Results: In-frame RAF1 fusion events were seen in 79,209 solid tumors. The most common tumor types included lung non-small cell (8 cases or which 7 were adenocarcinoma, 0.05% of all lung cancers), melanoma (9 cases, 0.5% of melanoma), skin (10 cases) pancreatic cancer (9 cases, of which 3 were pancreatic acinar carcinomas and 3 were neuroendocrine cancers), brain (8 cases), and prostate (4 cases). These fusions result from 8 unique breakpoints, almost all occurring in intron 7 of RAF1, and involved 44 unique fusion partners. An index patient with metastatic pancreatic acinar cancer refractory to standard therapy, that harbored a KANK4-RAF1 fusion, experienced dramatic clinical improvement with RECIST partial response and more than 40 per cent tumor shrinkage in target lesions when treated with sorafenib and the glutamate antagonist riluzole on an investigational trial. In vitro characterization of the KANK4-RAF1 fusion, including sensitivity to RAF kinase inhibitors will be presented.

Conclusions: In this large dataset, RAF1 fusions are present in multiple solid tumor types including lung adenocarcinomas, melanomas, pancreatic acinar carcinomas and prostate cancer. Cancers harboring RAF1 fusions may be exceptionally sensitive to treatment with RAF inhibitors. We propose further clinical investigation of RAF inhibitors such as sorafenib in pancreatic acinar and other cancers with RAF1 fusions.

Conflict of interest: Ownership: Siraj Ali, Alexa Schrock, Jeffrey Ross, Dean Pavlick, Philips Stephens and Vincent Miller are employees of Foundation Medicine.

446 Poster (Board P115)
Antitumor activity of the WEEl inhibitor AZD1775 as a monotherapy and in combination with the PARP inhibitor olaparib in patient-derived explant (PDX) models
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Background: Cancers are associated with G1/S checkpoint deficiencies, higher levels of endogenous DNA damage and replication stress. This can lead to a dependency on the WEEl kinase that during S phase deals with replication stress and provides a G2/M checkpoint to repair DNA damage before mitosis and cell division. AZD1775 is a highly selective small-molecule inhibitor of WEEl being developed to treat patients (pts) with advanced solid tumors. In an AZD1775 monotherapy study in 25 pts with refractory solid tumors (Do et al 2015), partial responses were seen in two pts with a BRCA1/2 mutation (BRCAm). We assessed AZD1775 in triple-negative breast cancer (TNBC) and ovarian cancer (OC) PDX models with and without a BRCAm.

Materials and Methods: TNBC models with varying sensitivities to platinum and olaparib (Lynparza™) were tested for response to different AZD1775 monotherapy doses and schedules. OC models were tested for response to olaparib and AZD1775 as monotherapy. Target engagement was assessed by PKPD analyses. The efficacy of AZD1775 in combination with olaparib was assessed in all models.

Results: Of three TNBC models, two had a BRCA2m: HBCx-10 was sensitive to olaparib but insensitive to AZD1775, HBCx-17 was relatively resistant to olaparib but very sensitive to AZD1775. The third model was resistant to olaparib and partially sensitive to AZD1775. In all models, AZD1775 plus olaparib led to enhanced effects on tumor regression and/or duration of response. All five OC models (whether BRCAm or BRCA wild type [wt]) were resistant to olaparib but responded to AZD1775; olaparib plus AZD1775 led to improved responses versus AZD1775 alone. Further genetic characterization of these models is ongoing.

Conclusions: AZD1775 showed a greater-than-expected level of single-agent antitumor activity, with 7/8 PDX models having significant sensitivity. Responses to AZD1775 were not seen in the remaining model (HBCx-10), which was the most sensitive to olaparib. In all models, AZD1775 plus olaparib led to improved responses versus the single agents alone. These data support the clinical assessment of AZD1775 as monotherapy, and combined with olaparib, in TNBC and OC in both BRCAm and BRCAwt pts.

Conflict of interest: Ownership: MJC, RO, AH, ZL, AJP, JY, SP, BD, JC, and PJ own stock in AstraZeneca. Corporate-sponsored Research: JE has received research funding from AstraZeneca, Janssen, Merck, Array and Veristem; UAM has received research funding from AstraZeneca and Tesaro; JFL is the site principal investigator of clinical trials sponsored by Genentech/Roche, AstraZeneca, Merck Pharmaceuticals and Atara Biotherapeutics. Other Substantive Relationships: MJC, RO, AH, ZL, AW, AJP, JY, SP, BD, JC, and PJ are employees of AstraZeneca; AJP holds patents/royalties/other intellectual property with AstraZeneca. JE is currently an employee of EMD Serono.

Poster Session – Molecular targeted agents II, Thursday 1 December 2016
A phase 1/1b study of RXDX-105, an oral RET and BRAF inhibitor, in patients with advanced solid tumors

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Background: RXDX-105 is a multikinase inhibitor that has demonstrated potent inhibition of RET and BRAF with biochemical IC50 values of 0.3−0.8 nM against RET rearrangements, 4 nM against RET M918T, and 54 nM against BRAF V600E. RET alterations can be oncogenic drivers in various types of cancer, including NSCLC and thyroid cancer. Similarly, acquired BRAF mutations can result in constitutive activation of the MAP kinase signaling pathway, which fuels cancer growth. Clinical inhibition of these targets has been associated with tumor regression.

Methods: Ph1/1b advanced solid tumors were enrolled in a Ph 1b basket study. RXDX-105-01. RXDX-105 was administered orally, once daily, in combination with PD-1 blockade (Powers, AACR 2016). During Phase 1 dose escalation in the first-in-human clinical trial (NCT02318329), a patient with urothelial cancer (UC) was treated with FPA144 at the 3 mg/kg dose level. This patient had a durable, complete response to FPA144. Here, we explore the potential utility of FPA144 in UC by assessing the frequency of FGFR2b overexpression in samples from UC patients.

Results: Normal bladder has weak staining of the transitional epithelium (<1+), while UC has stronger staining. The FPA144-responsive UC patient’s primary tumor sample showed FGFR2b is overexpressed in >10% of samples with expression intensity of at least 1+.

Conclusions: We have identified objective responses in gastric cancer patients with FGFR2b positive tumors and acquired mutations in FGFR2b with FPA144 overexpression to FPA144 and the positive IHC staining in additional samples suggest that UC may be an indication that is also sensitive to FPA144 treatment. Additional work is ongoing to understand the frequency of FGFR2b overexpression in primary and metastatic UC and the utility of combining FPA144 with PD-1/PD-L1 blockade in UC.

Conflict of interest: Ownership: FivePrime Therapeutics.
repeated cycles of chemo, demonstrating preservation of this essential cell type. Updated CBC data will be presented.

**Conclusions:** Persistent lymphopenia months after 1st-line SCLC chemo is a possible indicator of HSC damage and reduced immune system function. Preclinical studies show G1T28 administration with chemo decreases myeloid-biased differentiation and preserves immune system function. Clinical data suggest that lymphocyte numbers are preserved when G1T28 is given with chemo in SCLC patients. Preserving adaptive immunity with G1T28 may enhance the efficacy of chemo and immune checkpoint blockade, supporting clinical testing of novel combinations.

**Conflict of interest:** Ownership: Patrick Roberts, Jessica Sorrentino, John Blandy. Dr. Rajesh Malik are G1 Therapeutics employees and have equity ownership in the company, Norman Sharpless is a co-founder and has equity ownership in G1 Therapeutics. Advisory Board: Geoffrey Shapiro serves as G1 Therapeutics advisor.

### 440 Poster (Board P119)

**Improvement of pharmacokinetics and myeloid effector cell engagement in vivo by Fc-engineering of IgA antibody against the epidermal growth factor receptor**

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**Background:** Antibodies of IgA isotype play an important role in linking adaptive and innate immunity. Fc-redirected cytotoxicity (ADCC) has been identified in several cancer types, including thyroid, lung, and breast cancer. Furthermore, tyrosine kinase inhibitors (TKIs) of several TKIs have been identified in several cancer types, including thyroid, lung, and breast cancer. ADCC has been associated with increased anti-tumor efficacy, especially in myeloid cells. We have previously shown that Fc-engineering of IgA antibodies can significantly improve ADCC activity, making these antibodies attractive for cancer immunotherapy.

**Materials and Methods:** Recombinant IgA antibodies were engineered to improve the ADCC activity by Fc engineering. The resulting antibody was compared to wild type IgA2 regarding biochemical characteristics as well as Fab and Fc-mediated effector functions.

**Results:** The resulting antibody variant was shown to have improved ADCC activity, with a significant increase in the level of target cell killing compared to wild type IgA2. Additionally, the antibody variant showed improved stability and pharmacokinetic properties, making it a promising candidate for cancer immunotherapy.

**Conclusions:** We have demonstrated that Fc-engineering of IgA antibodies can significantly improve ADCC activity, making these antibodies attractive for cancer immunotherapy. Further studies are needed to fully understand the potential of these antibodies in clinical settings.

No conflict of interest.
and non-kinase targets. LOXO-195 is predicted to robustly inhibit both wild- 
type and resistant TRK fusions in patients at clinically tolerable doses, 
and therefore offers the potential to induce new responses in patients who 
have progressed due to acquired resistance from treatment with 1st 
generation TRK inhibitors. LOXO Oncology will be initiating clinical development of 
LOXO-195 in the coming months.

Conflict of interest: Ownership: Authors are employees of Loxo Oncology 
or Array BioPharma.

443 Poster (Board P122)
Preclinical evaluation of M-DGN549, a folate receptor alpha-targeting 
antibody-drug conjugate (ADC) with a DNA-alkylating payload 
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Background: Mirtentuximab soravtansine (IMGN853) is a folate receptor 
alpha (FRα)-targeting ADC that comprises a FRα-binding antibody conjugated to the potent tubulin-binding maytansinoid, DM4. In early 
clinical testing, IMGN853 demonstrated encouraging single-agent activity 
in platinum-resistant ovarian cancer patients and has now advanced into a phase III trial in this indication (FORWARD II). However, there remains 
an unmet need for ovarian cancer patients with FRα expression below the 
threshold for inclusion into FORWARD I, as well as those insensitive to 
IMGN853 or a maytansinoid (DM4) mechanism of action. Here we report 
on the preclinical findings with M-DGN549, an ADC that utilizes the same 
FRα-binding antibody as IMGN853 linked to a novel, DNA-alkylating IGN 
payload, DGN549, via a protease cleavable L-Asp-L-Ala linker.

Methods: The cytotoxicity of M-DGN549 was evaluated in multiple 
human cancer cell lines. FRα expression levels in these lines were 
quantitated using flow cytometry. Bystander killing activity was assayed 
by treating mixed cultures of FRα+ and FRα− cells. In vivo efficacy 
was assessed in immunocompromised mice bearing cell line or patient 
derived (PDX) xenograft models, where FRα expression was determined 
by IHC. Additionally, in vivo pharmacokinetic and tolerability studies 
were conducted in CD-1 mice.

Results: M-DGN549 demonstrated high antigen-specific cytotoxicity 
towards multiple cell lines, including those with low antigen levels that 
were insensitive to IMGN853. Of note, M-DGN549 was able to induce robust 
bystander killing with low FRα+ expressing cells. M-DGN549 was 
higher active at doses well below the maximally tolerated dose. Additionally, 
complete regressions were observed in multiple xenograft models – 
including models with low FRα expression and cell line-derived tumors 
that were insensitive to IMGN853 and/or DM4. M-DGN549 demonstrated a 
favorable PK and mass spectrometry analysis showed the peptide linker to 
be stable in circulation in mice.

Conclusion: M-DGN549 demonstrates potent in vitro and in vivo activity 
against multiple tumor types, including tumors with low FRα expression 
or insensitive to IMGN853 and/or DM4. This ADC has a favorable PK 
and tolerability profile and warrants further preclinical evaluation.

No conflict of interest.

444 Poster (Board P123)
A phase I dose escalation, safety and pharmacokinetic (PK) study 
of AZD5312 (IONIS-ARXs), a first-in-class Generation 2.5 antisense 
oligonucleotide targeting the androgen receptor (AR)
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Medical Oncology, Sarasota, USA; 4Sarah Cannon Research Institute, 
Medical Oncology, Nashville, USA; 5Sarah Cannon Research Institute 
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8AstraZeneca, Clinical Pharmacology, Macclesfield, United Kingdom, 
9AstraZeneca, Medical, Waltham, USA; 10AstraZeneca, 
Project Leadership, Waltham, USA; 11Sarah Cannon Research Institute/ University College, Medical Oncology, London, United Kingdom

Background: The androgen receptor (AR) remains a key target for the 
treatment of prostate cancer. Androgen receptor splice variants (ARV) and 
AR mutations contribute to treatment failure and resistance to AR targeted 
therapy. AZD5312 is a novel Generation 2.5 (constrained ethyl bicyclic 
nuclidic acid) antisense oligonucleotide designed to target full length, splice 
variant and mutated forms of AR. It suppresses AR expression and AR- 
regulated genes in prostate cancer cell lines and xenograft models. The 
objectives of this first-in-human study (NCT02144001) were to determine 
the safety, tolerability, PK and potential efficacy of AZD5312 in patients 
(pts) with metastatic castrate-resistant prostate cancer (mCRPC) who 
have previously failed standard of care treatments including chemotherapy, 
enzalutamide and abiraterone.

Methods: In an accelerated 3+3 dose escalation, pts were given AZD5312 
intravenously on days 1, 4, 8, 11, 15 and 22 of Cycle 1 and days 1, 8, 
15, and each subsequent 28 day cycle in each of 5 cohorts from 150− 
1150 mg. In mCRPC pts, pharmacodynamic response to AZD5312 was 
assessed at each cycle by PSA and circulating tumor cells (CTCs), and by 
immunohistochemistry (IHC) and gene expression in a limited number of 
tumor tissue biopsies.

Results: An MTD of 900 mg was established after treating 29 pts in 5 
cohorts. Pharmacokinetic analysis showed an apparent dose proportional 
increase in plasma concentration and a rapid distribution to tissues. 
Exposure was consistent with preclinical data for AZD5312. At 900 mg, 
pts received a median of 3 cycles (range 1−14). Dose limiting toxicity 
of increased liver function tests were seen at 900 mg (1/13 pts) and 1150 mg 
(27 pts). Pulmonary embolism events were seen at 900 mg (3/13 pts) and 1150 mg 
(1/7 pts). PSA50 responses accompanied by >50% reduction in 
CTCs were observed in 2/12 mCRPC pts in the 900 mg cohort. There 
was one PSA30 responder (1/7) in mCRPC pts in the 1150 mg cohort. 
In addition, there was 1 unconfirmed partial response (PR) with 
AZD5312 in one patient on study.

Conflict of interest: Ownership: Paul Elvin, Tanya Coleman, Humphrey 
Gardner, and Paul D. Lyne have employee and stock ownership with AstraZeneca.

445 Poster (Board P124)
Targeting Akt in oesophageal adenocarcinoma
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Oesophageal adenocarcinoma (OAC) is the predominant 
histological subtype of oesophageal cancer in North America and Europe 
and incidence rates have progressively increased by 600% in the past 3 
decades [1]. Despite neo-adjuvant chemotherapy and chemo-radiotherapy 
offering some benefit, the 5-year survival for all stages is poor at 13% and 
only 23−36% for patients with localized disease undergoing surgical 
resection [2, 3]. Consequently, there remains an unmet clinical requirement 
for the identification of effective targeted therapies to improve survival rates 
in OAC. PI3K/AKT is the oncogenic pathway most frequently affected by 
mutation in OAC [4] and is therefore an attractive therapeutic target in this 
disease. This preclinical study uses the novel allosteric Akt1/2 inhibitor, 
ALM301, to investigate the potential utility for Akt inhibition in OAC.

Materials and Methods: A comprehensive panel of 10 OAC cell lines 
was characterised for sensitivity to ALM301 using the CellTitre-Glo® viability 
assay. The effect of combining ALM301 with 5-fluorouracil (5FU), cisplatin 
(CDDP) or hypoxia was measured using MTT and clonogenic viability 
assays. Western blot analysis examined levels of phosphorylated Akt 
Serine 473 and Threonine 308, total Akt and cleaved PARP. Cell death 
was determined by propidium iodide/Annexin V flow cytometry.

Results: Pearson and Spearman statistical analysis showed no correlation 
between ALM301 IC50(72h) doses and densitometry analysis of total Akt 
or phosphorylated Akt. This indicates that phosphorylation of Akt does 
not confer sensitivity to ALM301 and is unlikely to be a biomarker for 
ALM301 sensitivity in these cell line models. Akt inhibition synergistically 
sensitised the OAC cell lines to SFU and CDDP (combination index <1.0). 
However, the chemo-sensitisation effect of ALM301 was context specific. 
Akt inhibition is reported to sensitize cancer cell lines with a p53 DNA 
binding domain (DBD) deficiency to hypoxia-induced apoptosis [5]. In 
agreement with this, ALM301 significantly sensitised mCRPC cell lines 
to hypoxia, resulting in increased apoptosis. This has major clinical 
greenance that 70% of OAC tumours are reported to harbour p53 mutations 
with 13% showing p53 copy number loss [4]. In addition, up to 80% of 
OAC tumours harbour hypoxic regions [6].
Conclusions: ALM301 sensitised p53 DBD mutant OAC cell lines to hypoxia-induced apoptosis. ALM301 also sensitised OAC cell lines to chemotherapy used in standard neo-adjuvant treatment of OAC. Together these preclinical findings support a potential clinical role for ALM301 in the treatment of OAC. However, given the context specific effect observed in this study, further investigations are required to determine the mechanisms underlying ALM301-induced sensitisation to 5-FU and CDDP which could inform a clinical strategy for patient stratification.

Conflict of interest: Ownership: Gerald Gavory is employed by Almac Discovery; Timothy Harrison is employed by Almac Discovery and Queen’s University Belfast; Richard Kennedy is employed as the Medical Director for Almac Diagnostics; Nuala McCabe is employed by Almac diagnostics.

446 Poster (Board P125)

Anti-tumor activity of a TBK1/IKBKE inhibitor in combination with a MEK inhibitor in KRAS mutant colorectal and non-small cell lung cancer models

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Background: One third of all human cancers have a mutated RAS allele and, regardless of indication, they are generally refractory to standard of care chemotherapy. The clinical activity of agents that target downstream of RAS, such as MEK inhibitors, have limited single agent activity and tumors become refractory to therapy by pathway reactivation. This emphasizes the need for combination therapy to treat RAS-driven cancers. TANK Binding Kinase 1 (TBK1) is required for the RAS-dependent transformation of murine embryonic fibroblasts and directly binds RALB, a key effector pathway downstream of RAS signaling. KRAS-dependent cancer cell lines are sensitive to TBK1 knockdown. I-Kappa-B Kinase Epsilon (IKBKE), a functionally similar homolog, can promote an autocrine cytokine loop that contributes to proliferation. Together, TBK1 and IKBKE, can promote RAS-driven tumorigenesis.

Methods: The activity of a novel and potent dual TBK1/IKBKE inhibitor was evaluated in clonogenic growth assays using KRAS mutant or KRAS WT cancer cell lines. The TBK1/IKBKE inhibitor, a MEK inhibitor, and the combination the two were evaluated in a patient-derived KRAS mutant colorectal cancer (CRC) xenograft model and a KRASLSL-G12D;p53flox/flox genetically engineered mouse model (GEMM) of non-small cell lung cancer (NSCLC). The dose of the MEK inhibitor was chosen to match reported clinical exposure levels of the compound. The primary endpoints included tumor burden, overall survival, and pharmacokinetics.

Results: In vitro, KRAS mutant cells were more sensitive to TBK1/IKBKE or MEK inhibition than KRAS WT cells. In KRAS mutant cells, inhibition of MEK resulted in TBK1 pathway activation as determined by Western blot for pTBK1. In the CRC patient-derived xenograft, the combination of the novel TBK1/IKBKE inhibitor and a MEK inhibitor resulted in significant tumor growth inhibition and an increase in overall survival compared to MEK inhibitor alone. In the NSCLC GEMM, the combination of TBK1/IKBKE inhibitor and MEK inhibitor resulted in significant tumor regression compared to MEK inhibitor alone.

Conclusion: These data suggest that the combination of a TBK1/IKBKE inhibitor with a MEK inhibitor may be beneficial in KRAS mutant CRCs and NSCLCs.

Conflict of interest: Ownership: All Gilead employees are stock holders. Corporate-sponsored Research: Kwok-Kin Wong, David Barbie, and Kimmie Ng are funded by Gilead Sciences for pre-clinical research. Ownership: Gerald Gavory is employed by Almac Discovery; Timothy Harrison is employed by Almac Discovery and Queen’s University Belfast; Richard Kennedy is employed as the Medical Director for Almac Diagnostics; Nuala McCabe is employed by Almac diagnostics.
sought. Sigma receptors (SR1 and SR2) are members of a class of unique receptors integrated in plasma, mitochondrial and endoplasmatic reticulum membranes of mammalian cells. They have received much attention in the drug-discovery field other than their role in several neurological disorders, also because of probable involvement in breast cancer cell proliferation and aggressiveness. Therefore, inhibitors or modulators are of great interest as novel therapeutic anti-cancer drugs. In the present work, 3-D primary cultures of GBM endowed with stemness features, were used to evaluate the antitumor activity of a novel sigma receptor modulator RC-106.

Materials and Methods: Primary cultures of hGBM have been isolated starting from surgical tumor samples and grown as monolayer or 3D-cell colonies and morphology monitored by open-source AnaSP and ReViSP software tools. The molecular analyses were performed by flow cytometry and qRT-PCR. Cell viability was measured using CellTiter-Glo® 3D Cell Viability and MTS Assays. The apoptosis and cell cycle were analyzed by flow cytometry.

Results: We established a number of cell lines with different growth properties and different stemness gene expression profile. In particular, we evaluated the expression levels of the target genes S1R and S2R, and of tumor propagating cells surface markers as EphA2, CD44 and CD133 by flow cytometric and qRT-PCR analysis. Notwithstanding the inter-tumor heterogeneity observed, the treatment with RC-106 significantly inhibited cell viability and caused a strong apoptosis induction in all cell cultures tested. Conversely, the exposure to different concentrations of temozolomide did not induce significant cytotxic effect. In particular, RC-106 (25mM) induced an impairment of in vitro clonogenic ability of GBM cultures tested. Conversely, the exposure to different concentrations of temozolomide did not induce any cytotoxic effect. Overexpression of S1R and S2R gene detected in patient’s tissues and in their derivative cell lines support the interest for these receptors as potential druggable targets also in GBM. Notably, RC-106 compound showed to be able to induce a strong cytotoxic effect in all the cell lines tested both actively proliferating and in low proliferation rate cells, and such effect persist until 42 days, the longest time tested.

Conclusions: The high level of expression of S1R and S2R gene detected in patient’s tissues and in their derivative cell lines support the interest for these receptors as potential druggable targets also in GBM. Notably, RC-106 compound showed to be able to induce a significant cytotoxic effect in all the cell lines tested both actively proliferating and in low proliferation rate in response to serum deprivation.

No conflict of interest.

449 Poster (Board P128)

Impact of circulating biomarkers in patients with metastatic colorectal cancer treated with first-line FOLFOX/afiblercept therapy.

Results of the GERCOR VELVET Phase II study

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Background: The combination of afiblercept to OPTIMOX (VELVET study) was evaluated in patients with previously untreated advanced colorectal cancer (Chibaudel B et al, J Clin Oncol 33, 2015 (suppl; abstr 3567)). A biomarker program was set-up to explore the expression of several biomarkers upon treatment cycles to identify the best monitoring biomarkers of the treatment strategy.

Methods: VELVET was a prospective, open-label, single-arm, phase II trial conducted on a Biocartis platform. Patients: Among 49 patients included in the VELVET study from May 2013 to May 2014, 44 (90%) patients were evaluable for circulating biomarkers expression. The proportion of patients with tumor response (CR or PR) was higher in patients with high baseline levels of sVEGFR2, sVEGFR, G-CSF, Prolactin and low baseline levels of VEGFA and MIF. Progression-free survival (PFS) was higher in patients with low baseline levels of sIL-6R (HR: 0.52, P = 0.045) and Osteopontin (HR: 0.53, P = 0.045) and in patients with high baseline levels of VEGF-C (HR: 0.45; P = 0.014) and sVEGF3 (HR: 0.50; P = 0.045). Overall survival was higher in patients with high sVEGF3 (HR: 0.36; P = 0.030) and IL-8 (HR: 0.32; P = 0.014) levels. In both responders and non-responders, sVEGF1 dramatically increased upon exposure to afiblercept and remained overexpressed for the all course of induction therapy. Induction therapy also comes with increased expression of VCAM (+42%), SDF-1 (+62%) and SP-D along with decreased expression of sVEGF-3, VEGF-C, Ang1, Ang2, PDGF and IL-8 Tumor expression of some of these markers will be presented at the meeting.

Conclusions: Exposure to afiblercept is associated with an increase of sVEGF1 at cycle 1. Eleven circulating biomarkers expression levels of on-target sVEGF3 predict favorable outcome in patients treated with afiblercept.

Conflict of interest: Advisory Board: Pr C. Tourniand (Sanofi); Pr C. Louvet (Sanofi, Roche, Celgene); Dr JB Bachet (Agen, Lilly, Celgene, Roche); Pr A. Thierry (Roche, Boeringher); Dr B. Chibaudel (Sanofi); Pr A. de Gramont (Sanofi). Other Substantive Relationships: Marielle Chiron is an employee of Sanofi.

450 Poster (Board P129)

Development of AVID100, a novel antibody–drug conjugate for the treatment of EGFR expressing solid tumors

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Background: AVID100 is a novel epidermal growth factor receptor (EGFR)-targeting antibody–drug conjugate (ADC). EGFR is an important oncogene overexpressed by many types of solid tumors, including lung, breast, head and neck, and others. Unlike currently marketed anti-EGFR therapies that depend on EGFR blockade for anti-cancer activity, AVID100 has an additional mechanism of action of direct cytotoxicity via conjugated payload. This is expected to result in significantly enhanced anti-cancer activity of AVID100 compared to currently marketed anti-EGFR agents. We conducted in vitro and in vivo studies to evaluate the anticancer activity of AVID100.

Methods: Effects of AVID100 were tested against EGFR+ cancer cell lines, including those resistant to marketed anti-EGFR agents. Activity of AVID100 against non-transformed EGFR+ keratinocytes was also evaluated. Pharmacology studies investigating the anti-cancer activity of AVID100 were performed in mice bearing human tumor xenograft models, including breast and head and neck cancers.

Results: AVID100 demonstrated potent and broad activity against multiple cell lines with IC50 values in the pM to nM range. The ADC was also active against cancer cells resistant to marketed to anti-EGFR therapies. In vivo, AVID100 demonstrated significant anti-cancer activity in multiple cancer models including complete remissions in breast and head and neck cancer xenografts. Importantly, AVID100 was demonstrated to be minimally toxic against normal EGFR+ keratinocytes. Skin toxicity is a class effect of anti-EGFR therapeutics and this result suggests AVID100 skin toxicity will be comparable to other agents in the class, despite significantly higher potency of AVID100 on tumors. Tolerability of AVID100 was subsequently confirmed in non-human primate studies.

Conclusion: AVID100 is a promising anti-cancer therapeutic for the treatment of EGFR expressing tumors, including tumor types resistant to currently marketed anti-EGFR agents. AVID100 is currently undergoing IND-enabling development with clinical trials planned for 2016.


451 Poster (Board P130)

Development of a novel chromogenic RNA in situ hybridization method for detection of somatic mutations

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Background: The identification of somatic mutations in tumors is becoming increasingly important for patient selection for targeted therapies. While high throughput sequencing technologies allow for comprehensive mutation-profiling, sequencing does not permit assessment of intratumoral heterogeneity or the association of genetic alterations with cellular morphology. In addition, DNA mutational status does not predict expression of the mutant allele which may provide information connecting genotype to phenotype. Therefore, a technology for mutation detection directly in the tumor context is desirable. We present a chromogenic in situ hybridization
(ISH) method for the detection of mutations directly in gene transcripts. Improving upon the double Z (ZZ) probe design strategy of RNAscope®, we have developed a next generation ISH technology to detect single nucleotide variations and small indels.

Materials: We selected 8 common mutations in the BRAF, KRAS, EGFR, APC, and PIK3CA genes (BRAF V600E; KRAS G12D & G12V, EGFR T790M & E746A750 del; APC 853G>T; PIK3CA H1047R & E545K) and designed double Z probes specific for each mutation and the corresponding wild-type (wt) sequence. We then performed chromogenic RNA ISH to test the probe specificity in FFPE sections prepared from known cell lines. We subsequently tested the assay’s ability to detect BRAF V600E mutations in blinded FFPE samples of human colorectal carcinoma (CRC) characterized by BRAF status by PCR.

Results: Mutation specific probes generated distinct chromogenic dot signals, representing individual mutant transcripts, in histologic sections from mutant but not wt cell lines. Probes for wt sequences at the same positions resulted in signals in wt and heterozygous mutant cell lines, but not in homozygous mutant cell lines. Blinded analysis of 10 CRC samples (5 mutant & 5 wt) correctly identified the BRAF V600E status consistent with PCR findings. Evaluation of the spatial distribution of mutant transcripts revealed similar signals across the tumors in 4 of 5 cases. One tumor showed a decrease in the signals for mutant transcripts in poorly differentiated areas of the tumor.

Conclusions: The described novel RNA ISH technology enables detection of somatic mutations as well as mapping of genetically distinct subpopulations within tumors. The technique also allows for direct visualization of the expression patterns of these subpopulations in tissue. This functionality has the potential to further our understanding of tumor heterogeneity and the evolution of resistance mutations. Furthermore, as the mutation status can be visualized chromogenically, this technology has the potential for incorporation into the current and future pathology workflow for high microscopic assessment of patients’ tumors for specific mutations that would permit selection for targeted therapies.

No conflict of interest.

453
Poster (Board P132)
Development of selective MELK kinase inhibitors for breast cancer treatment

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Maternal embryonic leucine zipper kinase (MELK) is an atypical member of the AMPK family of serine-threonine kinases that has been implicated in stem cell renewal, cell cycle progression, cytokinesis, mRNA splicing and apoptosis. Its activity is correlated with its phosphorylation level, cell cycle dependent, and maximal during mitosis although direct upstream regulators of MELK kinase activity are unknown. In recent years MELK has been identified as a novel oncogenic protein that is overexpressed in several types of solid cancers with low levels in normal tissues. MELK expression level correlates intensively with poor prognosis in colon, breast, ovary, lung, prostate cancer and glioblastoma. Recent findings underlying the oncogenic role of this kinase in the tripe negative breast cancer (TNBC) category of high-grade and invasive tumors. Due to the lack of estrogen and progesterone receptors TNBC remains difficult to treat with hormonal therapies. Additionally, therapies targeting HER2, such as Herceptin, are also inefficient in this type of tumors. Despite the fact that the exact MELK function is not known, selective targeting of this kinase may be an effective cancer treatment strategy. The knockdown of MELK decreases cell-cycle progression, proliferation and tumor growth. In this study we are reporting development of a series of selective MELK kinase inhibitors. Synthesized compounds exert excellent selectivity and potency in MELK inhibitions with the low nanomolar range. Therapeutic effect of the compounds was investigated in the panel of breast cancer cell lines with different genetic background as well as with different MELK kinase levels. We identified several cell lines in which MELKI induced cell death with nanomolar ED50 values. Potent MELK inhibitors exhibited significant tumor growth suppression in xenograft studies using breast cancer cell lines in mice. Taken altogether, the presented data supports our rationale of using MELK kinase inhibitors as a novel and interesting approach for the cancer therapy.

Conflict of interest: Ownership: Selvita S.A. Board of Directors: Selvita S.A.
**New Therapies with Pleiotropic Activity**

456 Poster (Board P135)

Small activating RNA to CEBPA as a novel therapeutic approach to treat patients with liver cancer

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**Background:** We have developed a small activating RNA, CEBPA-S1, that upregulates the transcription factor CCAAT/enhancer binding protein alpha (CEBPα) and formulated it in NVS340 SMARTICLES® (MTL-CEBPA) for liver delivery. CEBPA is a master regulator of normal liver function and also acting as a potential tumour suppressor. Its expression has been reported to be decreased in liver disease/liver tumours. Here we present data demonstrating the anti-tumour activity of CEBPA-S1 in liver tumour cell lines and the ability of MTL-CEBPA to improve normal liver function whilst inhibiting liver tumour growth.

**Methods:** Liver tumour cell lines (HepG2, Hep3B) were transfected with 20nm CEBPA-S1 using liposomal reagents. The amount of CEBPA mRNA and protein along with impact on tumour cell growth (WST-1 and SRB assays) was measured. The ability of MTL-CEBPA to improve normal liver function was examined in rats following i.p. injection of CCl4 for 8 weeks to induce liver cirrhosis. At week 8, rats (n=9) were treated with 20mg kg−1 of either PBS or a control oligonucleotide was also observed. Administration of MTL-CEBPA to CCl4 treated rats led to significantly decreased in liver disease/liver tumours. Here we present data demonstrating the anti-tumour activity of CEBPA-S1 in liver tumour cell lines and the ability of MTL-CEBPA to improve normal liver function whilst inhibiting liver tumour growth.

**Results:** Transfection of CEBPA-S1 into HepG2 or Hep3B cells after 72hr led to a significant increase in both CEBPA mRNA (1.7–2.5 fold by qPCR) and protein by western blot. A significant inhibition in cell growth compared to either PBS or a control oligonucleotide was also observed. Administration of MTL-CEBPA to CCl4 treated rats led to significantly increased levels of CEBPA mRNA in the liver post treatment accompanied by a significant increase in serum albumin (a direct target gene of CEBPA). Liver function was restored to near normal parameter as judged by the significant reduction in hydroxyproline, bilirubin and ammonia levels as well as prothrombin time. Markers of liver injury (ALT, AST) were also reduced to normal levels. DEN induced spontaneous tumour nodules were significantly decreased by 80% in MTL-CEBPA treated animals when compared to a control oligonucleotide SMARTICLES® formulation. MTL-CEBPA also significantly improved LFPs in the DEN tumour animals.

**Conclusion:** MTL-CEBPA represents a novel therapeutic approach to treating liver dysfunction. Its activity to improve liver function whilst reducing tumour burden makes it a unique and promising drug candidate. Phase 1 trials with MTL-CEBPA in liver cirrhosis patients have been completed.

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**Conflict of interest:** Ownership: David Blakey, Vikash Reebye, Jon Voutila, Robert Habib, Pal Saetrom, John Rossi, Nagy Habib are shareholders of Mina Therapeutics. Advisory Board: Hans Huber is a consultant for Mina Therapeutics. Board of Directors of MiNA Therapeutics. Corporate-sponsored Research: Kai-Wen Huang receives research sponsorship from MiNA Therapeutics.
mediated degradation

E.T.Oh1,2, H.J.Park 2,3.

NQO1 increases HIF-1α

S150 Poster abstracts Poster Session – New Therapies with Pleiotropic Activity, Thursday 1 December 2016

Background: Up-regulation of NAD(P)H:quinone oxidoreductase 1 (NQO1) has been known to correlate to poor prognosis in human cancers including breast, colon, cervix, lung, and pancreas. However, the molecular mechanisms underlying the pro-tumorigenic capacities of NQO1 have not been fully elucidated. Here, we report a previously undescribed function for NQO1 in stabilizing the hypoxia-inducible factor-1α (HIF-1α), a master transcription factor of oxygen homeostasis that has been implicated in the survival, proliferation, and malignant progression of several cancers.

Material and Methods: To investigate the correlation between NQO1 expression levels and clinicopathological features in colorectal cancer patients utilizing publicly available data sets. To validate specific up-regulation of HIF-1α in NQO1-expressing tumors, we analyzed HIF-1α protein levels in tumor tissues. We further assessed HIF-1α expression in different cell types with NQO1 gain-of as well as loss-of-function to investigate the role of NQO1-mediated HIF-1α signaling at the cellular level. The interaction between HIF-1α and NQO1 was determined by co-immunoprecipitation as well as NI-TA bead-based pulldown assays. To determine the functional contribution of NQO1 to HIF-1α expression and tumor growth in vivo, we evaluated the growth rate of NQO1-knockdown or overexpressing RKO xenografts in female BALB/c nude mice.

Results: We found that NQO1 prevents the polyubiquitination and proteasome-mediated degradation of hypoxia inducible factor-1α via directly binding to the oxygen-dependent degradation (ODD) domain of HIF-1α in the cytosol. Thus, the depletion of NQO1 resulted in increased interactions between HIF-1α and the regulatory proteins PHDs, which are involved in HIF-1α degradation. In addition, NQO1 knockdown in human colorectal and breast cancer cell lines suppresses HIF-1α signaling and tumor growth. Consistent with this pro-tumorigenic function for NQO1, high NQO1 expression levels correlate with increased HIF-1α expression and poor colorectal cancer patient survival.

Conclusion: These results collectively reveal a novel function of NQO1 in the oxygen-sensing mechanism that regulates HIF-1α stability in cancers.

No conflict of interest.

458 Preclinical evaluation of pitavastatin as a treatment for chemotherapy-resistant ovarian cancer

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Background: The emergence of drug-resistance in patients with ovarian cancer limits 5 year survival to 40% and new therapies are needed. Statins inhibit the production of mevalonate by HMG-CoA reductase (HMGCR), the rate-limiting step in the synthesis of cholesterol. HMGCR has been recognized as a “metabolic oncogene” which leads to the production of isoprenoids such as geranylgeraniol which are necessary for the membrane localization and activity of small G-protein oncogenes. We have shown that statins, especially lipophilic statins, potently induce apoptosis in ovarian cancer cells. However, hepatic metabolism of lipophilic statins leads to a short half-life in plasma. Many clinical trials evaluating statins in cancer have been poorly designed because they have failed to take this into account to ensure the continual inhibition of HMGCR. Pitavastatin is the statin most potent for cholesterol reduction in vitro and in vivo. Since platinum-based treatment was introduced over three decades ago, there has been considerable advances in understanding of cellular and molecular pathology of ovarian cancer. However, only modest improvements in overall survival have been achieved and new therapies are required. Due to the cost and long development time required for the development of new drugs, repositioning of existing medications offers a provocative alternative. Pitavastatin is the most commonly prescribed statin with a long half-life in plasma. We have found that pitavastatin is effective in drug-resistant disease and in support of this pitavastatin inhibited growth and in parallel assays was suppressed by the addition of geranylgeraniol or by organic solvent extracts from several foodstuffs suggesting that clinical trials of statins may have failed because of dietary sources of geranylgeraniol.

Materials and Methods: We conducted preclinical studies to assess pitavastatin as a potential treatment for ovarian cancer. The activity of pitavastatin was assessed using a panel of ovarian cancer cell lines by measuring cell growth, trypan blue exclusion, caspase activity, PARP cleavage and induction of xenograft growth.

Results and Discussion: The expression of HMGCR was higher in 8 ovarian cancer cell lines than normal epithelial cell lines. The expression of HMGCR was increased in cells expressing gain-of-function mutants of TP53 and repressed by siRNA directed to TP53. The growth of these cell lines was inhibited by pitavastatin in both monolayer (IC50 = 0.2–8 M) and 3-D spheroid cultures (IC50 = 0.6–4 M). The sensitivity of the cell lines to pitavastatin did not correlate with sensitivity to carboplatin. This suggested that pitavastatin may be effective in drug-resistant cancer and in support of this pitavastatin inhibited growth of ovarian cancer cell lines which had been derived from patients before and after the onset of clinical drug resistance. Pitavastatin induced apoptosis, evidenced by a sub G1 population, increased caspase activity, PARP cleavage and production of caspase-cleaved CK18. Strikingly, the activity of pitavastatin in these assays was suppressed by the addition of geranylgeraniol or by organic solvent extracts from several foodstuffs suggesting that clinical trials of statins may have failed because of dietary sources of geranylgeraniol. Ovcar-4 xenografts in nude mice regressed when treated with pitavastatin when animals were maintained on a controlled diet lacking geranylgeraniol but not in mice receiving dietary geranylgeraniol.

Conclusion: Our data explain why clinical trials with statins to date have mostly been unsuccessful and point to how these trials can be better designed to demonstrate the efficacy of pitavastatin in cancer.

No conflict of interest.

460 Phytoagent deoxylephalandin derivative DEDT inhibits triple negative breast cancer cell activity by modulating oxidative stress and paraposis-like cell death

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Breast cancer is most commonly diagnosed cancer diseases in women worldwide. The triple negative breast cancer (ER-PR-HER2- TNBC) is a highly metastatic breast cancer among the cancer subgroup and a thorny issue for clinical therapy owing to absent of efficient targeted therapeutic strategies. In the past decades, the medicinal plant-derived phyto compounds developed as chemotherapeutic or chemopreventive agent for treatment or prevention of human cancers have attracted...
462 Poster (Board P140)

A microbiome based model of anticancer intervention

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Abstract
Dysbiosis impacts in the natural history of cancer. A set of experimental data sustains the potential of how manipulations of the gut microbiome might improve the outcomes of cancer patients and can activate the activity of pleiotropic agents, through selective induction of Reactive Oxygen Species (ROS) in cancer cells. Robust experimental demonstrates that commensal bacteria promotes antitumor immunity and synergizes with PD-1/L and CTLA4 disrupting agents. Serum samples from consenting cancer patients (n = 92) and healthy individuals (n = 94) have been analyzed in an immune precipitation assay, panel of 30 strains of advanced bacterial, this technology platform permits the identification of a dysbiotic profile in serum of cancer patients vs healthy subjects. A galenic process has been developed to lead to a postbiotic extract, IG1806t, that contains secondary metabolites from commensal bacteria present in serum of cancer patients. A clinical plan, randomized double blind, is under implementation in solid tumors in combination with pleiotropic agents or with PD-1/ CTLA4 interacting agents. Early data from a compassionate program with IG1806t is going to advanced cancer as single agent or as maintenance therapy is available. 35 non selected consecutive patients with advanced solid tumors are the subject of this analysis. IG0608 is given orally, 300 mg total dose daily, on a continuous basis. IG1806t PS at entry = 2 (1-3), ninety-four % of the patients being Stage IV, 29% with liver metastasis, equal number with bone metastasis. 30 cases have received IG1806t for >2 years. Most common side effects are dysgeusia and fatigue. The program included a personalized assessment of patient’s quality of life (QoL) by using the EORTC QLC-C30 questionnaire at baseline and every other month (mo). An intrapatient baseline vs 2 mo QLC-30 information shows significant improvement in 12/35 patients, moreover the improvement pattern noted at 2 mo is confirmed at the 4 mo time point. The serum dysbiotic profile detectable at baseline becomes negative in most of the patients during IG1806 therapy. The median survival (Kaplan-Meier) is 25 mo (95% CL 17–) : the survival rates at 12, 24 and 36 mo are 82% (69–95), 57% (35–69) and 37% (17.5–56) respectively. An intrapatient analysis shows that statistical deviations, long lasting tumor control and improved survival, not justified by indolent disease or the play of oncogenic mechanisms but the presence of a gut dysbiosis, this is the case of neck squamous cell carcinoma, lung adenocarcinoma, breast adenocarcinoma, Prostate adenocar (3), NSCLC, pleomorphic sarcoma, high grade glioblastoma, esophageal ca and NHL, all of them surviving for >24 mo. A translational program on IG1806 molecular pharmacodynamics is ongoing. The impact of this microbiome anticancer intervention will be confirmed in the upcoming clinical plan.

Conflict of interest: Ownership: All authors are part or full time employees at Igen Biotech including stock ownership.

463 Poster (Board P142)

CB-5083, a first-in-class p97 inhibitor that disrupts protein homeostasis, exhibits anti-tumor activity in a panel of solid tumor xenografts


Background: CB-5083, a small-molecule inhibitor of the p97 ATPase, is a novel anti-cancer agent that demonstrates significant activity in vitro and in preclinical models, validated in multiple solid tumor xenografts and in other preclinical model systems. CB-5083 targets the ATPase activity of a heterogeneous group of p97/VCP homologs to induce macrovesicle formation and dissolution, ubiquitin protein response (UPR), endoplasmic reticulum-associated degradation (ERAD), and protein homeostasis [1]. The clinical success of ubiquitin proteasome system (UPS) inhibitors in multiple myeloma validates the approach of targeting protein homeostasis in high secretory disease such as cancer. CB-5083 is an inhibitor of the AAA-ATPase p97, which is involved in several facets of protein homeostasis upstream of the proteasome, including the unfolded protein response (UPR), endoplasmic reticulum-associated degradation (ERAD), and autophagy [3]. CB-5083 preferentially targets the autophagy and the unfolded protein response pathways in cancer cells, which correlate with CB-5083 sensitivity – these included activation of well-known cancer pathways such as MAPK [3]. Given these data, CB-5083 was tested in a range of cell-derived xenograft (CDX) models with said molecular background, and patient-derived xenograft (PDX) models with a secretory phenotype to observe anti-tumor efficacy.

Materials and Methods: CB-5083 efficacy was initially tested in 13 CDX models, including lines derived from colorectal cancer (CRC) and pancreatic neuroendocrine tumors (PNET), selected based on their in vitro proliferation (IC50). 20 PDX models from CRC as well as clear cell renal cell carcinoma (ccRCC) were also chosen based on the high
incidence of mutations in genes upstream of MAPK signaling in CRC, and the intrinsic secretory biology of ccRCC. Cells or PDX fragments were incubated subcutaneously in the hind flank of NCr nude mice, and when tumor volumes reached 100–200 mm³, mice received CB-5083 per os at the maximum tolerated dose (MTD) on a QD4/3ff regime. The percent of tumor growth inhibition (TGI) compared to vehicle control and the percent change in relative tumor volume (%RRTV) were calculated at the end of the study.

Results: After correlating the TGI obtained after CB-5083 administration with basal level of phospho-ERK1/2 as a readout of MAPK activation, we found that CDX models with high levels of phospho-ERK1/2 were more sensitive to CB-5083 with a TGI of >50% (CRC cell line HCT-116 and PNET cell line GPG-1). In CRC PDX models, 2 of 4 models with either Kras or Braf mutations were responsive to CB-5083, while 5 of 16 RCC PDX tumors responded. 1 RCC model in particular (RXF 1781) showed complete regression (%RRTV of ~100%).

Conclusions: Together these data suggest that cancer cells with activation of the MAPK pathway may rely heavily on p97’s function in protein homeostasis and thus be more sensitive to CB-5083. CB-5083 also shows promising anti-tumor activity in CRC with a high incidence of constitutive MAPK activation and in an all-comer panel of ccRCC PDX tumors.

Background: The Golgi apparatus plays an essential role in the transport, processing, and sorting of cell surface proteins including receptor tyrosine kinases (RTKs) expressed on the cell surface. We previously demonstrated that M-COPA (2-methylcyclopentanolamine, also called ‘AMF-26’), disrupted the structure of Golgi apparatus via inhibiting the activation of ADP ribosylation factor 1 (Arf-1), which plays an essential role in the formation of COP1 and/or Clathrin-coated transport vesicle from endoplasmic reticulum (ER) through Golgi to cell surface. In RTK-addicted cancers, cell growth and survival is dependent on aberrant RTK overexpression and/or activation, which is achieved by gene amplification or activating mutation.

In this study, we investigated the effect of M-COPA on cell surface expression of RTKs and on cell growth of RTK-addicted cancers.

Materials and Methods: Drug sensitivities in vitro were measured by sulforhodamine B assays after 48 h exposure of M-COPA. Cell surface expression of RTKs (MET, FGFR2, and EGFR) was analyzed by flow cytometry. Maturation/phosphorylation state of RTKs and activation status of downstream signaling molecules including Akt and S6 ribosomal protein were determined by immunoblot analysis. The in vivo efficacy of M-COPA (50 mg/kg, p.o., qd x 5) was examined using nude mice bearing xenografted human tumors.

Results: Upon M-COPA treatment with M-COPA, cell surface expression of MET was downregulated along with accumulation of its precursor form in MET-amplified GC cells. M-COPA also reduced levels of the phosphorylated form of MET and its downstream signaling molecules Akt and S6. Similar results were obtained in GC cell lines with amplification of the FGF receptor FGFR2 gene, and also in lung cancer cell lines with an activating mutation in EGFR gene. Interestingly, M-COPA was also effective for suppression of cell surface expression of EGFR carrying a secondary mutation that exhibits resistance to ATP-competitive TKIs such as gefitinib. Finally, murine xenograft experiments demonstrated the antitumor activity of M-COPA against MET-amplified GC and EGFR-mutated lung cancers in vivo.

Conclusions: Our results offer a preclinical proof of concept (POC) for the use of M-COPA as a candidate treatment option for RTK-addicted cancers, especially for those harboring TKI resistance, via an activating mutation of the processing and the transport of RTK protein onto the cell surface.

Conflict of interest: Corporate-sponsored Research: K. Yoshimatsu (Employee of Eisai Co., Ltd).

465 Poster (Board P144)
TAS4464, a novel highly potent NEDD8 activating enzyme inhibitor, demonstrates anti-tumor efficacy in rituximab-resistant double-hit lymphoma models

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Background: Double hit lymphoma (DHL) is an agresssive subtype of diffuse large B-cell lymphoma that carries concurrent MYC and BCL2 or BCL6 translocations. Disease prognosis with standard R-CHOP therapy is poor for DHL, and other MYC and BCL2 double-expressing lymphomas show similar prognosis to DHL. Therefore, more effective therapy is urgently needed for these types of lymphomas. We previously reported that the NEDD8 activating enzyme inhibitor TAS4464 showed broad activity against hematogenic cancer cell lines, and that TAS4464 downregulated MYC and BCL2 transcripts through NF-κB pathway inhibition. Here, we report the possible use of TAS4464 to treat DHL.

Material and Methods: To evaluate the biological activity of TAS4464, we used 4 different human DHL cell lines. Intracellular ATP levels were measured to assess in vitro cell growth. Effects on the cell cycle were analyzed by using fluorescence-activated cell sorting. The effects of TAS4464 treatment on protein and gene transcript levels were evaluated by Western blot analysis and qRT-PCR, respectively. The antitumor activities of TAS4464 alone and in combination with rituximab were evaluated in subhuman DHL xenograft models.

Results: TAS4464 led to growth arrest and cell death in DHL cell lines in the nanomolar range. Cell cycle analysis showed that TAS4464 induced apoptosis in DHL cell lines within 24 h. The activation of caspase-9 may contribute to the induction of apoptosis by TAS4464. TAS4464 inhibited NEDD8 conjugation of cullin ring ligases (CRLs) in DHL cell lines, leading to the inactivation of CRLs and the accumulation of substrate proteins, including CDT1, NRF2, and BCL2. Furthermore, TAS4464 enhanced the antitumor activity of rituximab in rituximab-resistant DHL xenograft models. Because the BCL2 inhibitor venetoclax showed limited antitumor activity in a model, the strong antitumor effect of single-agent TAS4464 likely is not dependent only on BCL2 down-regulation. The investigation of underlying mechanisms is ongoing.

Conclusions: TAS4464 leads to cell growth arrest and strong apoptosis induction via the regulation of apoptosis-associated factors. The results suggested that TAS4464 leads to NF-κB inactivation following accumulation of phospho-IκBα and consequently downregulation of MYC and BCL2 (other anti-apoptotic proteins) at the transcriptional level. In addition, TAS4464 demonstrates marked antitumor activity alone and in combination with rituximab in DHL xenograft models. Therefore, TAS4464 may be a valuable addition to current options for chemotherapy in DHL.

No conflict of interest.

466 Poster (Board P145)
Phase I study of ganetesib and ziv-afibercept in patients with advanced gastrointestinal carcinomas, non-squamous non-small cell lung carcinomas, urothelial carcinomas, and sarcomas

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Background: Ganetesib is a non-geldamycin synthetic inhibitor of Hsp90 that has demonstrated activity against multiple cell lines and xenografts. Inhibiting the Hsp90 chaperone complex results in the recruitment of ubiquitin ligases, polyubiquination, and proteasomal degradation of Hsp90 client proteins (VEGF, VEGFR, HIP-1, STAT-3); growth factor independence (Raf, EGFR, Her2, IGF2); resistance to anti-growth signals (CDK4); tissue invasion and metastasis (MMP, TIMP2); and avoidance of apoptosis (AKT, RIP, Survivin, Bcl-2). Combining Hsp90 inhibition with ganetesib and TKI anti-angiogenic therapy with ziv-afibercept, a soluble fusion protein with high binding affinity for VEGF-A, VEGF-B, and PIGF, presents a novel strategy for overcoming resistance to anti-angiogenic therapies.

Methods: Pts with progressive malignancies after standard therapy, ECOG PS of 0–2, and adequate organ function, no major surgery within 4 weeks, no radiation/chemotherapy within 3 weeks prior to enrollment were eligible. Pts must have recovered from toxicities of prior therapies. Ganetesib
was given IV weekly on days 1, 8, and 15 of a 28-day cycle, and ziv-aflibercept was given IV on days 1 and 15. The escalation portion of the trial was given IV weekly on days 1, 8, and 15 of a 28-day cycle, and ziv-aflibercept was given IV on days 1 and 15. The second patient the dose was deescalated due to grade 5 small bowel perforation down to DL-1 (Ganetespib 100 mg/m^2 IV D 1 & 15). 1 pt died on study of progressive disease and 1 patient died due to sudden death, cause not elucidated without attributable drug effect.

Conclusions: The combination of ganetespib and ziv-aflibercept may be too toxic on this schedule. A different schedule may need to be considered for development to move forward.

No conflict of interest.

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Poster (Board P146)

Findings across pre-clinical models in the development of PT-112, a novel investigational platinum-pyrophosphate anti-cancer agent

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PT-112 is a platinum-containing (Pt) new chemical entity designed to minimize DNA-repair drug resistance and certain toxicities associated with Pt chemotherapies.

A previous report noted PT-112 was more potent than cis-Pt in A549 lung cancer cells (IC50 0.9 vs. 2.8 μM) and had significantly lower Pt accumulation in whole cells and DNA. Here, in an anti-proliferation panel PT-112 was the most potent in the majority of cell lines vs. cis-, carbo- and oxali-Pt. PT-112’s median IC50 value was 2.6 μM, with nM potency again found in lung cancer.

Unlike cis-Pt, PT-112’s IC50 did not differ significantly between HCT116 DNA repair-deficient cells and their isogenic DNA repair-proficient counterparts. At IC50 PT-112 caused modest effects on Ku70 and H2AX protein levels in HCT116 colorectal cancer cells, further indicating limited DNA damage/repair effects. Cell cycle inhibition was observed at the G1/S checkpoint, with upregulation of p16, p21 and Rb, and potent inhibition of transcription factors CDK1/4 and E2F3. Activity on the MDM2-p53 complex was confirmed, along with immunogenic cell death potentiation via release of HMGB1 and CEA/CEACAM expression, and suppression of STAT3.

PT-112 is active in vivo. In the GFP / luciferase PANC1 pancreatic model, a durable response was observed (mean ΔT/ΔC 2.5%) including cases of regression. Sustained tumor growth delay was observed in the CFX 280 colon PDX and Panc1 pancreatic models.

In vivo pharmacokinetics demonstrated significant unbound PT-112 Pt levels in plasma AUC (72.75%). An LC-MS/MS analytical method developed to detect parent PT-112 revealed its relative predominance, indicating stability in plasma. Unlike cis-Pt, which caused morbidity, rodents tolerated 7-fold higher concentration of PT-112 with minimal effects on renal function parameters creatinine, BUN and GFR. In a mouse model of acute neurotoxicity, a single dose of PT-112 was indistinguishable from untreated controls when given at twice the concentration of oxali-Pt, which induced significant cold-pain response. After repeat doses, PT-112 caused minimal accumulation of Pt in dorsal root ganglia nerve tissue and no significant loss in nerve conduction velocity, while oxali-Pt demonstrated dose-dependent loss indicative of chronic neuropathy.

PT-112 bears hallmarks of pleiotropic anti-cancer activity, representing a new model of well-tolerated, multi-targeted pharmacological intervention. Various experimental systems indicate PT-112 sensitivity is not significantly affected by DNA repair function, that it affects other cancer signaling targets and has a positive therapeutic index. Neither acute nor cumulative renal or neurotoxicity are likely major components of PT-112 treatment limitations. These findings suggest a clinically relevant departure from the paradigm associated with approved Pt agents. Further validation is ongoing in translational and clinical development.

Conflict of interest: Ownership: Dr. Ames and Dr. Jimeno hold a financial interest in Phospholamin Therapeutics, sponsor of some of the research.

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Poster (Board P147)

Investigating a naturally occurring small molecule, EBC-46, as an immunotherapeutic agent to help treat cancer

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Background: EBC-46 is a novel diterpene ester currently being developed by QBiotics Ltd. and researchers at the QIMR Berghofer Medical Research Institute for the local treatment of a wide range of solid tumours in humans and companion animals. The compound, isolated from an Australian tropical rainforest plant, shows structural similarity to the prototypical protein kinase C (PKC) activator, phorbol 12-myristate 13-acetate (PMA). We have performing studies to understand how EBC-46 functions as an anti-cancer compound and to investigate its potential as an immunotherapeutic agent.

Materials and Methods: Several animal studies were performed in xenograft/syngeneic models of cancer (tumour size and survival). Treated tissues were analysed by immunohistochemistry. Veterinary data was acquired from companion animal studies performed by QBiotics Ltd.

Results: EBC-46 showed remarkable efficacy in ablating cutaneous and subcutaneous tumours in both preclinical mouse models (xenograft/syngeneic) and veterinary cases (spontaneously occurring cancers), resulting in local cure following a single intrasquamous injection in ~75% of cases. EBC-46 causes rapid inflammation around the injection site, after which the tumour swells and bruises. This resolves over the next 1–2 weeks, resulting in permanent ablation of tumour with regeneration of normal skin and hair. Histochemical analysis showed that treatment induces vascular disruption (CD31 staining), followed by red blood cell extravasation into the surrounding stroma with tumour cell necrosis, the latter of which was confirmed through ex vivo clonogenic assays. It also has direct effects on tumour cell lines, where it induces eIF2α phosphorylation (a marker of ER stress) and rapid cancer cell necrosis via a ROS-dependent mechanism. In contrast to PMA, EBC-46 activates a limited subset of PKC isoforms (PKC ι > θ > γ > α). Interestingly, microarray data suggests that EBC-46 induces a transcriptional profile in tumour stroma with the characteristics of a Th1 immune response (IL-17, TNF, IFNγ, IL-6 pathways). Furthermore, treatment of syngeneic mouse models of melanoma (B16-F1 and B16-F10-OVA) with EBC-46 lead to the development of vitiligo at the lesion site.

Conclusions: Together, these observations suggest that in addition to affecting tumour vasculature and inducing tumour cell necrosis, EBC-46 may also have immunostimulatory effects that could be important for efficacy. We are currently investigating if EBC-46 can induce immunogenic cell death (ICD) in various cancer cell lines and whether combinatorial treatment strategies with the immune checkpoint inhibitors (PD-1/CTLA-4) can synergize to improve efficacy and help treat metastatic disease in immunocompetent mouse models.

Conflict of interest: Corporate-sponsored Research: The work described within was funded by QBiotics Ltd. under commercial contract with the Drug Discovery Group at QIMR Berghofer. Dr. Jason Cullen, Ms. Pei-Yi Yap and Prof. Peter Parsons positions are fully funded by EcoBiotics Ltd. (QBiotics parent company) and QBiotics Ltd.
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