Conclusions: In this study, we evaluated the effect of MCT1 inhibition in cervical cancer cells and observed a significant decrease in total cell biomass which may be a result of inhibition of cell viability, cell proliferation and/or induction of apoptosis. Further studies are needed to better comprehend the mechanisms by which MCT inhibition exerts its effect on cervical cancer cells, however, by the results herein presented a promising therapeutic target can be anticipated for this type of tumours.

352 POSTER

Involvement of Grb2 adaptor protein in nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) mediated signaling and anaplastic large cell lymphoma growth

L. Riera[1], E. Lasorsa[1], C. Ambrogio[1], N. Surrenti[1], C. Voena[1], R. Chiarle[1]. [1]*University of Torino, Biomedical Science and Human Oncology CeRMS, Torino, Italy*

Background: Most Anaplastic Large Cell Lymphoma (ALCL) express oncogenic fusion proteins derived from chromosomal translocations or inversions of the Anaplastic Lymphoma Kinase (ALK) gene. Frequently ALCL carry the t(2;5) translocation that fuses ALK gene to Nucleophosmin (NPM1) gene. NPM-ALK mediated transforming activity induces different pathways that control lymphoma cells proliferation and survival. Grb2 adaptor protein is thought to play an important role in ALK-mediated transformation, but its interaction with NPM-ALK and its functions in ALCL is still unclear.
In this work we focused on Grb2 binding to NPM-ALK, its phosphorylation by the fusion protein and its role in regulating signaling pathways and proliferation of ALCL cells.
Methods: Human embryonal kidney cells HEK-293T were transfected with different Grb2 and/or NPM-ALK constructs and immunoprecipitation and immunoblot analysis were performed. All Grb2 and all kinase mutants were generated by PCR-based mutagenesis. Inducible ALK and Grb2 shRNA SU-DHL-1 and TS cells were obtained by co-transduction with pLV-tTRKRAB (TTA) vector and pLVTHM vector containing the H1 promoter shRNA cassette. NPM-ALK or Grb2 silencing is achieved when 1μg/mL of doxycyclin is added to the medium for 72 hours. To generate Grb2 shRNA-resistant constructs, wild-type or Y160F Grb2 were mutated in 4 bases in the sequence corresponding to the shRNA (Grb2INT3/4). Co-culture and proliferation experiments were performed.
Results: In this study we demonstrate that Grb2 binds to active NPM-ALK and is phosphorylated in human ALCL cells. We identified Y160 as major phosphorylation site of Grb2 by NPM-ALK. We found that Y160 of Grb2 is phosphorylated also by other oncogenic fusion tyrosine kinases such as TPR-MET, BCR-ABL and TEL-JAK2, as well as by wild-type receptor tyrosine kinases, such as ALK and MET. Further, we show that NPM-ALK combined mutations in Y152–156, Y567 and P415–417 almost completely abrogated Grb2 binding and Y160 phosphorylation. Finally, shRNA knock-down experiments showed that Grb2 is essential for SHP2 activation in ALCL and is required for sustained ALCL cell growth.
Conclusions: Grb2 silencing in ALCL cells strongly impaired cell proliferation, suggesting that Grb2 is fundamental for the full activation of a signaling cascade that involves Shc and SHP2 and assures lymphoma cells proliferation. Thus, Grb2 could represent a potential target to control cell proliferation in NPM-ALK mediated lymphomas.

353 POSTER

Integrating alternative splicing studies as a tool for innovative therapeutical interventions: focus on novel drug targets and novel epitopes

A. Casagrande[1], M. Pando[1], L. Desire[1]. [1]*Exonhit Therapeutics, Therapeutic division, Paris, France*

Alternative RNA splicing is a key molecular mechanism for the generation of functional protein diversity. Abnormal alternative splicing can occur in cancer, resulting in the production of novel transcript variants or in an imbalance between mRNA isoforms. In both cases, it can affect the global pattern of protein expression within a cell, sustain tumour growth or affect drug response. Therefore, identification of cancer-associated alternative splicing variants may represent a significant step forward and potential source of new clinical diagnostic, prognostic and therapeutic strategies.
Results: ExonHit has generated discovery engines aimed at studying alternative splicing and is currently building libraries of alternative splicing events that are deregulated in cancer and in cases of therapy resistance. Here, using ExonHit's Genome Wide SpliceArray™ microarray, we investigated patterns of alternative splicing in diverse human cancers as well as in drug resistance models. Distinct splicing patterns were evidenced using principal component analysis and through statistical analysis of differential splicing. We show that a number of already targeted genes, as well as drug resistance genes, in fact undergo alternative splicing, which can ultimately affect drug response. We also implemented bioinformatic processes and selectivity filters that allowed to identify (1) alternatively splicing variants with altered druggable domains and (2) splicing variants that generate novel cell surface epitopes. Using this strategy, we show for example how the small GTPase Rac1 which is subjected to alternative splicing to generate the self-activated variant Rac1b, can be targeted through isoform-selective medicinal chemistry programs. In addition, we illustrate how such strategy can help to identify a number of other alternatively spliced transcripts containing novel amino acid sequences that can be used as novel epitopes. These novel epitopes-containing variants are now being used to target monoclonal antibodies for therapy.
Conclusion: Alternative RNA splicing offers a currently underexploited source of novel disease targets. Our results demonstrate a significant contribution of splicing to cancer development and drug response. Platforms dedicated to studying alternative splicing can be integrated into discovery processes to allow identification of novel targets for drug discovery which can then be subjected to innovative therapeutical interventions based on splicing variant-selective drug design, antisense- or antibody-based therapies.

354 POSTER

Investigations on organic anion-transporting polypeptides 1A2, 1B1 and 1B3 in colon cancer as potential targets for cancer therapy

V. Kounnis[1], E. Ioachim[2], M. Svoboda[3], I. Sainis[1], C. Ausch[4], G. Hamilton[5], T. Thalhammer[3], E. Briasoulis[1]. [1]*University of Ioannina, Cancer Biobank Center, Ioannina, Greece;* [2]*Hatzikosta General Hospital, Pathology Department, Ioannina, Greece;* [3]*Medical University of Vienna, Department of Pathophysiology and Allergy Research, Vienna, Austria;* [4]*Medical University of Vienna, Clinic for Surgery, Vienna, Austria;* [5]*LBI-Cluster for Translational Oncology, Vienna, Austria*

Background: Organic Anion-Transporting Polypeptides (OATP) selectively facilitate the uptake of endogenous substrates and drugs into cells. Previous studies suggest that certain OATPs are expressed in several cancers, which profiles them as potential target candidates for novel cancer therapeutics (I. Sainis *et al.*, *Mar Drugs* **8**, 629, 2010).
Materials and Methods: We investigated the expression of OATPs 1A2, 1B1, and 1B3 in human colon cancer. We studied their expression at the mRNA level by TaqMan real-time RT-PCR and at protein level by immunohistochemistry, in human colon cancer material and in four colon cancer cell lines (Caco, C205, HT29 and L174T). For immunohistochemistry we used the following antibodies: polyclonal rabbit SSV anti-OATP1A2, monoclonal mouse ESL anti-OATP1B1 and monoclonal mouse MDQ anti-OATP1B1/1B3.
Results: Using frozen samples from cancerous and adjacent non-cancerous colon tissues, we found OATP1B3 mRNA significantly expressed in 18/20 cancerous samples (where it reached 8.3-fold levels over control), as was also in the HT-29 and L174T cell line (4.7-fold enrichment). It normal colon OATP1B3 mRNA levels were undetected. Interestingly, OATP1A2 mRNA expression was also detected in the CaCo cell line, while HT29 expressed OATP1B1. The immunohistochemical study revealed that OATP1A2 and 1B1 were expressed in all studied cases while1B3 (by using the mMDQ antibody which also recognizes an epitope shared by 1B1) was expressed in 27/30 samples. Interestingly in positive cases, almost all cancer cells were stained positive. Furthermore, OATP1A2 protein expression was intense in 3/4 of the cases studied while 1B1 and 1B1/1B3 expression was weak in 40% and 63.3% respectively. Investigation of cancer-associated mutations of 1B3 is under way and will be presented.
Conclusions: Organic Anion-Transporting Polypeptides 1A1, 1B1 and 1B3 are differentially expressed in colon cancer. We suggest that further investigation of OATPs in colon and other cancers is warranted, in search of new cancer targets that may offer perspectives for the development of novel targeted cancer therapies.

355 POSTER

Identification of an inhibitor for melanoma cell migration through the inhibition of Pirin

I. Miyazaki[1], S. Simizu[1], H. Osada[1]. [1]*RIKEN, Chemical Biology, Saitama, Japan*

Background: Bcl3 was originally identified as a putative proto-oncogene which frequently rearranged in chronic B cell lymphocytic leukemia. Bcl3 interacts with NFkB by binding to p50 and p52. The overexpression of Bcl3 was reported to enhance cell survival, proliferation and tumor malignancy. Pirin is known to be bound to Bcl3, however, the exact roles of Pirin in tumor cells have not been clarified.
Materials and Methods: To discover the Pirin ligands, we have carried out a screening with the aid of a chemical array. In this screening system, small-molecules are covalently immobilized on the glass slides through a

photo-affinity linker and the cell lysates that overexressed DsRed or DsRed-fused Pirin were used.
Results: We identified a small-molecule that binds to Pirin by using the chemical array screening method. The K_d value of the small-molecule to Pirin was 614 nM by isothermal titration calorimetry experiments. The pull-down assay showed that the small-molecule inhibited the interaction of Pirin with Bcl3 *in vivo* and *in vitro*. To get a insights into the effect of the small-moleclue against cells, we examined the cytotoxic activity against a number of different human cancer cell lines. As a result, the small-molecule did not show potent cytotoxic activity. In contrast, the inhibition of migration in melanoma cells was observed in the dose-dependent treatments of the small-molecule, but not the treatment of the negative control that does not bind to Pirin. We found that the treatment with the cultured tumor cells with the small-molecule or Pirin-targeted siRNA resulted in suppression of migration, but not cell proliferation.
Conclusions: We have discovered the first inhibitor of Pirin, functions in cellular system is unclear. The inhibitor disrupted the interaction of Pirin with Bcl3 *in vivo* and *in vitro*. By use the inhibitor and siRNA, it is suggested that Pirin regulates migration of the tumor cells, and the inhibitor of Pirin might be a new chemotherapeutic potential for invasive tumors.

356 POSTER

An innovative platform technology accelerates drug screening

F. Becker[1], S. Hannus[1], K. Hansen[1]. [1]*Intana Bioscience GmbH, Assay Development, Planegg, Germany*

Fluorescence Correlation Spectroscopy (FCS) and Fluorescence Cross-Correlation Spectroscopy (FCCS) are biophysical techniques that enable precise and sensitive analysis of molecular interactions in solution. FCCS allows analysis of reactions in realtime at nanomolar concentrations and can be applied to the monitoring of binding events in cellular lysates and living cells.
FCS and FCCS are single molecule sensitive fluctuation analysis methods; in contrast to other fluorescence techniques, the parameter of primary interest is not the emission intensity itself, but rather spontaneous intensity fluctuations caused by minute deviations from thermal equilibrium. Consequently, FCS/FCCS can only function properly if concentrations and observation volumes are reduced such that only few molecules are simultaneously detected. For this, the observation volume is defined by strongly focused, overlapping excitation laser beams and a confocally arranged optical setup. Fluorescent molecules entering and leaving this open, illuminated spot of below 10^{-15} liters volume by diffusion emit photons, as long as they dwell in the observation focus. In combination with single photon sensitive detectors the photon count trace is recorded and subjected to a mathematical operation, the auto- and cross correlation analysis, to extract information on particle number, brightness, size and interactions.
The authors will demonstrate the application of FCCS addressing several relevant cancer targets and reference inhibitors. Esp. the quantitative determination of binding constants within cellular lysates enables the efficient and sensible characterization of kinase inhibitors with long residence time.

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Antitumor triazoloacridinone C-1305 as a potent FLT3 tyrosine kinase inhibitor in human acute myeloid leukemia (AML) cells

E. Augustin[1], A. Skwarska[1], J. Koprowska[1], I. Pelikant[1], J. Konopa[1]. [1]*Gdansk University of Technology, Pharmaceutical Technology and Biochemistry, Gdansk, Poland*

Background: An internal tandem duplication (ITD) in the juxtamembrane domain of Fms-like tyrosine kinase 3 (FLT3) is the most common molecular defect associated with acute myeloid leukemia (AML) and predicts for poor prognosis. This mutation causes constitutive activation of the FLT3 receptor in the absence of natural FLT3 ligand (FL). Here, we investigated whether antitumor triazoloacridinone C-1305, selected for extended preclinical trials, exhibits inhibitory activity toward FLT3 kinase.
Materials and Methods: C-1305 activity was evaluated in human leukemia cell lines, MV4;11 (FLT3 ITD) and RS4;11 (FLT3 WT). The inhibitory effect of C-1305 on FLT3 receptor activation was determined by analysis of protein phosphorylation by ELISA. Flow cytometry for annexin V/PI assay, caspase-3 activation and sub-G1 DNA fraction was applied to identify apoptosis. DAPI staining was used to analyse cellular morphology.
Results: Ligand-independent phosphorylation of FLT3 in MV4;11 cells was inhibited by C-1305 with an IC_{50} of approximately 7 μM. Treatment of MV4;11 cells with the drug for 72 h suppressed cell proliferation with EC_{50} concentration equal to 0.2 μM. To determine whether blocking of FLT3 activity modulates cellular response upon C-1305 treatment, MV-4;11 cells were exposed to various drug concentrations. C-1305 was found to exerts its cytotoxic effect by the induction of apoptosis as evidenced by chromatin condensation, phosphatydilserine externalization, caspase-3 activation and presence of sub-G1 fraction. In all tests, the number of apoptotic cells increased after treatment with C-1305 in a time- and dose-dependent manner and reached about 80% after 72 h of drug exposure. Importantly, apoptotic cell death was observed at concentration of drug required to block FLT3 phosphorylation, suggesting that inhibition of FLT3 kinase by C-1305 may account for its cytotoxic activity in MV4;11 cells. Since majority of AML harbor WT-FLT3, we further tested the effect of C-1305 on RS4;11 cells. Significantly higher concentration of the drug (EC_{50} 1,8 μM) was required to inhibit the growth of RS4;11 cells, compared to MV4;11, suggesting potent selectivity of C-1305 towards FLT3 ITD cells.
Conclusions: Our results indicate that C-1305 shows high potency and selectivity toward acute leukemia cells harboring FLT3 ITD activating mutations as evidenced by inhibition of cellular proliferation, induction of apoptosis and inhibition of FLT3 autophosphorylation in these cells.

358 POSTER

Study of cetuximab and panitumumab activity in an 80 cell line panel to evaluate specificity of small molecule EGFR inhibitors

I. Ivanov[1], P. Amon[1]. [1]*Oncolead GmbH & Co KG, Research & Services, Martinsried/Munich, Germany*

Developed and broadly applied in the Developmental Therapeutics Program of NCI/NIH, multiple cell line panels became essential platforms for anticancer drug discovery and development efforts. An activity profile of an anticancer agent in a large cell line panel can lead to hypotheses about its mode of action when compared with reference agents. Antibodies with naturally inherited specificity of action could be better reference molecules, but they are hardly ever used for these purposes. This can be explained by a number of factors. First, antibody responses cannot be described by a common sigmoid dose response curve. Therefore, statistical correlation methods cannot be applied for comparison as performed with small molecules. Secondly, antibody activity *in vivo* is linked to secondary responses, e.g. Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC), which partially explain only minor effects of antibodies on the growth of tumor cell lines *in vitro*.
We will present an approach to implementing activity profiles of antibodies for studying the mode of action of small molecules. We tested cetuximab and panitumumab in a panel of 80 cell lines *in vitro*. Both agents were applied to cells in a range of 0.01 μg/ml to 100 μg/ml for 72 hours, and their activity was measured by the sulforhodamine B assay. Based on the dose response curve we selected three different groups of cell lines. The first group included cell lines, e.g.NCIH292, most probably dependent on EGFR activation, and very sensitive (already at 0.1 μg/ml) to the antibody action. The second group comprised cell lines, e.g. A431, that were less sensitive to the antibodies. Resistant cell lines were combined into the third group. Activity profiles of small molecules were used to match activity patterns of these three groups. Among more than 300 reference anticancer agents, the strongest similarity was observed for EGFR inhibitors, e.g. lapatinib or erlotinib. Less, but still significant similarity was observed for agents known to be involved in the EGFR pathway: some Akt, MEK and PI3K inhibitors. These results indicate that antibodies can be applied to study small molecule's mode of action. In addition, we observed that the molecular profile of cell lines sensitive to the antibodies correlated well with the known clinical outcome for these antibodies. This fact suggests that larger sets of cell line models should be considered for pre-clinical evaluation of antibodies independently from an ADCC study.

359 POSTER

Effect of the TGF-beta 2 specific antisense oligodeoxynucleotide trabedersen on TGF-beta 2 and -beta 1 expression in human glioma cells: Cross-regulatory loops regulate TGF-beta isoform expression

F. Jaschinski[1], M. Kielmanowicz[1], T. Rothhammer[1], A. Schneider[1], K.H. Schlingensiepen[1]. [1]*Antisense Pharma, Preclinical Research & Development, Regensburg, Germany*

Background: Transforming growth factor-beta (TGF-beta) plays a key role in cancer progression, by regulating crucial cancer mechanisms. Three mammalian isoforms, i.e. TGF-beta 1, 2, and 3, have been isolated. TGF-beta 2 plays a particular role in several cancers, and our newly developed therapeutic approach based on the specific inhibition of TGF-beta 2 synthesis by the antisense oligodeoxynucleotide trabedersen (AP 12009) has been successfully tested in clinical trials in patients with advanced stage glioma, pancreatic cancer or malignant melanoma.
Apart from TGF-beta 2, most tumors also express considerable amounts of TGF-beta 1. Since auto-induction and cross-regulation of different TGF-beta isoforms has been reported in some tissues, we were investigating, if this phenomenon is also present in human tumor cells and