116 POSTER

R1507, a human monoclonal antibody targeting IGF-1R (insulin like growth factor receptor) is effective alone and sensitizes small cell lung cancer cell lines to chemotherapy and radiation in vitro

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Introduction: Insulin like growth factor receptor-1 (IGF-1R) inhibition could be an important therapeutic approach in small cell lung cancer (SCLC), given the activation of multiple autocrine loops in the pathogenesis of this disease, one of them involving the co-expression of IGF-1R and one of its ligands IGF-1 or IGF-2. Besides IGF-1 is a potent stimulator of PI3K-Akt signaling in SCLC.
Materials and Methods: In this study we evaluated the importance of IGF-1R axis in SCLC by assessing IGF-1R expression and Akt activation in human SCLC tissue specimens. In parallel we evaluated *in vitro* the efficacy of R1507, an IgG1 fully human monoclonal antibody directed to IGF-1R. We performed Western blotting to assess the expression of IGF-1R and the effect of R1507 on downstream signaling cascades using three small cell lung cancer cell lines: H69, H146 and H526. The *in vitro* cytotoxicity of R1507 alone and in association with cisplatin or ionizing radiation was evaluated by WST-1 cell proliferation assay and by clonogenic survival. To assess apoptosis induction we evaluated cell cycle distribution. We also tested concomitant targeting of MEK and IGF-1R on H146 cells.
Results: We demonstrated in vitro efficacy of R1507 in two of the three cell lines examined, as well as a synergistic effect with cisplatin and radiotherapy in H526 cell line. Efficacy was dose dependent, becoming more evident at concentrations above 100 nM. The inhibition of PI3K-Akt pathway was correlated with treatment response. The effect of IGF-1R blockage on MAPK pathway showed a high variability among the three cell lines examined. R1507 was able to induce cell cycle arrest in H526 cell line. Concomitant inhibition of MEK and IGF-1R in H146 cell line did not have synergistic effect. IGF-1R was mostly overexpressed (55% showing a more than 1+ expression level) in surgical specimens originating from patients suffering an intervention for limited stage SCLC. We noticed a high level (79%) of concomitant expression of IGF-1R and pAkt in the same samples.
Conclusions: R1507 has a single agent activity and remarkable chemo- and radiosensitizing effect in defined small cancer lung cancer cell lines in vitro. Efficacy is dose-dependent and related to the capacity to inhibit PI3K-Akt signaling pathway.

117 POSTER

Vorinostat induces acetylation of BH3-only gene promoters triggering their expression and leading to apoptosis in mantle cell lymphoma

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Background: Current therapies for the B-cell neoplasm mantle cell lymphoma (MCL) have shown limited efficacy. Recently, Histone Deacetylase Inhibitors (HDACis) have been successfully introduced for the treatment of hematological malignancies. Accordingly, tumor suppressor genes are frequently epigenetically silenced in these entities, due to histone deacetylation in their promoters. Therefore, our purposes were to evaluate the antitumoral properties of the HDACi suberoylanilide hydroxamic acid (Vorinostat; Merck & Co) in MCL, and to describe the molecular mechanisms involved in HDACi signaling in this disorder.
Materials and Methods: 8 MCL cell lines and 10 primary MCL samples were used. HDAC activity was measured using a colorimetric HDAC Assay Kit. Flow cytometry was used to determine sensitivity to vorinostat through measurement of AnnexinV binding, and to analyze apoptosis features. Vorinostat anti-tumoral signaling was evaluated by RQ-PCR determination of gene transcription, western blot analysis and acetyl Histone H4 ChIP assays.
tightenup>**Results:** Vorinostat exhibited a heterogeneous cytotoxic effect among MCL cell lines, with a median LD_{50} of 6.6 μM after 24-hour incubation. Nevertheless, cytotoxicity increased notably after 48 h of exposure to the drug with LD_{50} ranging from 0.4 to 5.3 μM. Interestingly, 7 out of the 10 MCL primary samples tested were extremely sensitive to the compound (with a median LD_{50} of 2.2 μM after 24-hour incubation). Vorinostat increases the acetylation of H3 and H4 histones, as well as inhibits global HDAC activity in just 1 hour of incubation. The drug notably decreases cyclin D1 protein levels while induces upregulation of the proapoptotic BH3-only proteins Bmf, Bim and Noxa, triggering the mitochondria-dependent cell death and activation of the caspases cascade. Acetyl Histone H4 ChIP assays showed that vorinostat increases acetylation of *BMF*, *BIM* and *NOXA* gene promoters, consequently up regulating Bmf, Bim and Noxa mRNA levels.
Conclusions: This study suggests that vorinostat could define an attractive therapeutic approach for the treatment of MCL. We identify *BMF*, *BIM* and *NOXA* as target genes of HDAC inhibitors in MCL cells, promoting the induction of mitochondria-mediated apoptosis.
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118 POSTER

Responses of human pancreatic tumour cells to treatment with anti-EGFR mAb ICR62 and the irreversible EGFR/HER1 and HER2 tyrosine kinase inhibitor BIBW2992

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Aberrant expression of epidermal growth factor receptor (EGFR) family members has been reported in a wide range of epithelial tumours and, in some patients, has been associated with poor prognosis and resistance to therapy. Currently, a number of monoclonal antibodies (mAbs) and reversible EGFR and/or HER-2 tyrosine kinase inhibitors (TKIs) have been approved for treatment of human cancers. Of these the reversible EGFR TKI erlotinib, in combination with gemcitabine, has gained FDA approval for the treatment of patients with pancreatic cancer. However, while the EGFR inhibitors improve survival in pancreatic cancer patients, the duration of response is often limited. The aim of this investigation was to evaluate the growth response of a panel of human pancreatic tumour cell lines (Capan-1, Panc-1, FA-6, BxPc-3, PT-45, AsPc-1 and Miapaca-2) to treatment with our anti-EGFR mAb ICR62, BIBW2992 which is an irreversible EGFR/HER1 and HER-2 TKI and gemcitabine, using the SRB colorimetric assay. We reported previously that, at concentrations above 3.2 nM, mAb ICR62 inhibits completely the growth of the EGFR overexpressing DiFi cells *in vitro* and induces apoptosis. However, at maximum concentration of 200 nM used in this study, we found that mAb ICR62 had no effect on growth of the human pancreatic tumour cell lines. Interestingly, of the 7 human pancreatic tumour cell lines examined, BXPC3 cells were highly sensitive to treatment with BIBW2992 with an IC_{50} value of <10 nM. The growth of other human pancreatic tumour cells was also inhibited by BIBW2992 with IC_{50} values ranging from 247 nM (ASPC1) to 821 nM (FA6). Using FACS analysis, we found that the mean fluorescence intensities (MFIs) for EGFR expression in these tumour cell lines ranged from 32 (Miapaca-2) to 184 (PT45). In contrast, the levels of HER-2 expression in these cell lines were much lower and the MFI for HER-2 expression ranged from 11 (Panc-1) to 33 (Miapaca-2). Interestingly, all the human pancreatic cell lines tested were found to be negative for the expression of HER-3 and HER-4. We did not find any clear association between the expression levels of the EGFR family members and the response to treatment with BIBW2992 and ICR62. Taken together, our results presented here underlie the need for further investigation on the anti-tumour activity of the BIBW2992 as a single agent and in combination with gemcitabine and/or other targeted therapies in pancreatic cancer.

119 POSTER

ARQ 087: A potent ATP-independent fibroblast growth factor receptor (FGFR) kinase inhibitor showing in vivo anti-tumor activity in FGFR2-driven tumors

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Dysregulation in the FGFR tyrosine kinase family has been implicated in a number of human cancers, including gastric, breast, endometrial, and bladder carcinomas. We have previously described the discovery of a chemical series of FGFR kinase inhibitors employing a proprietary structure-based design approach. ARQ 087, a lead candidate for clinical development, inhibits FGFR1, 2, and 3 with IC_{50} values in the 2−4 nM range, with FGFR4 being inhibited 10-fold less potently. ARQ 087 displayed inhibition kinetics that were ATP-independent, while showing a 20-fold preference for inactive FGFR2 in biochemical assays. ARQ 087 showed potent inhibition of FGFR2 phosphorylation in KATO III (IC_{50} value = 150 nM) and SNU-16 (IC_{50} value = 45 nM) human gastric carcinoma cells with comparable antiproliferative potencies by MTS assay (KATO III

[GI_{50} value = 130 nM] and SNU-16 [GI_{50} value = 690 nM]). Concentration-dependent inhibition of phosphorylation of downstream FGFR signals (FRS, MEK, ERK, and AKT) is evident in response to ARQ 087 treatment. In addition, growth of SNU-16 gastric carcinoma, AN3CA endometrial cancer, and FGFR2-transfected Ba/F3 tumor xenografts in athymic mice was markedly suppressed after daily oral administration. Finally, ARQ 087 shows favorable pharmaceutical properties that warrant its consideration as a candidate for future clinical development.

120 POSTER

Primary tumor derived preclinical model mimics human colon cancer: a novel platform to study cancer biology and to evaluate anti-cancer drugs

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Background: Colon cancer is a major cancer in developed and developing nations for which, the underlying mechanism of initiation, maintenance and progression is relatively unknown. The preclinical models used to understand the biology of colon cancer were, till recently, less valuable largely due to lack of consistency in maintaining intra-tumor heterogeneity and tumor microenvironment. At present, established cancer cell lines and cell line based allograft or genetically engineered mouse models are being used for testing personalized therapeutics. However, these models often fail to mimic the real disease and therefore have had limited success as predictive platforms. In order to understand the biology of cancer pathways and testing novel anti-cancer agents, we have developed a novel organotypic explant culture and xenograft models using primary tumors from treatment naïve patients.
Materials and Methods: In this organotypic culture we used paracrine growth factors or ligands for receptors that were derived from the same patient. Extensive profiling of these tumors was performed using transcriptomics and phospho proteomics to provide a mechanistic insight of this system. As proof of concept, advanced stage colorectal cancer explants were treated with oxaliplatin at different concentrations for 4 days. Further freshly isolated treatment naïve primary tumors from patients was propagated in immune compromised mice and treated with oxaliplatin. Anticancer effect of oxaliplatin was evaluated by immuno-histochemical and biochemical analysis.
Results: Data indicate that presence of autologous human ligands significantly enhance the survival and viability of tumor cells due to signal induced activation of key oncogenic pathways. Dose and time dependent effect of oxaliplatin was observed in these models. Molecular profiling and histological data from both primary and xenografted tumor maintained in mice are very similar which suggests that these models preserve the pathological characteristics of primary tumors.
Conclusion: Our data indicate that signaling pathways responsible for tumor growth require human ligands for the activation of downstream signaling network in ex vivo setting. Anti-tumor efficacy of oxaliplatin in explant and primary human derived xenograft models correlates with the clinical outcome which suggest that these models might be useful to predict the treatment options for patients.

121 POSTER

Functional role of CD133 in glioblastoma multiforme

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Background: Glioblastoma multiforme (GBM) is a cytologically malignant tumour of the central nervous system, associated with poor prognosis and fatal outcome (5 year survival, <6%). Such tumours are believed to be initiated and maintained by a subpopulation of cells, which resemble normal adult stem cells. Cancer stem cells (CSCs), may contribute to the chemo-/radio-resistance exhibited by these tumours and can be identified using the immunocytochemical marker CD133. This pentaspan membrane protein is associated with increased tumorigenicity, chemo-/radio-resistance and poor prognosis. In this study we investigated the functional role of CD133 in the progression of GBM to elucidate any therapeutic benefits of modulating CD133 expression.
Materials and Methods: CD133-specific siRNA and siPORT-Amine transfection reagent were used to achieve knockdown of CD133 in GBM cell lines. Gene and protein expression were measured over time using real-time PCR and FACS, respectively. GBM cell lines were cultured in 1% oxygen to induce hypoxia. Transient knockdown of hypoxia-inducible factors (HIF) was achieved using HIF-specific siRNA, in hypoxic conditions. Biological functions of CD133 were assessed by performing wound-healing assay to investigate migration; MTT to measure the rate of proliferation; neurosphere formation to assess tumorigenicity; and etoposide drug challenge to assess chemo-resistance.
Results: Hypoxia upregulated CD133 expression by 4-fold ($p < 0.001$; $n = 3$) compared to cells cultured in normoxia. Knockdown of hypoxia inducible factors resulted in the downregulation of CD133 in hypoxia. For example, in GBM cell line U251, HIF2-α knockdown resulted in a significant reduction in CD133 gene expression (60% downregulation; $p < 0.01$; $n = 5$). CD133-specific siRNA successfully knocked down gene expression of CD133 (85% knockdown; $p < 0.0001$; $n = 3$) leading to significantly reduced migration ($p < 0.001$; $n = 3$); increased susceptibility to chemotherapeutic agent etoposide ($p < 0.05$; $n = 3$); and reduced neurosphere forming potential ($p < 0.05$; $n = 3$) in GBM cell lines. No change in cell proliferation was noted.
Conclusions: Hypoxia, via HIF-2a, increases CD133 expression in GBM cells. CD133 expression alters important biological properties of GBM cells with CD133 knockdown reducing their migration ability, tumorigenicity and sensitivity to chemotherapeutics. Therefore, using CD133 targeted therapies, in combination with established standards-of-care may improve GBM patient outcome.

122 POSTER

Novel class I PI3K inhibitor CH5132799: potential clinical application in rational combination with molecular targeted therapeutics

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Background: The phosphatidylinositol 3-kinase (PI3K) pathway regulates various cellular processes, such as proliferation and apoptosis. Class I PI3K is a heterodimer, consisting of a regulatory and a p110 catalytic subunit, which transduces signals from receptor tyrosine kinases (RTKs). One of four p110 isoforms, p110α is known to be actively mutated in various human cancers. CH5132799 is a potent class I PI3K inhibitor with a novel structure, which will be presented in an accompanying poster. We will also present data showing that PI3K pathway-activated tumors, particularly the PIK3CA-mutated tumors, are sensitive to CH5132799. Here, we describe the preclinical efficacy in combination with current standard therapeutics, including RTK-targeted drugs.
Results: The trastuzumab-insensitive breast cancer cell line KPL-4, which harbors Her2 amplification and PIK3CA mutation (H1047R), showed tumor regression by CH5132799 monotherapy. The combination of CH5132799 with trastuzumab induced remarkable antitumor efficacy, resulting in the disappearance of the xenografted tumors. This suggests that CH5132799 can overcome trastuzumab insensitivity in PIK3CA mutants through PI3K inhibition. With lapatinib, *in vitro* cell growth inhibition and apoptosis was enhanced in Her2-amplified breast cancer BT-474 cells. Consistently, this combination enhanced tumor growth inhibition in the BT-474 xenograft model. These data indicate potent compatibility of CH5132799 with Her2-targeted drugs.
Combined administration of CH5132799 with erlotinib was also examined. In NSCLC NCI-H292 cells, erlotinib treatment suppressed EGFR-driven Erk phosphorylation with weak suppression of Akt phosphorylation, whereas CH5132799 could completely suppress Akt phosphorylation. When the drugs were combined, phosphorylation of Erk and Akt were efficiently suppressed concomitantly. Using this rationale, the combination of CH5132799 and erlotinib achieved enhanced antitumor efficacy in a H292 xenograft model.
In addition to RTK-targeted drugs, CH5132799 combined with paclitaxel induced more prolonged tumor growth inhibition than each alone.
Conclusions: CH5132799 showed enhanced efficacy when combined with current therapeutics including RTK-targeted drugs and paclitaxel. These results suggest potential clinical applications of CH5132799 in combination therapy with molecular targeted agents and cytotoxics. CH5132799 is progressing toward phase I clinical trials.

123 POSTER

Anti-tumor activity of CXR1002, a novel anti-cancer clinical phase compound that induces ER stress and inhibits PIM kinases: Human tumor xenograft efficacy and in vitro mode of action

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Summary: CXR1002 is an ammonium salt of perfluorooctanoic acid. It has a unique pharmacokinetic, pharmacologic and toxicity profile and induces cell death in a wide range of human tumor cells *in vitro* and *in vivo*. CXR1002 causes an ER stress response, acts as a fatty acid mimetic,