ErbB receptor interactions were tested by coimmunoprecipitation, western blot and by FRET analysis.
Results: Analyses of ErbB receptor indicated that ErbB2 and ErbB 3 were expressed in the four cell lines studied.
These two receptors dimerize upon ligand binding, and this ligand-mediated interaction was long lasting. Overexpression of ErbB2 did not cause stable Her2-Her3 dimers.
The action of six different TKI on receptor activation and dimerization was analyzed. We observed that irreversible drugs more potently inhibited ErbB phosphorylation. Unexpectedly, we saw that some TKI interfere with oligomerization whereas others do not.
To gain insights into the mechanism by which distinct TKI differently affect ErbB dimerization, we compared crystal structures of EGFR tyrosine kinase domain, available at the Protein database (PDB) bound to four of the drugs. We found that drugs interfering with ErbB dimerization, bind to the close conformation of the tyrosine kinase domain.
Conclusions: NRG stimulation is compulsory for ErbB2/3 dimerization in breast cancer cell lines.
Some TKIs are able to interfere with ErbB2/3 dimerization while others do not, and this disparity is not due to their reversibility.
TKIs capable of disrupting ErbB dimerization can only bind to the closed ErbB tyrosine kinase domain conformation. Thus, ErbB kinase domain, plays a key role on ErbB dimerization.
In addition to offer information about the impact of distinct TKI on ErbB receptor dimerization, our results demonstrate a role of the open/close states of ErbB receptors in regulating stabilization of receptor-receptor complexes.

338 POSTER

Preclinical profile of novel and potent c-Met kinase inhibitors

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Background: *c-Met* is a proto-oncogene that encodes the protein Met with intrinsic tyrosine kinase activity. Aberrant Met kinase activity triggers a series of unwarranted phosphorylation events and signalling processes that ultimately lead to the development of cancer. Alteration of the Met kinase signalling cascade represents an attractive approach aimed at blocking invasion and metastasis of cancer cells. Herein, we describe the biological and pharmacokinetic properties of representative molecules from a series of novel and small molecule c-Met kinase inhibitors with scope to be further developed as clinical candidates for various cancers.
Methods: Met Kinase activity of the test compounds was determined using using an HTScan® recombinant human c-Met Kinase Assay Kit (Cell Signaling Technology, Beverly, MA) with modifications. Hepatocyte growth factor (HGF) induced cell proliferation assay (MTT) was conducted to determine the growth inhibitory effect of the compounds on the high Met kinase expressing sk-LMS-1 cell line. Inhibition of HGF induced Met kinase phosphorylation in LMS-1 cells was measured in an ELISA assay. Metabolic stability of the compounds was evaluated in microsomes obtained from mouse, rat, dog, monkey, and human. Pharmacokinetic behaviour of compounds in plasma after single dose oral administration or IV injection was determined in female Balb/c mice.
Results: Among the compounds evaluated, RP1088 and RP1101 demonstrated remarkable potency against the purified Met kinase by inhibiting enzyme activity at low nanomolar concentrations (K_i <6 nM). In addition, the compounds caused a significant reduction in HGF-stimulated proliferation (IC_{50} <100 nM) and phosphorylation (IC_{50} <50 nM) in sk-LMS-1 cells. Pharmacokinetic studies in female Balb/c mice indicated good oral absorption with peak plasma concentrations reaching above 2 μM. Further, the compounds were metabolically stable across the species studied.
Conclusions: Our findings demonstrate that RP1088 and RP1101 are potent Met kinase inhibitors with a favourable pharmacokinetic profile and IC_{50} values comparable to existing Met kinase inhibitors in development. Besides Met kinase, these compounds have the potential to inhibit the anaplastic lymphoma tyrosine kinase (ALK) and are currently being evaluated in relevant cell assays. The compounds are also being tested for *in vitro* and *in vivo* efficacy across various cancer cell lines and xenograft models besides selectivity against other receptor tyrosine kinases.

339 POSTER

Fibroblast growth factor receptor 4 (FGFR4) G388R polymorphism in colorectal cancer

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Introduction: Fibroblast Growth Factors (FGFs) and their receptors (FGFRs) play a crucial role for cell proliferation, differentiation, and migration. In tumorigenesis their expression and activity is frequently deregulated. A genetic polymorphism has been described in the transmembrane domain of FGFR4 (G388R) and has been correlated with enhanced tumor aggressiveness in several tumour types. In colon cancer its role is under dispute.
Materials and Methods: Tissue specimens of human colon cancer patients were collected and allelic expression of FGFR4 was measured. In addition the expression of FGFR4 in different colon cancer cell lines were analyzed. Cell lines specifically overexpressing FGFR4-G388 (G388) and FGFR4-R388 (R388) were constructed and the biological impact of transgene expression on cell viability, proliferation, clonogenicity, migration, and anchorage independent growth was tested in vitro. The transfected cells were injected subcutaneously into SCID-mice and tumor growth was measured during a period of 4−9 weeks. Tumors and lungs of the mice were harvested and evaluated by immunohistochemistry. Furthermore the consequences of FGFR4 knock down on the biological characteristics of the tumor cells were assessed.
Results: Presence of the R388 allele was predominant in higher grade human tumors and metastatic lesions suggesting a role for this allele in invasion and metastasis. In vitro data support this assumption. R388 overexpression strongly stimulated cell migration but decreased clonogenicity while G388 had the reverse effect. In clonogenicity and anchorage independent growth G388 demonstrated a strong stimulatory effect.Tumorigenicity in vivo was differentially affected by the G388 and R388 alleles with G388 enhancing local tumor growth, while R388 overexpressing cells had a higher tendency of metastasis to the lung. SiRNA mediated knock down showed downregulation of viability, migration and colony formation in all tested cell lines.
Conclusion: Based on the results of this study both forms of FGFR4 have to be regarded as oncogenes and relevant targets for therapy in colorectal cancer. While R388 overexpression was correlated with higher tumor aggressiveness in vivo, mediated by upregulation of cell migration, overexpression of G388 stimulated malignant cell growth in vitro and enhanced local tumor growth in vivo.

340 POSTER

Inhibition of aldehyde dehydrogenase (ALDH) reduces chemotherapy and radiation resistance of stem-like ALDHhiCD44+ breast cancer cells

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Breast cancer is a leading cause of death in women, due primarily to the ineffective treatment of metastatic disease. In order to reduce mortality from breast cancer, it is essential to learn more about the biology of the metastatic process, specifically what makes metastases so resistant to current cancer treatments. Recently, we discovered stem-like $ALDH^{hi}CD44^{+}$ cells in several different breast cancer cell lines that demonstrated significantly increased metastatic behavior both *in vitro* and *in vivo* when compared to $ALDH^{low}CD44^{-}$ cells. The objectives of the current study were to determine the response of $ALDH^{hi}CD44^{+}$ stem-like breast cancer cells to standard cancer therapy and to test the hypothesis that differentiation therapy with All-*trans* Retinoic Acid (ATRA) would sensitize these cells to therapy. $ALDH^{hi}CD44^{+}$ (stem-like) and $ALDH^{low}CD44^{-}$ (non stem-like) populations were isolated by FACS from MDA-MB-231 and MDA-MB-468 breast cancer cells lines and were plated onto 24-well dishes. The cells were then exposed to vehicle, chemotherapy (doxorubicin [0.1−0.5 μM] or paclitaxel [0.1−0.4 μM]) or radiation [2 × 3−5 Gy] in the presence or absence of ATRA. After 72 hours of treatment, cells were harvested and viable cells were quantified using trypan blue exclusion, or 1000 viable cells were re-plated into 6-well dishes and colonies were counted after 2 weeks. Significantly more $ALDH^{hi}CD44^{+}$ cells survived chemotherapy when compared to $ALDH^{low}CD44^{-}$ cells ($p < 0.001$). Western blots were performed in order to identify proteins that may be contributing to this therapeutic resistance. Glutathione-*s*-Transferase pi (GSTpi) and p-glycoprotein (Pgp) were found