dye labeled secondary antibodies. Finally, the protein expression levels of NSCLC tumors were associated with their response to specific drugs.
RPPA analysis was performed for 78 proteins in 53 xenograft models. Statistical analysis indicated significant associations between the expression of distinct proteins and the response rate to certain drugs (Erlotinib, Cetuximab, Paclitaxel, Carboplatin). We revealed an association between higher phospho-p38 expression and increased Paclitaxel response rate. ERK1/2 downregulation was observed upon Cetuximab treatment in responders.

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Tropomyosin Tm5NM1: A novel target for cancer therapy

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Background: The actin cytoskeleton is an important regulator of a variety of cellular functions including cell motility, adhesion, and proliferation. Changes in these processes are fundamental to cellular transformation making the actin cytoskeleton a long sought after chemotherapeutic target. Drugs developed to date have been unsuccessful due to their lack of specificity which ultimately causes unacceptable cardiac and respiratory toxicity. We have previously shown that tropomyosin (Tm), an integral component of the actin cytoskeleton, defines functionally distinct populations of actin filaments. We have identified a specific Tm isoform common to all tumour cells which regulates cell proliferation and have designed a new class of compounds to target this filament population.
Summary of results: The role of Tm5NM1, a ubiquitously expressed low molecular weight (LMW) Tm isoform, was investigated using both overexpression and knockdown neuroblastoma cell systems. Using clonogenic and proliferation assays we ascertained that elevated levels of Tm5NM1 accelerated cell proliferation. Conversely, siRNA knockdown of Tm5NM1 resulted in decreased cell growth. We have developed a novel class of anti-Tm compounds that target LMW Tm5NM1 containing filaments. Our lead compound, TR100 targets the actin cytoskeleton and is effective against a panel of neuroblastoma and melanoma cell lines (average LC50 ~2−3 uM). TR100 inhibited survival and growth in a 3D melanoma model, which simulates the tumour microenvironment, and significantly reduced tumour growth in the B16/F10 melanoma mouse model. In vivo data from the drug treated animals also showed no evidence of cardiac damage as measured by blood Troponin I levels and no obvious hypertrophy as measured by intraventricular septum thickness.
Conclusions: We have demonstrated for the first time a novel class of chemotherapeutic compounds which specifically target an actin filament population required for cell growth. This has enormous implications for the treatment of a variety of cancers.

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CD133+ or CD44+CD166+ cells from human colorectal cancer cell lines do not display cancer stem-cell features nor increased drug resistance

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Background: Tumour initiation and maintenance is caused by rare tumour cell subsets, defined as “cancer stem cells (CSCs)”, endowed with self-renewal and differentiation capacity. CSCs have a number of properties permitting them to survive conventional cancer chemotherapy and radiotherapy. The development of alternative therapeutic approaches specifically targeting CSCs is urgently needed. Primary screening of novel anti-cancer compounds is conventionally conducted on established tumour cell lines, easy to propagate in vitro and amenable to high throughput studies. However, whether they do actually comprise CSC populations resembling those of primary tumours remains highly debated.We performed phenotypic and functional characterization of putative CSC populations in established cell lines of human colorectal carcinoma (CRC) and evaluated their suitability for predicting efficacy of anti-cancer therapies.
Material and Methods: A panel of 10 established human CRC cell lines was studied. Expression of putative CSC markers, including CD133 or CD44/CD166 molecules, was evaluated by flow cytometry. CD133+ or CD44+ CD166+ cells were sorted from individual cell lines by flow cytometry and evaluated for CSC properties in comparison to their negative counterparts or to the parental cell line. Spheroid formation ability, clonogenicity, stemness-related gene expression, aldehyde-dehydrogenase (ALDH)-1 activity, side population (SP) phenotype, in vitro invasiveness, chemo-resistance and tumorigenicity upon injection in NOD/SCID mice were assessed.
Results: None of the putative CSC phenotypes analyzed was found to be significantly associated with functional features of CSC. Importantly, neither CD133+ nor CD44+ CD166+ cells showed significantly increased resistance to chemotherapeutic drugs currently in use for CRC treatment, as compared to their negative counterparts.
Conclusions: On established CRC cell lines, the expression of putative CSC markers does not correlate with CSC functional features. Our findings question the adequacy of established CRC cell lines for screening of CSC-specific therapies and underline the urgency to develop novel platforms for anti-cancer drug discovery.

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Splicing factors as novel therapeutic targets in ovarian and breast cancers

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We have reported earlier that two splicing factors, polypyrimidine tract binding protein (PTB) and SRp20 are overexpressed in ovarian tumors, compared to matched controls (*He et al, Clin Cancer Res 10:4652−60, 2004*). PTB is a widely-expressed RNA binding protein belonging to the heterogeneous nuclear ribonucleoprotein family (and is also known as hnRNP I) whose molecular functions include regulating internal ribosomal entry site (IRES)-mediated translation and, importantly, alternative splicing. SRp20 is a member of the serine/arginine-rich (SR) protein family with multiple functions in RNA processing such as polyadenylation and alternative splicing. Immunostaining of tissue microarrays revealed that both PTB and SRp20 are expressed differentially between benign tumors and invasive EOC, and between borderline/Low Malignant Potential tumors and invasive EOC. Our staining results reveal that expression of PTB and SRp20 is associated with malignancy of ovarian tumors but not with stage of invasive EOC (*He et al, ms submitted*). In addition to these clinical observations, we found that both of these splicing factors are highly expressed at the earliest stages of transformation in ovarian and breast tumor cell lines. Importantly, at least for PTB, there is little or no expression in normal ovarian surface epithelial cells and normal blood precursors. We established stable ovarian (A2780) and breast (MCF7) cell lines carrying doxycycline (Dox)-inducible PTBshRNA or SRp20shRNA. Knockdown by shRNA of either of these splicing factors in ovarian or breast tumor cell lines led to decreased cell growth. Moreover, we observed decreased colony formation and invasiveness in the A2780-PTBshRNA cells (*He et al, Oncogene 26:4961−8, 2007*). Further, knockdown of SRp20 in A2780 ovarian and MDA-MB-231 breast tumor cells by ~90% led to apoptosis that was associated with caspase-3, -7, and -9 activation and decreased expression of Bcl-2. Last, feeding Dox to mice bearing A2780 xenografts stably infected with Dox-inducible PTBshRNA led to suppression of tumor growth, compared to controls. Overall, our results suggest that both PTB and SRp20 may be novel therapeutic targets. Accordingly, we have begun to develop a cell-based two color assay to screen for small molecules that will inhibit these splicing factors, with the goal of bringing a new small molecule to the clinical treatment of ovarian and possibly breast cancer.
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Differential action of ErbB kinase inhibitors on receptor oligomerization

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Background: ErbB tyrosine kinase receptors participate in several physiological processes and their deregulation is involved in the pathophysiology of cancer. Two main types of agents have been developed against them: monoclonal antibodies and small tyrosine kinase inhibitors (TKIs). We have studied the action of some TKI on ErbB activation and receptor interactions.
Material and Methods: Four breast cancer cell lines were used as models: MCF7 and T47D, BT474 and SKBR3. EGF and NRGb1 were employed as ErbB ligands.
Six TKI were used in this work, three reversible (erlotinib, gefitinib, and lapatinib), and three irreversible (canertinib, pelitinib and neratinib).

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.

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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.

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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.

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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