to be over-expressed in the $ALDH^{hi}CD44^{+}$ population compared to the $ALDH^{low}CD44^{-}$ population ($p < 0.05$), indicating that these proteins may play a significant role in the therapy resistance in these cells. Pre-treatment of cell populations with ATRA or diethylaminobenzaldehyde (DEAB, an ALDH inhibitor) had no effect on $ALDH^{low}CD44^{-}$ cells, but resulted in a significant sensitization of $ALDH^{hi}CD44^{+}$ cells to doxorubicin, paclitaxel, and radiation initially ($p < 0.01$); however, only cells that were pre-treated with DEAB maintained this sensitization over the long term and resulted in fewer colonies being formed after 2 weeks in culture compared to the cells only control ($p < 0.01$). In summary, the results of this study demonstrate that stem-like $ALDH^{hi}CD44^{+}$ cells may play an important role in therapy resistance, potentially via increased expression of Pgp and GSTpi. In addition, pre-treatment with the differentiation agent ATRA appears to convey an initial sensitization of $ALDH^{hi}CD44^{+}$ cells to both chemotherapy and radiation; however, DEAB was able to sensitize the $ALDH^{hi}CD44^{+}$ cells for much longer, indicating that ALDH may play a more important role in therapy resistance than was first thought. Ongoing experiments are aimed at clarifying the mechanisms behind this sensitization.

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Targeting NEK2 kinase in drug resistant multiple myeloma with small molecule inhibitors

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Background: Drug resistance is a major obstacle in cancer therapy. The molecular mechanisms of drug resistance still remain largely elusive. Microarray analyses on paired primary myeloma samples at baseline and after therapy or at relapse showed that NEK2 was one of the most up-regulated genes in myeloma cells after high-dose chemotherapy or at relapse. Additionally, high expression of NEK2 was associated with a shorter event-free and overall survival in multiple cancer types.
Materials and Methods: Three-dimensional models of Nek2 were generated using the crystal structures of Nek2 (PDB ID: 2JAV and 2W5A). High throughput virtual screening was carried out using ICM and GOLD docking programs. A biochemical kinase assay was performed using a TR-FRET approach.
Results: Our studies indicate that over-expressing NEK2 in cancer cells resulted in enhanced cell proliferation and drug resistance, whereas knockdown of NEK2 by RNAi induced significant cancer cell death and growth inhibition. In order to identify novel small molecule inhibitors of NEK2, we utilized three-dimensional models of the NEK2 crystal structure and computer-aided, structure-based drug design. These tools allowed us to virtually screen 2 million compounds in our computational database, which we followed up by screening just 18 compounds in a biochemical kinase assay. These efforts yielded a chemical lead containing a substituted benzo[*d*]imidazole scaffold with sub-micromolar activity (IC50 = 0.6 microM) in a NEK2 biochemical kinase assay and good cell-based activity in drug resistant myeloma cell line models. Lead optimization around this chemical series is ongoing in efforts to generate a drug-like compound that can be advanced into myeloma animal studies and eventually into clinical evaluation.
Conclusion: We conclude that NEK2 represents a predictor for drug resistance and poor prognosis in cancers and could be a potential target for cancer therapy. Furthermore, small molecule NEK2 kinase inhibitors based on the benzo[d]imidazole scaffold show promise as therapeutic agents targeting treatment-refractory myeloma.

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Characterization of cancer cell killing mechanisms of targeted hybrid peptide that binds to transferrin receptor

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The transferrin receptor (TfR) is a cell membrane-associated glycoprotein involved in the cellular uptake of iron and the regulation of cell growth. Various studies have shown the elevated expression levels of TfR on cancer cells compared with normal counterparts, and the extracellular accessibility of this molecule make it an excellent antigen for the treatment of cancer. Recently, we generated series of molecularly-targeted drugs named hybrid peptide, which is chemically synthesized peptide composed of target-binding peptide and lytic peptide containing cationic-rich amino acids components that selectively disintegrates the cancer cell membrane. In this study, we designed TfR binding peptide connected with the newly designed lytic peptide and demonstrated the selective cytotoxic activity and the characterization of cancer cell killing mechanisms of this molecule.
Cytotoxic activity of TfR-lytic peptide to various cancer cell lines was as low as 4.0 to 9.3 mM of IC_{50}. On the other hand, normal cells were less sensitive to this TfR-lytic peptide (IC_{50} >30 mM). It was found that cytotoxic activity was correlated well with the expression levels of TfR on the cell as assessed by flow cytometry. Competitive assay using TfR antibody or knock down of this receptor by siRNA confirmed the specificity of TfR-lytic peptide to TfR. In addition, it was revealed that this molecule can penetrate cell membrane to make the pore on the T47D cancer cell surface within 10 min to effectively kill these cells. Finally, the cell death mechanism of TfR-lytic peptide assessed by annexin-V binding, caspase activity, and JC-1 staining were assessed, and it was found that this molecule induces approximately 80% of apoptotic cell death via caspase 3&7 activation and cytochrome C discharge but not to normal cells.
Taken together, we concluded that TfR-lytic hybrid peptide may provide potent and selective anticancer therapeutic options to TfR-expressing cancer.

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Targeting p21-activated kinase 1 (PAK1) to induce apoptosis of squamous NSCLC cells

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Background: P21-activated kinases (PAKs) are serine/threonine protein kinases that serve as important mediators of Rac and Cdc42 GTPase function. PAK1 has been implicated in proliferation and survival signaling by growth factor and hormone receptors, in addition to morphogenetic processes that control cell polarity, invasion and actin cytoskeleton organization.
Results: To better understand the role of PAK1 in tumorigenesis, PAK1 expression was assessed via immunohistochemistry for approximately 600 tumors comprising multiple indications. Strong PAK1 expression was prevalent in specific tumor subtypes. For instance, elevated PAK1 expression was observed in 64% (cytoplasmic staining) and 30% (nuclear staining) of primary squamous cell lung carcinomas and prognostic significance was assessed using patient survival data. The functional requirement for PAK1 in tumor cell proliferation, survival and migration was further investigated via small molecule inhibitors and RNA interference, and in vivo anti-tumor efficacy was assessed for inhibition of PAK1 and PAK2 both individually and in combination. To better understand how PAK1 inhibition could be utilized for treatment of squamous NSCLC, a screen was performed testing a panel of compounds with well-characterized mechanism of action in isogenic lines with or without PAK1 knockdown. In accordance with our data for PAK1 effector signaling, strong combinatorial activity was observed for PAK1 knockdown and inhibitor of apoptosis (IAP) protein antagonists, a class of inhibitors that are currently in clinical development.
Conclusions: Taken together, our extensive preclinical validation of PAK1 inhibition renders this target attractive for future clinical investigation.

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Pharmacological and pharmacokinetic profile of AI1008, a novel and potent inhibitor of abl-kinase activity

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Background: Chronic Myelogenous Leukemia (CML) is a myeloproliferative disorder characterized by an increased proliferation and reduced apoptosis of malignant cells especially WBCs. The cancer involves a reciprocal translocation of the BCR and ABL genes resulting in a BCR-ABL fusion known as the *Philadelphia chromosome*. The objective of this study was to investigate the pharmacological and pharmacokinetic properties of AI1008, a novel small-molecule inhibitor of abl-kinase.
Methods: Abl-kinase activity of AI1008 was determined using using an HTScan® recombinant human abl Kinase Assay Kit (Cell Signaling Technology, Beverly, MA) with modifications. Viability assay (MTT) was conducted to determine the growth inhibitory effect of the compound on the *bcr-abl* overexpressing K562 cell line. Metabolic stability of the compound was evaluated in microsomes obtained from mouse, rat, dog, monkey, and human. Pharmacokinetic behaviour of AI1008 in plasma after single dose oral administration or IV injection was determined in female Balb/c mice.

Results: AI1008 demonstrated remarkable potency against the purified abl kinase by inhibiting enzyme activity at low nanomolar concentrations (IC_{50} = 3.1 nM). Additionally, the compound caused a significant reduction in viability of K652 cells (IC_{50} = 1.3 nM). Pharmacokinetic studies in female Balb/c mice indicated good oral absorption of AI1008 with a C_{max} value of 1.6 μM. Further, the compound had an elimination half-life of 1.5 hr with a clearance value of 26.9 ml/min/kg.
Conclusions: Our results demonstrate that AI1008 is a potent abl-kinase inhibitor with a favourable pharmacokinetic profile and comparable IC_{50} values to existing abl-kinase inhibitors. AI1008 is currently being tested for *in vitro* and *in vivo* efficacy across various cancer cell lines and selectivity against other receptor tyrosine kinases. Besides CML, xenograft models of gastrointestinal stromal tumors are currently underway to evaluate the efficacy of AI1008.

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Preclinical profile of novel, potent, and selective PI3 kinase delta inhibitors

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Background: Phosphoinositide-3 kinase (PI3K) belongs to a class of intracellular lipid kinases that phosphorylate the 3 position hydroxyl group of the inositol ring of phosphotidylinositol. The PI3K pathway is frequently activated in human cancers and thus represents an attractive target for small molecule inhibitors. Pan-PI3K inhibitors currently in development have been associated with adverse side-effects such as insulin resistance, thus necessitating the need to develop isoform specific inhibitors of PI3K. Herein, we describe the biological and pharmacokinetic properties of representative molecules from a series of novel and small molecule PI3Kδ inhibitors with scope to be further developed as clinical candidates for hematological malignancies such as acute myeloid and lymphoblastic leukemias.
Methods: Activity of test compounds on individual PI3K isoforms was determined by a Homogenous Time Resolved Fluorescence assay (Millipore, Billerica, MA) with modifications. Cell viability assay was conducted to determine the growth inhibitory effect of the compounds on the high PI3Kδ expressing THP-1 cell line. Inhibition of Akt phosphorylation was determined by Western blotting using monoclonal antibodies (Cell Signaling Technology, Beverly, MA) directed against the Ser473 and Thr308 residues. CYP inhibition potential of the compounds was evaluated in human liver microsomes.
Results: Among the compounds evaluated, RP5057, RP5066 and RP5067 inhibited PI3Kδ with IC_{50} values of 11.2, 7.4, and 23.3 nM respectively. Besides, the compounds displayed a high degree of selectivity over the alpha (>170–400 fold), beta (>40–120 fold), and gamma (>100–500 fold) isoforms. In addition, the compounds caused a dose-dependent reduction in viability of THP-1 cells. While total Akt remained unaffected, the compounds caused a significant reduction in phosphorylation at both the Ser473 and Thr308 sites. Further, the compounds showed no significant CYP inhibition in human liver microsomes.
Conclusions: Results demonstrate the PI3K delta selective nature of RP5057, RP5066, and 5067 along with an ability to suppress proliferation of cancer cells. *In vitro* selectivity and potency data indicate the therapeutic potential of the compounds in hematological cancers without the deleterious effects commonly associated with the Pan PI3K inhibitors. The compounds are currently being tested for *in vitro* and *in vivo* efficacy across various cancer cell lines and xenograft models besides selectivity against other kinases.

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Activated RON tyrosine kinase drives cellular proliferation, migration and invasion with corresponding tumor growth and metastasis in mice

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The receptor tyrosine kinase RON (MST1R) is closely related to cMet, but its role in cancer biology remains as yet poorly defined. Activating mutations of RON appear to be rare in human cancer, hence autocrine or paracrine stimulation by its unique ligand MSP (macrophage stimulating protein) are likely to be required to drive its tumorigenic activity *in vivo*. Since murine MSP does not bind to human RON, most human xenografts lacking MSP expression do not activate RON in mouse models, and thus the effect of RON signaling on tumour growth has likely been underestimated in murine xenograft models. We developed an engineered tumour model that can be driven by RON in the absence of a functional MSP signal, which was used to characterize the effects of RON activity *in vitro* and *in vivo*. NIH3T3 cells were retrovirally transfected with a constitutively active form of the human RON kinase to derive stable clones (termed 3T3 caRON) which were extensively characterized for signaling and phenotypic effects.
3T3 caRON cells show strong constitutive phosphorylation of RON *in vitro*, with concomitant activation of pAKT and pERK. This phosphorylation can be further increased by MSP, demonstrating a substantial, although incomplete, activation of RON in these cells. 3T3 caRON cells show enhanced migration, invasion, proliferation and anchorage-independent growth compared to the parental NIH3T3 cells, these effects being further stimulated by the addition of MSP. Similar signaling and oncogenic phenotypes were observed after MSP-stimulation of non-engineered tumour cell lines, demonstrating that the 3T3 caRON cells recapitulate bona fide RON-dependent activities. Importantly, 3T3 caRON cells grow aggressively as tumour xenografts and metastases in mice, whereas parental NIH3T3 cells are not tumorigenic, demonstrating that activated RON is required for tumour growth in this model.
In conclusion, 3T3 caRON cells can be used to model the consequences of RON activation and effects of RON-directed therapeutic intervention in RON-dependent tumours in mice *in vivo*, which has not previously been possible due to the inability of mouse MSP to activate human RON.

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Blockade of fatty acid synthase causes ubiquitination and degradation of PI3K effector proteins in ovarian cancer

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Phosphatidylinositol-3 kinase (PI3K) controls proliferation, differentiation, tumorigenesis, and apoptosis. Many ovarian carcinomas harbor aberrations within this pathway. The PI3K target AKT phosphorylates mTOR. mTOR activates S6 via p70S6K. S6 is involved in translation control. mTOR also phosphorylates eukaryotic translation initiation factor 4E (eIF4E) inhibitor binding protein 1 (4EBP1), which also controls translation. In addition, many ovarian carcinomas harbor aberrations of the ErbB1 or -2 receptor. Although PI3K transmits ErbB-derived signals and stimulates cancer growth clinical studies yet reveal that ErbB1 or -2 drugs are largely inefficient in ovarian carcinoma. Therefore, novel targeting strategies are urgently needed. Fatty acid synthase (FASN) being overexpressed in ~80% of ovarian tumors is a marker for poor prognosis. It supports formation of membrane lipid rafts, which accommodate growth factor receptors incl. ErbBs. FASN thus facilitates signal generation and inhibition of FASN delays ovarian cancer progression. We reported that the FASN inhibitor C75 downregulates ErbB1 and -2 in ovarian cancer and sensitizes the cells against ErbB drugs (Grunt et al., BBRC, 385, 454). We now show that C75 abrogates A2780 ovarian cancer cell growth. This correlates with reduced phosphorylation of AKT, mTOR, p70S6K and 4EBP1 in Western blots caused by specific deactivation and by ubiquitin-mediated degradation of these PI3K effectors. In contrast, specific activation of the mitogen-activated protein kinase ERK1/2 is increased, although ERK1/2 steady-state levels are concurrently decreased by C75. In comparison, the PI3K inhibitor LY294002 blocks phosphorylation while concurrently upregulating steady-state levels of AKT, mTOR, p70S6K, and 4EBP1, and it activates ERK1/2. This suggests that PI3K/AKT cross-inhibits ERK1/2, which is abrogated by PI3K/AKT silencing, and that PI3K, but not ERK1/2, is crucial for growth arrest of ovarian cancer cells. We demonstrate for the first time that C75-mediated silencing of PI3K reduces phosphorylation and protein stability by increasing ubiquitination. Thus, C75 provides additive anticancer action, when compared to the LY294002, which directly targets PI3K and downstream signaling, but does not stimulate protein degradation. In summary, FASN is a promising drug target, which should be further developed for use in ovarian carcinoma.
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