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.