likewise were sensitized to the combination. Selective downmodulation of protein and mRNA transcript levels of Wee1 and Chek1 were confirmed and little off target toxicity was seen in the RNAi assays. To move findings into the clinic we confirmed our observation with the first-in-class oral Wee1 inhibitor (MK-1775) which is currently in phase I clinical trials in solid tumors and well tolerated (Schellens et al, ASCO 2009). MK-1775 (www.axonmedchem.com/product/1494mk1775) +/− AraC was tested in a panel of 8 leukemia cell lines and exhibited extremely potent sensitization across various AML and ALL (acute lymphoid leukemia) and BCR-ABL positive CML (chronic myeloid leukemia) cells (K562), with a range of sensitization from >2 of up to 12 times. Ex-vivo validation with primary blasts is ongoing in preparation for a clinical trial. Wee1gene expression increased progressively in samples from AML, ALL and CML patients compared to normals (Oncomine) and together with a recent paper in solid tumors, strongly suggests that Wee1 expression in advanced myeloid and even more so in lymphoid diseases represents a genomic context of vulnerability that can be exploited for parallel biomarker development.
Conclusion: The presented data strongly suggests the potential to combine AraC with novel inhibitors against Wee1 kinase in clinical trials, based on identification of Wee1 as the most potent sensitizer kinase of the human kinome in RNAi screens, potent in vitro and ex vivo sensitization to the first in class Wee1 inhibitor and an underlying genomic context of vulnerability. We are currently designing and developing a clinical trial combining AraC + Wee1 inhibitor to improve outcome of patients with acute leukemias, including patients with ALL and advanced CML.

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TG02, a multi-kinase inhibitor with potent single agent and chemosensitization activity against solid tumors

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Kinase inhibitors have found applications in multiple oncology settings due to their ability to target key signaling pathways in many different cancers. In general, the broad-spectrum kinase inhibitors have yielded better clinical outcomes than more selective ones because they block more than one pathway critical for tumor growth. We describe herein the pharmacological profile of TG02, a multi-kinase inhibitor being developed in the clinic by Tragara Pharmaceuticals, which combines CDK inhibition with activity against kinase targets involved in antiapoptotic signaling & other aspects of the malignant phenotype.
Effects on cell proliferation were determined by CellTiter-Glo or MTT assay and cell cycle & apoptosis analyses were performed by PI & Annexin V staining and analyzed by FACS. *In vitro* drug synergies were explored using a caspase 3/7 ELISA and the PK, PD & *in vivo* activity of TG02 were tested in nude mice bearing established xenografts.
TG02 inhibits the cell cycle regulatory CDK1 and CDK2 and the transcriptional regulatory CDK9 with IC_{50} values around 10 nM, as well as other kinases implicated in malignant progression, including JAK2 and the emerging oncogenic MAP kinase ERK5, with similar potency. TG02 potently inhibits proliferation across a broad panel of human solid tumor cell lines (n = 29, IC_{50} from 30 to 504 nM). This potency exceeded that of other CDK inhibitors currently in clinical development (SNS-032 and seliciclib) and a JAK2 inhibitor that lacks CDK activity (TG101348), suggesting that the unique spectrum of kinases inhibited by TG02 may provide enhanced antitumor activity in solid tumors. TG02 induced G2/M cell cycle arrest that rapidly progressed to robust apoptosis in HCT-116 cells & synergized with doxorubicin in pancreatic and breast cancer cell lines, and with gemcitabine in ovarian carcinoma (OC) cells. TG02 was cleared from the blood within 8 hours of oral administration but was retained in tumor masses at supratherapeutic levels for 24−48 hours. Accordingly, pathway-related biomarkers were markedly suppressed for 24−72 hours after dosing. TG02 significantly inhibited tumor growth in a range of human xenograft models and synergized with SOC drugs. Chemosensitization pathways under investigation include CDK9/Mcl-1 in SCLC, JAK2/Bcl-2 in OC and ERK5 in breast cancer.
TG02 is a multi-kinase inhibitor with a previously unreported spectrum of targets, that shows promising preclinical activity for the treatment of solid tumors in man.

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Preclinical characterization of GDC-0068, a novel selective ATP competitive inhibitor of Akt

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Background: Akt is one of the most frequently activated protein serine/threonine kinases in human malignancies. As a central node of the PI3K-Akt-mTOR pathway, Akt plays a critical role in cancer initiation, progression and therapeutic resistance. From high-throughput screening and medicinal chemistry approaches, we discovered GDC-0068, a novel, selective, orally bioavailable small molecule inhibitor against this important and attractive therapeutic target.
Methods: The effect of GDC-0068 on cell proliferation and viability was evaluated in human cancer cell lines of various genetic backgrounds and its effect on xenograft tumor growth was assessed in nude mice. The inhibitory activity of GDC-0068 on Akt signaling was also characterized employing specific biomarkers of the Akt pathway both in vitro and in vivo.
Results: GDC-0068 is an ATP-competitive kinase inhibitor that is active against all 3 Akt isoforms with enzymatic IC50 values of 5−30 nM. It is highly selective against other protein kinases, with >100-fold selectivity over the closely related Protein Kinase A. GDC-0068 blocks Akt signaling both in cultured human cancer cell lines and in xenograft tumors as evidenced by dose-dependent loss of downstream target phosphorylations. As expected from its specific inhibition of Akt activity, GDC-0068 blocks cell cycle progression and inhibits the viability of cancer cell lines driven by Akt signaling. Dose-dependent and reversible increases in blood glucose and insulin levels were also observed in animal models treated with GDC-0068, consistent with its ability to inhibit Akt-mediated insulin signaling. In multiple cancer xenograft models, GDC-0068 is well tolerated and induces dose-dependent anti-tumor responses, ranging from tumor stasis to regression, when administered orally.
Conclusions: GDC-0068 is a novel, highly selective, ATP competitive Akt kinase inhibitor that demonstrates pharmacodynamic inhibition of Akt signaling and robust anti-tumor activity in human cancer cells in vitro and in vivo. These preclinical findings provide compelling evidence in support of clinical development of GDC-0068 as an anti-cancer agent.

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Branched peptides as targeting agents for tumor imaging and therapy

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Identification of new tumor-selective targets, which might allow either cancer cell tracing or therapy, is a crucial issue in cancer research. Membrane receptors for endogenous peptides such as neurotensin, somatostatin, bombesin and many others are over-expressed in different human cancers and could therefore be targeted as tumor-specific antigens. Peptide-receptor targeting might offer the advantage of contemporary providing both tumor targets and selective targeting agents, in the form of peptide ligands. The drawback, which has limited development of peptide drugs in oncology, is their short half-life caused by peptidase and protease hydrolysis.
We demonstrated that oligo-branched peptides can retain binding efficiency of corresponding linear sequence and become resistant to peptidase degradation. Our goal is to produce branched peptide molecules which can be used both for a specific receptor-tracing and for therapy or *in vivo* imaging, by carrying and delivery of either chemical tracers or chemotherapeutics to tumor cells that over-express peptide receptors.
We had found that tetra-branched neurotensin (NT) retains receptor-binding activity and becomes resistant to proteolysis by serum enzymes. We developed modular tetra-branched NT peptides (NT4), which can be used as 'theranostics', for both diagnosis and therapy, with no modification of the tumor targeting sequence, but only by addition of different functional units to a conserved branched core. Fluorophore-conjugated NT4 allow discrimination between tumor and healthy tissue in human surgical samples from colon and pancreas adenocarcinomas. Tumor versus healthy peptide binding was measured in each patient by quantitative analysis of confocal microscopy images, which also allowed statistical analysis and validation of NT4 targets. Drug-armed branched peptides were synthesized with different conjugation methods, resulting either in uncleavable adducts or drug-releasing molecules. Human cell lines from colon (HT-29), pancreas (PANC-1) or prostate (PC-3) carcinoma were treated with NT4 conjugated to several different chemotherapy drugs. We found that conjugation to NT4 switches drug internalization to a peptide-receptor mediated mechanism,

which greatly increases drug selectivity toward cancer cells and also might allow by-passing drug cell resistance, when this is generated by mechanism of drug internalization or drug export. Moreover, modularity of drug-armed NT4 allows tailoring of drug-armed peptides on the basis of sensitivity of cancer cells to different drugs.
NT4 armed with 5-fluoro-deoxyuridine was used for *in vivo* experiments in HT-29-xenografted mice and produced a 50% reduction of tumor growth with respect to animals treated with equal amount of the un-conjugated drug.
In vitro and *in vivo* results indicated that branched peptides are valuable tools for tumor selective targeting.

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Potential clinical application of a novel Heat Shock Protein 90 inhibitor CH5164840: preclinical efficacy in mono therapy and combination therapy

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Background: HSP90 is a molecular chaperone and plays an important role in protein folding and stability. In tumor cells, HSP90 is activated by forming super chaperone complexes with co-chaperones. Inhibition of HSP90 function leads to degradation of multiple oncogenic client proteins, resulting in loss of signal transduction, growth inhibition, cell death, and anti-angiogenesis. This unique feature is expected to overcome the problem of resistance to TKIs. Thus, targeting HSP90 is considered to be an attractive strategy for anticancer therapy.
Results: We have identified CH5164840 as an HSP90 inhibitor with a novel chemical structure through virtual screening based on 3D-structure. CH5164840 binds to an ATP-binding pocket of HSP90 comparable to that of ansamycins, 17-AAG and 17-DMAG. Treatment with CH5164840 showed marked degradation of multiple clients in a dose- and time-dependent manner. Consistent with its selective binding to HSP90 in the super chaperone complex, longer pharmacodynamic duration and tumor retention profiles, CH5164840 shows tumor-selective degradation in vivo and therefore exhibits potent antitumor efficacy with a wider therapeutic range in NCI-N87, a Her2 positive gastric cancer model. Further extended efficacy studies with oral daily administration of CH5164840 in many xenograft models revealed that CH5164840 is sensitive to RTK-addicted tumors that occur when EGFR and HER2 are mutated or dysregulated. Moreover in combination therapy, CH5164840 enhances the anti-tumor efficacy with current standard RTK inhibitors.
Conclusion: CH5164840 is a novel, orally available, synthetic HSP90 inhibitor and shows highly potent antitumor efficacy in mono- and combination-therapies with standard-of care-agents. These profiles support the clinical development of CH5164840 for the treatment of RTK-addicted tumors, including tumors with overexpression and mutation of RTKs whose growth and survival depend on HSP90.

82 POSTER

Pharmacokinetic–pharmacodynamic modeling of the effect of GDC-0152, a selective antagonist of the inhibitor of apoptosis (IAP) proteins, on monocyte chemotactic protein-1 (MCP-1) indicates species differences in MCP-1 response

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Inhibitor of apoptosis (IAP) proteins are believed to suppress apoptosis and are overexpressed in a variety of cancers. GDC-0152 is a potent and selective antagonist of the IAP proteins that was developed as a potential treatment of tumors that are resistant to chemotherapies or radiotherapy. Monocyte chemotactic protein-1 (MCP-1) is a chemokine that is expressed during an inflammatory response. Based upon preclinical studies, antagonism of IAP proteins has been shown to induce MCP-1 expression via cIAP degradation and activation of NF-kB signaling. The objective of this study was to investigate species differences in MCP-1 response to GDC-0152 in rats, dog, and humans using pharmacokinetic/pharmacodynamic (PKPD) modeling. Briefly, dogs (n = 40) and rats (n = 20) were given intravenous (IV) doses of GDC-0152 ranging from 0.3 to 15 mg/kg and 20 to 120 mg/kg, respectively. A two compartment model was used to characterize the pharmacokinetics of GDC-0152 in both dogs and rats. A semi-mechanistic population PKPD model incorporating transit compartments was used to characterize the MCP-1 response to GDC-0152. Estimated parameters from the described model indicate that lower concentrations of GDC-0152 are required to trigger an increase in MCP-1 plasma levels in dogs when compared to rats. Simulations were performed with pharmacodynamic (PD) parameters estimated from rat and dog using human pharmacokinetic parameters and select doses. In simulations performed using dog PD parameters, an approximately 4-fold increase in MCP-1 plasma concentration was estimated at a dose of 0.76 mg/kg. In contrast, similar simulations using rat PD parameters suggest little or no change in MCP-1. Humans given intravenous doses $\geqslant$0.76 mg/kg showed no evidence of MCP-1 increase. Thus, the dog appears to be more sensitive to GDC-0152 (in terms of MCP-1 increase) when compared to rats and humans.

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Effect of TG02, a kinase inhibitor targeting Erk5, on triple negative breast cancer cells

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Background: Breast cancer is the most common neoplasia in women. Mitogen-activated protein kinases (MAPK) play important roles in tumorigenesis. Formerly, we reported that one of them, Erk5, is linked to the proliferation of breast cancer cells in vitro, is commonly overexpressed in primary breast tumors, and that its overexpression is an independent negative prognostic marker for disease-free survival. In addition, inhibition of Erk5 sensitized cells to treatments commonly used in the breast cancer clinic. Therefore, Erk5 may represent a novel therapeutic target in breast cancer.
Here we studied the effect of TG02 (a multikinase inhibitor that targets Erk5) on a panel of cell lines representing the basal-like or triple negative subtype of human breast cancer (TNBC).
Materials and Methods: The expression of Erk5 in a panel of TNBC cell lines and the mechanism of action of TG02 on Erk5 were analyzed by immunoprecipitation and Western blotting, using antibodies directed against Erk5. Effects on cell proliferation were determined by MTT assay and cell cycle and apoptosis analyses were performed by propidium iodide DNA staining and FACS analysis. In vitro drug synergies were explored using a caspase 3/7 ELISA and the in vivo activity of TG02 was tested in nude mice bearing established MDA-MB-231 xenografts.
Results: The TNBC cell lines analyzed showed high levels of Erk5 expression, and Erk5 was active under resting conditions in some cases. Cell proliferation studies indicated that the TNBC cells were very sensitive to the action of TG02 at low concentrations (IC_{50} $\leqslant$100 nM) and short exposure times (24−48 hrs). TG02 also induced cell cycle arrest at the G2/M transition leading to apoptotic cell death.
As Erk5 is a target of TG02, we explored whether Erk5 activity was affected by drug treatment. The kinase activity of Erk5 was compromised even though TG02 did not affect the Erk5 upstream activating kinase Mek5, or other upstream activating kinases. In vivo studies indicated that TG02 exerted strong antitumor activity in mice bearing MDA-MB-231 xenografts as a single agent and synergized with the standard of care drug doxorubicin.
Conclusions: TNBC cells are very sensitive to TG02, both in vitro and in vivo. TG02 induced cell cycle arrest at the G2/M transition, causing cell death alone and in synergy with doxorubicin, perhaps via inhibition of Erk5. These preclinical studies establish the bases for the clinical development of this compound for the treatment of TNBC.

84 POSTER

Identifying statins and dipyridamole as a novel drug combination showing efficacy in multiple myeloma and acute myelogenous leukemia

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Statins are drugs that have been utilized for years to treat hyperlipidemia via inhibition of the rate-limiting enzyme of the mevalonate (MVA) pathway, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR). Preclinical