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.

**78** POSTER

**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.

**79** POSTER

**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.

**80** POSTER

**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,