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Novel class I PI3K inhibitor CH5132799: disruption of the activated PI3K signaling in PIK3CA mutants confers potent antitumor efficacy

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Background: The phosphatidylinositol 3-kinase (PI3K) pathway regulates various cellular processes, such as proliferation and apoptosis. Class I PI3K is a heterodimer, consisting of a regulatory and a p110 catalytic subunit, which transduces signals from receptor tyrosine kinases (RTKs). One of four p110 isoforms, p110α is known to be actively mutated in various human cancers. CH5132799 is a potent class I PI3K inhibitor with a novel structure, which will be presented in an accompanying poster. We describe here the preclinical pharmacology of CH5132799 against activated PI3K signaling in PIK3CA-mutated cancer. In another poster we will present the preclinical efficacy in combination with current standard therapeutics including RTK-targeted drugs.
Results: CH5132799 is a class I PI3K inhibitor with a novel chemical structure. In cell-free enzyme assays, CH5132799 inhibited class I PI3Ks and acted most potently on PI3Kα and its mutants; E542K, E545K and H1047R. Treatment with CH5132799 suppressed the PI3K/Akt pathway in PI3K-mutated cancer cells, resulting in G1 arrest and apoptosis induction. Cancer cell panel analysis of antiproliferative activities revealed that the PI3K pathway-activated cell lines, including PIK3CA mutants in breast, ovarian, prostate and endometrial cancer, are sensitive to CH5132799. Moreover, in expanding tumor types, CH5132799 appeared to be significantly more effective against PIK3CA mutant cell lines than against cells without mutation. The higher sensitivity of PIK3CA mutants is also demonstrated in the xenograft models. Daily oral administration of CH5132799 exhibited remarkable antitumor efficacy in several models with PI3Kα mutation or activated PI3K signaling. A pharmacodynamic response was confirmed by suppression of phosphorylated Akt in the grafted tumors with PIK3CA mutation.
In addition, we demonstrated that in the H1047R mutant model, CH5132799 induced regression of a grafted tumor which had re-grown during continuous treatment with everolimus. Abrogated phosphorylation of Akt and its downstream molecules in the grafted tumors supported the efficacy in this everolimus-refractory model.
Conclusion: CH5132799 is a class I PI3K inhibitor and has potent antitumor efficacy, *in vitro* and *in vivo*, on PI3K pathway-activated tumors, especially on PI3K mutants. From these observations, CH5132799 offers a potent therapeutic strategy for the treatment of PI3K-mutated tumors.

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The Aurora A kinase inhibitor MLN8237 in combination with docetaxel induces synergistic antitumor activity in triple-negative breast cancer xenograft models

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Small molecule inhibitors of Aurora A are being pursued for use as anti-mitotic therapy in a broad array of cancer indications. One such molecule MLN8237, a selective Aurora A inhibitor, is being evaluated in multiple phase I and II clinical trials. Here, the antitumor activity of MLN8237 in combination with docetaxel was tested in three xenograft models of triple negative breast cancer, including two primary human transplant breast tumor models. Mice were dosed for three weeks with MLN8237 orally either continuously (QD) or intermittently (3 days on/ 4 days off) and with docetaxel intravenously once a week (QW). In all three tumor models, single agent anti-tumor activity was observed in both MLN8237 continuous and intermittent dosing regimens and with docetaxel; however, tumors re-grew upon treatment termination. In the MLN8237 and docetaxel combination treatment arms, additive or synergistic anti-tumor activity was observed and significant tumor growth delay occurred relative to the single agent arms after discontinuing treatment. Importantly, the higher combination doses resulted in complete cures with no tumor re-growth in several animals. In combination with docetaxel, the intermittent dosing of MLN8237 yielded equivalent antitumor activity as continuous dosing in all three models. Body weight loss (BWL) was observed in the combination arms in a dose dependent manner in an acceptable range (maximum ~10%); however body weight recovered quickly after terminating treatment. Interestingly, the body weight loss was less severe when MLN8237 was dosed intermittently than when dosed continuously, though the antitumor activity was similar. The plasma and tumor PK profiles of MLN8237 and docetaxel in the MDA-MB-231 model were similar when dosed as single agents or in combination demonstrating no drug-drug interaction. Histopathological assessment of tumors after multiple days of treatment revealed increase in cell size in tumors treated with both MLN8237 and docetaxel but not with either agent alone, consistent with previous studies demonstrating the Aurora A inhibition overrides the spindle assembly checkpoint induced by microtubule perturbing agents. The robust and durable antitumor activity of MLN8237 combined with docetaxel has provided a rationale for evaluating the safety and antitumor activity of Aurora A inhibitors combined with taxanes in clinical studies.

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Design and functional analysis of a novel hybrid TPR peptide targeting Hsp90

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Despite a better understanding of the molecular biology of tumor cells, the treatment of most cancers has not significantly changed for the past three decades, and drugs that do not discriminate between tumor cells and normal tissues remain mainstays of anticancer therapy. Heat shock protein (Hsp90) has attracted considerable interest in recent years as a potential therapeutic target for the identification and development of a new generation of anticancer drugs to treat several cancer and malignancies [1]. Hsp90 is a molecular chaperone that participates in the quality control of protein folding. Hsp90 and Hsp70 cooperate with numerous cofactors containing so-called tetratricopeptide repeat (TPR) domains. TPR domains are composed of loosely conserved 34-amino acid sequence motifs that are repeated 1−16 times per domain. The TPR cofactors of the Hsp70/Hsp90 multi-chaperone system interact with the C-terminal domains of Hsp70 and Hsp90. The N-terminal TPR domain, TPR1 specifically recognizes the C-terminal seven amino acids of Hsp70 (PTIEEVD), whereas TPR2A recognizes the C-terminal five residues of Hsp90 (MEEVD). Hsp90 is typically involved in proliferation and survival of cell. This is thought to play a key role in cancer, and the stress response recognition of Hsp90 may help promote tumor cell adaptation in face of unfavorable environments.
In this study, we engineered a cell-permeable peptidomimetic, termed Antp-TPR hybrid peptide, modeled on the binding interface between the molecular chaperone Hsp90 and the TPR2A domain of its cofactor protein p60/Hop. It was demonstrated that the Antp-TPR hybrid peptide inhibits the interaction of Hsp90 with the TPR2A domain of Hop, inducing cell death of breast, pancreatic, renal, lung, prostate, and gastric cancer cell lines *in vitro*. In contrast, Antp-TPR peptide has less cytotoxicity to normal cells. In addition, analysis *in vivo* revealed that Antp-TPR peptide displayed significant antitumor activity in a xenograft model of human pancreatic cancer in mice. These results indicate that Antp-TPR hybrid peptide could provide potent and selective anticancer therapy to cancer patients.

References

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RNAi lethality screening in acute leukemias identifies wee1 inhibition as potent sensitizer to cytarabine und uncovers a genomic context in lymphoid malignancies

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Background: Therapy and outcome for patients with acute myeloid leukemias (AML) has not changed for decades and new therapies are urgently needed. To identify novel rational combinations of Cytarabine (AraC), the most active drug in AML, that have the potential to improve responses and therapy outcome for AML patients we performed a first-in-class RNA interference (RNAi) lethality screen of the human kinome in AML.
Materials and Methods: Using lipid-based reverse transfection in a High-Throughput RNAi (HT-RNAi) format for transient siRNA gene silencing in myeloid suspension cells, 572 kinases of the human kinome were silenced in combination with AraC. Proliferation/viability was measured using a luminescence-based assay 48 hours after AraC treatment (total 72−96 hrs). siRNA dose drug response curves (siDDR) were used for secondary validation with si and inhibitors against the target of interest. Immunoblotting and RT-PCR were performed according to standard protocols.
Results: Of 572 kinases that were individually silenced in combination with AraC, only 1−2% significantly increased sensitivity to AraC. The Wee1 family of kinases, PKMYT and especially Wee1 showed the most potent sensitizing activity to AraC, in some cell line systems even more potent than Chek1 inhibition. Validation studies confirmed ~2−10× sensitization to AraC across a broad panel of AML lines by either siRNA gene knockdown or using commercial Wee1 inhibitors. Ex-vivo primary leukemia cells

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,