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The design and synthesis of a novel orally available Hsp90 inhibitor CH5164840

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Background: Hsp90 is a constitutively expressed molecular chaperone that governs the maturation and stability of many regulatory factors that are key to cell growth and survival. Since many of the client proteins are implicated in tumor cell growth and survival, Hsp90 inhibitors could induce the simultaneous blocking of multiple signaling pathways. Accordingly, Hsp90 is attracting significant research interest as a target for anticancer agents. Although several Hsp90 inhibitors including geldanamycin derivatives (GM) and non-geldanamaycins are currently under clinical trials, no clear clinical efficacy against solid tumors has been demonstrated yet, probably due to safety, physicochemical properties and/or DMPK issues. We aimed to identify a novel non-geldanamycin type and orally available Hsp90 inhibitor that has better drug-like properties and shows stronger antitumor efficacy than GMs.
Material and Methods: Virtual screening (using docking software FlexX, followed by measurement of ATPase activity) and fragment screening (by surface plasmon resonance (SPR)-based binding assay, followed by hit validation by NMR to confirm their binding to ATP sites) were conducted in parallel to identify lead compounds. X-ray crystal structures of the complexes between the hits and Hsp90 were solved. Structure-guided inhibitor design and optimization were carried out by software Moloc and GRID.
Results: Several weak inhibitors having aminopyrimidine, aminotriazine and indazole moieties as core templates were identified. The X-ray structures of the Hsp90 complex with validated hit compounds revealed important interaction sites in the ATP binding pocket of Hsp90, namely, Asp93, and a hydrophobic pocket formed by Phe138 and Leu107. Starting with these templates, we designed and synthesized a series of simplified macrocyclic analogs with the aim of enhancing both binding affinity and cytotoxicity, in such a way that an inhibitor could form strong hydrogen bonds with crystal water and Lys58. Finally, a potent Hsp90 inhibitor CH5164840 was identified: Kd (Hsp90α) = 0.52 nM, IC_{50} (NCI-N87/Her2$^+$ GC) = 66 nM. Oral CH5164840 had fairly good PK profiles (mice: T1/2 = 1.6 hr, BA = 70.8%), and showed greater antitumor activity than 17-DMAG in various human cancer xenograft models (*e.g.* NCI-N87: TGI 160%; 50 mg/kg, 11 q.d.).
Conclusions: We identified a novel, orally available small molecule Hsp90 inhibitor, CH5164840, which has potent efficacy in human cancer xenograft mouse models.

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Selective inhibition of PI3K alpha using a novel covalent compound

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Background: The PI3K Class I family consists of four isoforms: a, b, g, and d. PI3K a and b are ubiquitously expressed, whereas g and d are more restricted. The PI3K pathway is activated in many cancers through either PTEN inactivation or through activating mutations in the gene that encodes the PI3Ka. Several PI3K inhibitors are currently in clinical development, but most are pan-PI3K inhibitors. Tumor biology data suggests that targeting PI3Ka specifically should be efficacious, and there may be advantages to not disrupting other members of the complex PI3K signaling cascade. Using structure-based drug design (SBDD), we have generated a targeted small molecule inhibitor of PI3Ka that acts through selective and irreversible covalent bond formation. Covalent inhibitors have many advantages including improvements in potency, selectivity, prolonged duration of action, and translational biomarker opportunities.
Materials and Methods: SBDD was used to generate selective small molecule inhibitors of PI3Ka. Mass spectrometry verified covalent bond formation to PI3Ka. PI3K enzyme activity was measured using an HTRF assay. PI3Ka inhibition was evaluated in SKOV-3 cells by measuring P-Akt^{Ser473} levels. Washout experiments were performed to assess prolonged duration of action in cells. SKOV-3 xenograft studies were performed in mice to measure P-Akt^{Ser473} inhibition and tumor growth inhibition *in vivo*. A biotinylated covalent probe molecule specific for PI3Ka was used to verify and quantitate target occupancy by the covalent inhibitor, both *in vitro* and *ex vivo*.
Results: Using mass spectrometry, we verified that CNX-1351 bonds specifically to an amino acid which is structurally unique to PI3Ka, and it does not bond to the other isoforms. CNX-1351 potently inhibits PI3Ka enzyme activity and inhibits P-Akt^{Ser473} in SKOV-3 cells (EC_{50} ~100 nM). PI3Ka activity continued to be inhibited after compound removal, confirming the mechanism of action. CNX-1351 demonstrated *in vivo* inhibition of PI3Ka and tumor growth inhibition in an SKOV-3 xenograft model. A biotinylated covalent probe molecule, which bonds specifically to PI3Ka, was used to verify and quantitate target occupancy, both *in vitro* and *ex vivo*.
Conclusions: CNX-1351 is a specific and potent irreversible inhibitor of PI3Ka that demonstrates prolonged duration of action, and activity *in vivo*. This approach should yield a first-in-class selective covalent PI3Ka inhibitor with numerous clinical advantages.

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PDL192, a humanized anti-Tweak receptor monoclonal antibody, mediates antitumor effects in primary human breast carcinoma xenografts

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Background: PDL192 is a humanized IgG1 monoclonal antibody that binds the human TWEAK receptor (TweakR). TweakR, a member of the TNFR (Tumor Necrosis Factor Receptor) superfamily, is overexpressed in several human cancers including breast cancer (BC). In BC, it may also play a role in the invasive and metastatic potential of the disease (Willis et al, Mol Cancer Res 2008). In TweakR-expressing cancer cell lines or mouse xenograft models, PDL192 has a potent antitumor effect (Culp et al, CCR 2010). All these data therefore suggest that anti-TweakR targeting could be a promising new therapeutic approach for human BC patients.
Material and Methods: TweakR expression was assessed by IHC (immunohistochemistry) on 3 Tissue-Micro-Array (TMA) banks of BC samples (basal-like, ERBB2, and luminal A/B), and on 25 primary human BC xenografts (HBC$\times$). The cut-off of positivity was defined as at least 25% cells with membraneous or cytoplasmic staining or by a combined score of percentage of positive staining cells $\times$ intensity $\geqslant$50. The *in vivo* antitumor effect of PDL192 was then assessed on 7 TweakR-positive models (10 mg/kg thrice a week for 3 weeks by intraperitoneal route).
Results: TMA analyses showed that TweakR was expressed in 16/37 basal like BC (43%), 23/37 ERBB2-positive BC (62%), and 38/71 luminal BC (54%). Evaluations of possible correlations between TweakR expression and the clinico-biological characteristics of the tumors, as well as the outcome of the patients, is currently ongoing. Moreover, 13/25 xenografts have been found to be TweakR-positive (52%). Seven human BC models have been treated with PDL192, with 3 models (43%) showing a tumor growth inhibition (TGI) greater than 50%. No correlation has been observed between TweakR expression and *in vivo* TGI.
Conclusions: TweakR is expressed in 77/145 human BC samples (53%). In *in vivo* experiments, PDL192 showed potent TGI in 3/7 models. All these data therefore support the use of anti-TWEAK receptor monoclonal antibodies in the treatment of TweakR-positive BC patients. Further therapeutic combinations should also be evaluated.

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Therapeutic targeting the loss of the Birt–Hogg–Dubé suppresser gene

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Background: Birt–Hogg–Dubé (BHD) disease, an autosomal dominant familial cancer, is associated with increased risk of kidney cancer. BHD syndrome is caused by loss-of-function mutations in the folliculin (FLCN) protein. In order to develop therapeutic approaches for renal cell carcinoma (RCC) in BHD syndrome, we adopted a strategy to identify tumour-selective growth inhibition in a RCC cell line with *FLCN* inactivation.
Material and Methods: The COMPARE algorithm was used to identify candidate anticancer drugs tested against the NCI-60 cell lines that demonstrated preferential toxicity to low *FLCN* expressing cell lines.
Results: Fifteen compounds were selected and detailed growth inhibition (SRB) assays were performed in paired BHD RCC cell lines (UOK257 derived from a patient with BHD). Selective sensitivity of FLCN-null over FLCN-wt UOK257 cells was observed in seven compounds. The most selective growth-inhibitory sensitivity was induced by mithramycin which demonstrated a ~10-fold difference in GI50 values between FLCN-null

(64.2±7.9 nM, n = 3) and FLCN-wt UOK257 cells (634.3±147.9 nM, n = 4). Differential ability to induce caspase 3/7 activity by mithramycin was also detected in a dose dependent manner. Clonogenic survival studies showed mithramycin to be ~10 fold more cytotoxic to FLCN-null than FLCN-wt UOK257 cells (200 nM). Following mithramycin exposure, UOK257-FLCN-null cells were mainly arrested and blocked in S and G2M phases of the cell cycle and low dose of rapamycin (1 nM) potentiated mithramycin sensitivity (1.5 fold in G2M population and 2 fold in G2M period time, 2 × GI50, 48 hrs).

Conclusions: These results provide a basis for further evaluation of mithramycin as a molecularly-targeted therapy for RCC associated with BHD.

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Discovery of BMS-817378: a novel prodrug of the dual Met/VEGFR-2 inhibitor BMS-794833

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Met receptor tyrosine kinase (RTK) is expressed predominantly on epithelial and endothelial cells, and serves as the only known high-affinity receptor for the mesenchyme-derived ligand, hepatocyte growth factor (HGF). Met activation can occur via ligand binding, receptor overexpression, and/or activating mutations. Subsequent signal transduction leads to complex biological responses, such as cellular proliferation, motility, migration, invasion, survival, morphogenesis and angiogenesis. Dysregulated Met-HGF signaling promotes tumor formation, growth, proliferation and metastasis, and thus, has been implicated in a wide array of human malignancies. Several agents that modulate the Met-HGF signaling axis have progressed to various stages of clinical development, including the potent and selective Met "superfamily" kinase inhibitor BMS-777607. In an effort to identify a second development candidate with a broader spectrum of antitumor activity relative to BMS-777607, compounds within the 2-aminopyridine series were screened against additional kinase targets, particularly those that play a role in tumor angiogenesis. The potent ATP-competitive Met/VEGFR-2 kinase inhibitor BMS-794833 was identified, which demonstrates enhanced activity versus both Met-dependent and Met-insensitive tumor lines. BMS-794833 also inhibits Ron (Met family), Axl (phylogenetically related Axl/Tyro3/Mer subfamily) and Flt-3 with IC_{50} values <3 nM. The compound was selective versus a panel of >200 additional RTKs, non-RTKs and serine/threonine kinases based on biochemical or Ambit binding assays. In cell culture, BMS-794833 inhibited the proliferation of human tumor cell lines containing constitutively activated Met receptor (GTL-16 gastric carcinoma). Tumor cell lines whose growth is stimulated by HGF (U87 glioblastoma) were also effectively inhibited by BMS-794833. *In vivo*, BMS-794833 demonstrated dose-dependent tumor growth inhibition following oral administration in the GTL-16 and L2987 lung carcinoma (Met-insensitive) xenograft models. Despite the impressive antitumor activity, BMS-794833 showed dissolution rate-limited absorption from solid dosage forms. The phosphooxymethyl prodrug, BMS-817378 was found to effectively liberate BMS-794833 in various *in vitro* and *in vivo* systems. On the basis of its desirable pharmacological profile, acceptable *in vitro* ADME and safety characteristics, and favorable pharmacokinetic properties in multiple species, BMS-817378 was selected for clinical development. The preclinical profile of BMS-794833 and the prodrug strategy leading to BMS-817378 will be presented.

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In vivo anti-tumor structure–activity relationships of Judemycin C and E, small molecule modulators of the spliceosome

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Background: The spliceosome is an emerging target for cancer therapy that remains to be significantly exploited. We have recently reported the concise synthesis of Judemycin C, C1 and E (Figure 1), spliceosome modulators that show *in vivo* anti-tumor activity without notable toxicity. We are currently developing new analogs in this series that modulate splicing activity with the goal of yet further improving their anti-tumor efficacy.

Materials and Methods: Splice variants for many genes have been identified in mammalian cells, and in some instances the presence of these variant transcripts in tumors correlates with poorer clinical outcome (e.g. *MDM2*). By RT-PCR, we observed the modulation of *MDM2* splicing following exposure of the Rh18 rhabdomyosarcoma cell line to 0.1, 1, or 10 μM Judemycin C1 for up to 24 hours.

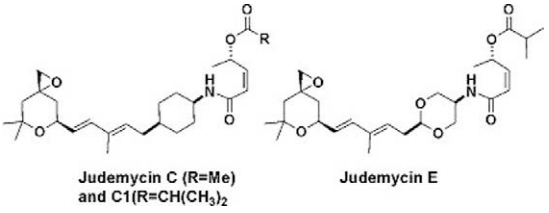

Figure 1. The structures of the lead compounds used in this study.

These compounds have also been tested in XTT cytotoxicity assays against a number of cancer cell lines and several sensitive lines have been identified, that possess IC_{50}s in the 80–200 nM range to these small molecules. Sensitive cancer cell lines (e.g. the JeKo-1 mantle cell lymphoma line) have been selected for *in vivo* xenograft anti-tumor efficacy studies in a NOD/SCID murine model. For our JeKo-1 animal studies, mice were treated with saline, vehicle, Judemycin E, or the proteasome inhibitor drug bortezomib for 5 consecutive days with 2 days off, for a total of 4 weeks. We will present new details on these extended dosing studies with Judemycin E.

Results: Our spliceosome modulatory compounds Judemycin C1 and E are cytotoxic against several cancer cell lines with IC_{50}s in the nanomolar range. In addition, we have observed modulation of *MDM2* splicing following treatment with the Judemycin compounds, resulting in shorter, alternatively spliced transcripts in drug-treated cells as compared to control samples. We have also demonstrated superior growth inhibition of the JeKo-1 mantle cell lymphoma xenograft *in vivo* as compared to the clinically approved mantle cell lymphoma drug bortezomib. Taken together, these new results are consistent with the hypothesis that Judemycin C1 and E alter splicing of *MDM2* (and other genes to be discussed in the presentation), presumably by interacting with the SF3b subunit of the spliceosome, resulting in the formation of aberrant mRNAs. We will also report on the structure–activity relationships of unpublished new Judemycin analog compounds prepared in our laboratory.

Conclusions: Judemycin C1 and E are examples of a new promising class of anti-cancer compounds. We have found that modulation of the alternative splicing of *MDM2* is a useful and sensitive marker for monitoring the splicing-modulatory effects of these compounds *in vivo*. This method is reproducible, requires small numbers of cells, and potentially could be used to document drug exposure and activity *in vivo* for a variety of tumor types. Studies to assess the latter possibility are currently underway.

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AEZS-129, an orally active PI3K inhibitor in preclinical development

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The phosphatidylinositol 3-kinase (PI3K)/Akt pathway plays a critical role in the regulation of cell growth, proliferation and survival in cancer. In many tumor types, the PI3K/Akt pathway is frequently activated through either the amplification/mutation of PIK3CA or the loss of the tumor suppressor phosphatase PTEN. Inhibition of the PI3K pathway therefore represents a high therapeutic value for patients with breast, colon, ovary, liver and other tumors.

AEZS-129 inhibits PI3Ka with an IC_{50} value of 28 nM with high specificity and proved to be a potent inhibitor of Akt phosphorylation in cellular assays. Mode-of-action studies showed that AEZS-129 acts as an ATP competitive compound. The *in-vitro* anti-proliferative activity against different human tumor cell lines (MDA-MB 468, U87, Hct116, PC-3, A549 and others) was determined, with EC_{50} values in the nanomolar range. AEZS-129 demonstrates favorable properties in early *in-vitro* ADMET screening including Caco-2 permeability, microsomal stability, plasma stability and testing against cardiac ion-channels.

Oral dosing of AEZS-129 results in high plasma levels in *in-vivo* pharmacokinetic experiments. Oral treatment with AEZS-129 is well tolerated and leads to significant tumor growth inhibition in multiple mouse xenograft cancer models, including colon (Hct116), lung (A549), prostate (PC-3) and endometrium (Hec1B) at 45 mg/kg repeated daily administration.

Based on these data, AEZS-129 was selected as preclinical development candidate.