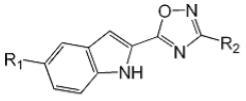
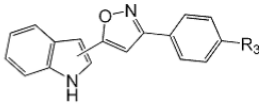
targets. Among the oxadiazole series, compound 21 showed the best activity. The pro-apoptotic activity testing of all novel compounds is in progress. Based of the docking studies and biological data, SAR analysis and structural modifications could result in better selective pro-apoptotic leads.

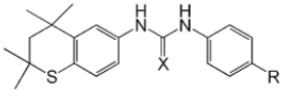


Indole-based 1,2,4-oxadiazoles

21: R_1= OCH_3, R_2= 4-$OCH_3C_6H_4$



Indole-based isoxazoles



Flex-Hets

X= O, S

R= NO_2, $COOCH_3$, SO_2NH_2

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142 POSTER

Interaction of chlorambucil and intercalating aniline mustards with defined DNA sequences using MALDI and ESI mass spectrometry

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Chlorambucil, like all nitrogen mustards, is prone to hydrolysis and is inclined to alkylate proteins in addition to DNA. This results in low dose-potency in the clinical setting. An additional practical difficulty is that the crosslinking effectiveness of nitrogen mustards is limited, so that the majority of adducts are monofunctionally bound to DNA, which provides a platform for mutagenesis and the later induction of tumours in long-term cancer survivors. As with the minor groove-directed alkylating agents, one way to overcome these deficiencies, so as to augment the specificity for alkylating DNA *per se*, is to navigate aniline mustards to DNA by appending them to a reversible-binding carrier such as an intercalating agent. We have explored the use of matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF) and electrospray ionisation time-of-flight mass spectrometry (ESI-TOF) to study the DNA complexes of DNA-directed alkylating cytotoxins. We have investigated the binding of intercalator-directed acridine mustards, and chlorambucil to the 2 dodecanucleotides CGCGAATTCGCG (A2T2) and ATATGGCCATAT (G2C2). Alkylation of purines at the N3 and N7 positions quaternises the base, imparting a positive charge and weakening the glycosidic bond to hydrolysis. As a result, apurinic sites are generated which lead to phosphate hydrolysis and breakage of the DNA backbone at the alkylated base. For the intercalating acridine mustards binding to A2T2 and G2C2, we find that they alkylate purines surrounding their intercalation sites with enhanced potency compared to chlorambucil, but, unlike chlorambucil, they are unable to form crosslinks. Directing the alkylating group to DNA with an intercalating moiety enhances the reactivity of the alkylating agent by some 100-fold. Both chlorambucil and the acridine mustards alkylate the same adenines and guanines on A2T2 and G2C2, but, whereas chlorambucil forms a variety of inter-strand and intra-strand crosslinks involving both adenine-guanine and guanine-guanine linkages, the bifunctional intercalating mustard failed to form crosslinks of any variety.

143 POSTER

Ex-vivo plasma protein binding and in vitro evaluation of AP5346 (ProLindac TM; PL), a novel polymer-bound platinum: Evidence showing that >72 h DACH-platinum (Pt) release may play a major role in cytotoxicity

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Background: PL displays activity in a wide variety of solid tumors in preclinical models and clinical trials. PL is designed to selectively release DACH Pt into the acidic tumor environment. PL has a cytotoxic profile similar to that of oxaliplatin in our human cancer cell line panel. We investigated PL binding to plasma proteins and the kinetics of Pt release.
Materials and Methods: Plasma protein binding and Pt release were evaluated ex-vivo in plasma at 300 and 30 μg/mL (concentrations representing the therapeutic range, Cmax and Cmin) at 37ºC with adjusted pH (7.35–7.4) using Ultra-4 filters (Amicon) with 50 and 3 kDa cut-off. Reversibility of binding was investigated by protein precipitation with acetonitrile. Pt levels were measured by atomic absorption. Antiproliferative effects were evaluated in HT29 and HCT116 human cancer cell lines by MTT assay after 1−72 h of exposure.
Results: Both PL and oxaliplatin bind plasma proteins. PL induces non-covalent protein binding: addition of acetonitrile caused dissociation of all weakly bound ligands. PL binding to proteins was sustained for up to >144 h (6 days). In these experiments, PL protein binding was about 94% immediately after PL addition. Unbound Pt was 2.96% (6.3 μg/mL) and 5.73% (1.7 μg/mL) for Cmax and Cmin, respectively. Interestingly, Pt release from plasma-protein bound PL-polymer increased progressively over time reaching a steady-state at >72−96 h. This slow Pt release was consistent with exposure cytotoxicity kinetics. In vitro, PL also displayed time-dependent cytotoxicity in HT29 and HCT116 colon cancer cells, PL exposure >72 h showing higher antiproliferative effects than shorter exposures (<24 h). At equimolar concentrations, oxaliplatin was slightly more active than PL for short exposure durations (<48 h). Conversely, for duration of exposure >72 h, PL displayed IC50 ranging from 0.3–0.5 μM in colon cancer cells while oxaliplatin IC50 ranged from 0.5–0.9 μM. Similarly, PL-induced Pt DNA incorporation was time-dependent, with a higher level of Pt bound to DNA observed for exposure >72 h in human cancer cells.
Conclusions: Together, our data strongly suggest that protein-bound PL polymers progressively release free-Pt in plasma, reaching a sustained steady state after >72 h, resulting in sustained exposure to Pt. Considering that extended duration of exposure is essential for PL cytotoxicity, our data may help optimize dosing schedules in the design of future combination clinical trials.

Heat shock proteins

144 POSTER

XL888, a novel, synthetic, orally bioavailable inhibitor of Hsp90

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Background: Hsp90 is a component of a molecular chaperone complex that promotes the conformational maturation and stabilization of many tumor-promoting oncoproteins. A hallmark of Hsp90 inhibition is the corresponding degradation of client proteins and loss of tumor cell growth and survival. XL888 is a novel, synthetic small molecule inhibitor of Hsp90 identified from a biochemical library screen coupled with extensive medicinal chemistry to optimize its drug like properties. We describe key aspects of its preclinical activity profile here.
Materials and Methods: Biochemical and x-ray crystallographic methods were used to determine the binding characteristics of XL888 to Hsp90. Proliferation IC50s were performed using a BrdU-incorporation ELISA. Client protein degradation, pathway inhibition, and heat shock induction responses in tumor cell lines and xenograft tumors were determined by Western blot. Human tumor xenografts were grown in nude mice for PD and efficacy studies.
Results: XL888 is a potent and selective ATP-competitive inhibitor of Hsp90. It binds in a manner that is structurally distinct from 17-AAG and other small molecule Hsp90 inhibitors. XL888 treatment inhibited the proliferation of a broad panel of human tumor cell lines with IC50 values ranging from 0.1 to 45 nM, and resulted in marked degradation of client proteins including HER2, MET, mutant BRAF and mutant EGFR. Client protein degradation correlated with attenuation of receptor signaling, with significant loss of phospho-receptor, phospho-S6 and phospho-ERK

signals by Western blot. XL888 was highly active in multiple mouse xenograft tumor models, including the HER-2 overexpressing gastric cancer model, NCI-N87. Consistent with the pharmacodynamic duration of action of XL888 in human xenograft tumors, intermittent oral dosing was equally as effective as chronic oral dosing at inhibiting tumor growth in multidose studies. The pharmacokinetic profile of XL888 in rodent and non-rodent species supports clinical development of XL888 as an oral inhibitor of Hsp90 and demonstrates that XL888 is preferentially retained over time in tumors relative to plasma and liver.

Conclusion: XL888 is a novel and potent inhibitor of Hsp90 in vitro and in vivo. The activity profile of XL888 in animal models is supportive of its clinical development for the treatment of cancers driven by Hsp90 oncoprotein clients.

145 POSTER

Hsp90 inhibitors target addiction to mutant oncoproteins in colorectal cancer

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Inhibitors of the molecular chaperone Hsp90 induce the simultaneous combinatorial depletion of >100 client proteins, including some that regulate the hallmarks of cancer. A key challenge in the application of these inhibitors is the understanding of the mechanisms governing the responses of tumours and especially to determine which client proteins are critical. One attractive hypothesis is that cancer cells become dependent on oncoproteins activated by mutation, and these gain of function mutants are unable to fold properly in the absence of Hsp90 and so acquire the ability to transform at the expense of greater dependence on Hsp90. Here we have used a panel of 29 colorectal cell lines to determine factors that influence response to Hsp90 inhibition. We find that basal expression of the Hsp90 complex components does not influence response to 17-AAG (tanespimicin), an Hsp90 inhibitor currently under clinical study. Cells with low expression of NQO1 exhibited reduced sensitivity to 17-AAG. Significantly, when the low NQO1 lines were excluded the cells most sensitive to 17AAG treatment were those with mutations in specific oncogenic kinases. Cells with these mutations were also the most sensitive to Hsp90 inhibitors that are not substrates for NQO1. MAPK signalling was also highly sensitive to inhibition by 17-AAG in cells with mutant kinases. These observations suggested that lines carrying mutant kinases might be dependent on these mutant oncoproteins for proliferation or survival. Consistent with this hypothesis, these lines were also highly sensitive to treatment with PD385901, a specific MEK inhibitor. siRNA targeting regulators of MAPK or PI3K signalling also supported this hypothesis as lines harbouring a mutant kinase were particularly dependent on that kinase for proliferation. Tumour xenografts of a mutant kinase line were sensitive to 17-AAG at doses that did not affect growth of xenografts of a tumour line deleted for PTEN or with a KRAS and PI3K mutation. Overall our observations support the hypothesis that proliferation or survival of tumour cells driven by mutated oncoproteins that depend on Hsp90 for stability or activity will be highly susceptible to Hsp90 inhibition. This also implies that clinical studies of Hsp90 inhibitors may benefit from strategies enriching or stratifying for tumours carrying certain kinase mutations.

146 POSTER

CU-0305, a novel synthetic Hsp90 inhibitor with unique pharmacology properties

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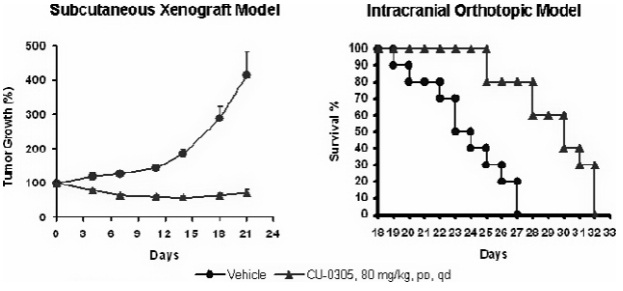
The molecular chaperone heat shock protein 90 (Hsp90) regulates the folding and degradation of key signaling molecules (client proteins) involved in cancer. CU-0305 is a novel synthetic Hsp90 inhibitor with potency in an Hsp90 binding assay similar to several leading drug candidates in development. CU-0305 has potent anti-proliferation and apoptosis-inducing activity against a broad range of cancer cell lines (IC50: 0.04–0.7 μM). Pharmacokinetic studies show that CU-0305 has high oral bioavailability (63% in mouse), and reaches much higher concentrations than reference compounds in tumor tissues with a half-life of more than 48 hours while being rapidly cleared from normal tissues. Furthermore, CU-0305 crosses the blood–brain barrier and reaches much higher brain concentrations than reference compounds. CU-0305 up-regulates Hsp70 and suppresses client proteins which involve PI3K, MAP kinase signaling, cell cycling and apoptosis. CU-0305 displays excellent efficacy after oral or IV administration in several tumor models. Tumor regression has been observed in N87 gastric cancer (Her2+), H1975 NSCLC (EGFR double mutations, EGFRi resistant) and U87MG glioblastoma (figure, left panel, $P < 0.0001$) subcutaneous xenograft models following oral administration of CU-0305 at 80 mg/kg daily. CU-0305 also significantly prolongs the survival of mice with orthotopic U87MG glioblastoma (figure, right panel, $P < 0.001$). Additionally, CU-0305 displays a favorable safety profile. CU-0305 is therefore a potential drug candidate for further evaluation in the treatment of cancers, especially primary and metastatic brain tumors.



Anti-tumor activities of CU-0305 in U87MG glioblastoma mouse models.

147 POSTER

AT13387, a fragment derived clinical candidate is active in lung and melanoma models

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Hsp90 is involved in the folding, maturation and stabilisation of key signalling molecules involved in cell proliferation, survival and transformation. Inhibition of Hsp90 can therefore simultaneously affect multiple signalling pathways required to maintain cellular transformation and as such is an attractive target for anti-cancer drug design. Recently, Hsp90 inhibition has been found to be of benefit in pre-clinical models of lung cancer and melanoma that depend on EGFR mutations, MET amplification and B-RAF mutations.

Astex Therapeutics has applied its fragment-based screening approach (PyramidTM) which employs a range of biophysical techniques, including X-ray crystallography and NMR (nuclear magnetic resonance) spectroscopy, followed by structure based drug design to discover AT13387. This compound has now been progressed into phase I clinical trials. AT13387 has prolonged tumour pharmacokinetics and pharmacodynamics in animal models.

Table 1

Cell	Line type	Cell line IC50
Lung	NCI-H1975	14 nM
Lung	A549	22 nM
Lung	Calu-6	32 nM
Melanoma	A375	54 nM
Melanoma	SK-Mel-28	46 nM

The effects of AT13387 have been investigated in several model systems including lung and melanoma models that have proved to be particularly sensitive to the agent. NCI-H1975 and A549 non small cell lung cancer and SKMel-28 and A375 melanoma cell lines have been characterised in detail for their sensitivity to AT13387 (see table above). In a more extensive 100 cell line panel screen multiple small cell lung cancer and non small cell lung cancer lines proved to be the most sensitive to AT13387. Both the NCI-H1975 (non small lung cancer) and the A375 (melanoma) xenograft models were demonstrated to be sensitive to single agent activity of AT13387 with concomitant modulation of pharmacodynamic markers. Furthermore, standard of care chemotherapies for both diseases in combination, in vitro and in vivo were tested against lung and melanoma models successfully.

Since AT13387 is progressing through dose escalation experiments in clinical trials, this combination work provides a unique opportunity to add AT13387 therapy to standard of care treatments in lung and melanoma diseases.