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.

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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.

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

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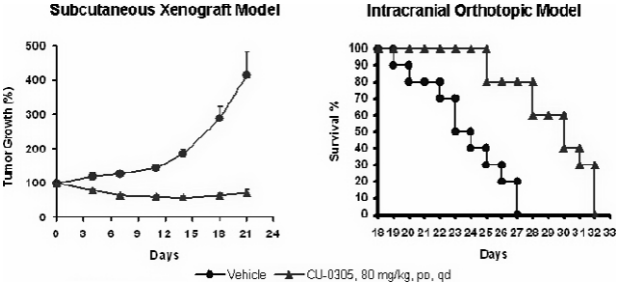
The molecular chaperone heat shock protein 90 (Hsp90) regulates the folding and degradation of key signaling molecules (client proteins) involved in cancer. CU-0305 is a novel synthetic Hsp90 inhibitor with potency in an Hsp90 binding assay similar to several leading drug candidates in development. CU-0305 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.

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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.