

# AT13387, A Fragment-Derived Clinical Candidate is Active in Lung Cancer and Melanoma Models

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## INTRODUCTION

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. Astex Therapeutics has applied its fragment-based screening approach (Pyramid™) 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. Here we show that AT13387 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. The effects of AT13387 have been investigated in several model systems including lung and melanoma models several of which are particularly sensitive to the agent. NCI-H1975 and A549 non small cell lung cancer and SK-Mel-28 and A375 melanoma cell lines have been characterised in detail for their sensitivity to AT13387 (see table 1). 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 cell 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 cancer and melanoma.

## CELLULAR DATA



Figure 1: Client Protein Knockdown in Melanoma and Lung Cancer Cell Lines

- 1µM of AT13387 was incubated with cells for 18 hours and lysates were blotted against antibodies to HSP70, GAPDH and CDK4 as well as EGFR and c-Met for lung lines and B-Raf for melanoma

Tumour Type	Cell Line	IC50	Mutation
NSCLC	NCI-H1975	14nM	EGFR mut
NSCLC	A549	22nM	EGFR wt
NSCLC	Calu-6	32nM	K-ras mut
Melanoma	A375	54nM	B-raf
Melanoma	SK-Mel-28	46nM	B-raf

Table 1: AT13387 Inhibits the Proliferation of Melanoma and Lung Cancer Cell Lines

- Cell Proliferation assays tested the sensitivity of lung and melanoma lines to AT13387
- Cells were plated and incubated with varying concentrations of AT13387 during 72 hours

## PHARMACOKINETIC PROFILING

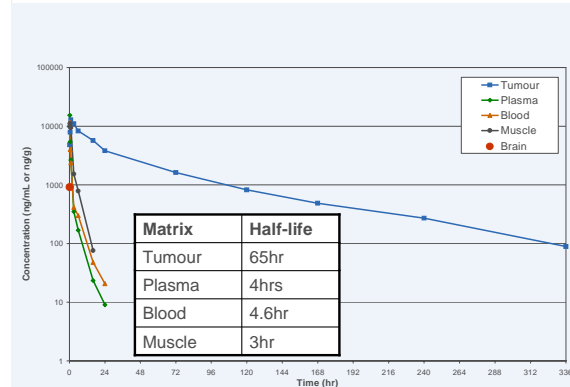


Figure 2: Tumour Levels of AT13387 in HCT 116 Tumour Bearing Mice

- Pharmacokinetic profiling of AT13387 in plasma, blood, brain muscle and tumour after a single 60mg/kg IP dose

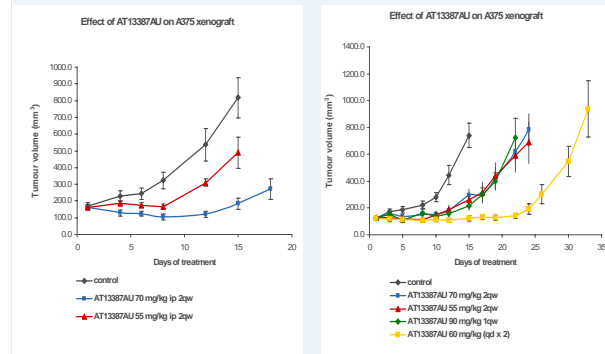


Figure 3: Efficacy of AT13387 in a B-Raf V600E Melanoma Model (A375)

- A375 tumour cells were injected s.c. into the flank of male nude mice.
- Treatment was started when tumours reached an average of 100-150 mm<sup>3</sup>
- A treatment group consisted of 6-8 animals.
- AT13387 was dissolved in 17.5% Hydroxypropyl-beta-cyclodextrin. All drugs were given at 10 ml/kg ip
- Results were plotted as mean ± SEM

## IN VIVO ACTIVITY

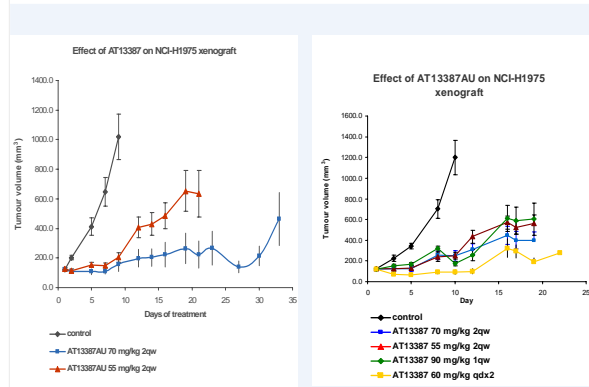


Figure 4: Efficacy of AT13387 in an EGFR Inhibitor Refractory Lung Model (NCI-H1975)

- NCI-H1975 tumour cells were injected s.c. into the flank of male nude mice.
- Treatment was started when the tumours reached an average size of 100-150 mm<sup>3</sup> (6-8 animals per group).
- AT13387 was dissolved in 17.5% Hydroxypropyl-beta-cyclodextrin. All drugs were dosed at 10 ml/kg ip.
- Results were plotted as mean ± SEM.

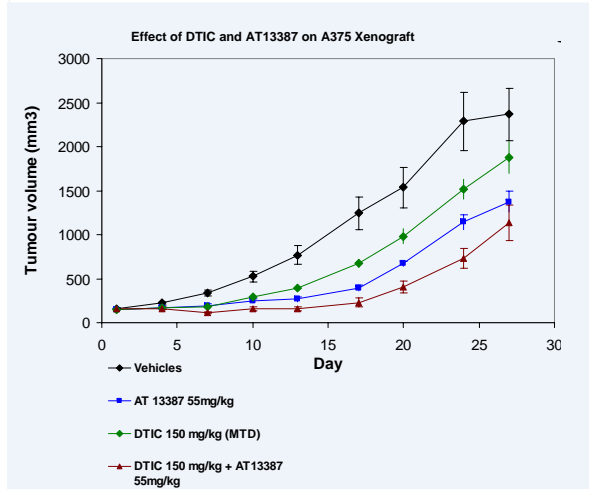


Figure 5: Efficacy of AT13387 and Dacarbazine in a B-Raf mutant Melanoma Model

A375 s.c. xenograft was established in nude mice. Dacarbazine-Dome was dissolved in saline and given ip on Days 1, 4, 10 and 13. AT13387 was given ip on Days 2, 5, 11 and 14. AT13387 monotherapy and combination therapy resulted in significant tumour growth inhibition between Days 13 and 24 (P<0.05) while dacarbazine monotherapy did not. Mean tumour sizes in combination groups were smaller than that in AT13387 monotherapy group, but the difference failed to reach statistical significance.

## LUNG CANCER COMBINATION DATA

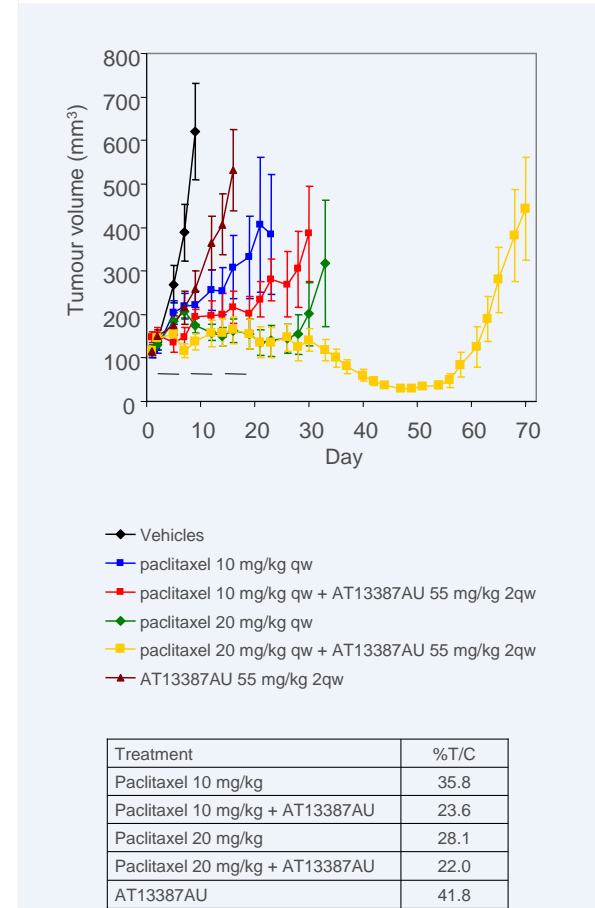


Figure 6: Efficacy of AT13387 and Paclitaxel in an EGFR Inhibitor Refractory Lung Cancer Model

NCI-H1975 s.c. xenograft was established in nude mice. Paclitaxel was dissolved in 10% Cremophor, 10% ethanol and 80% saline and was given ip once a week (Days 1, 8 and 15). AT13387 was given ip twice a week (Days 2, 5, 9, 12, 16 and 19). Tumours were measured three times a week. (A) Mean tumour sizes. All treatment resulted in significant tumour growth inhibition on Day 9 (P<0.001).

## PHARMACODYNAMIC RESULTS

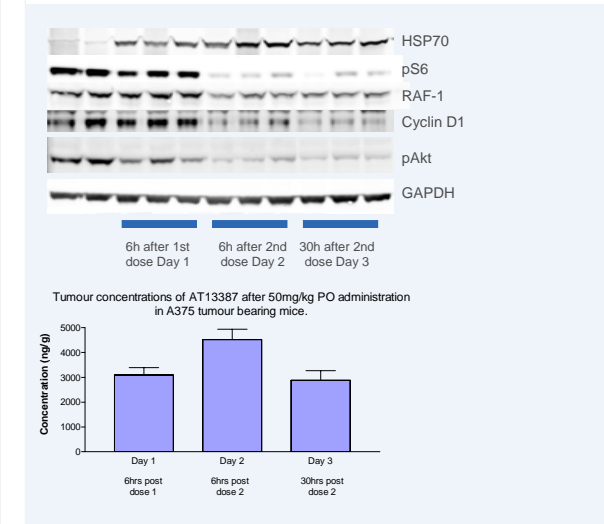


Figure 7: Tumour Levels and Biomarker Knockdown After Oral Dosing of AT13387 in B-Raf Mutant Melanoma Xenograft

50mg/kg of AT13387 was dosed daily by the oral route in mice for two days to ensure saturation kinetics and samples were taken at different times for analysis to test for the kinetics of biomarker knockdown. HSP70 and pAkt were affected within 6 hours of dosing and Cyclin D1 and pS6 required a further 24 hours before complete client protein knockdown was apparent

## CONCLUSIONS

- Astex Therapeutics has applied its fragment-based screening approach (Pyramid™) followed by structure based drug design to discover AT13387, a non-ansamycin inhibitor of HSP90 activity
- AT13387 induces degradation of client proteins in each of the transformed cancer lines
- AT13387 inhibits the growth of lung cancer and melanoma lines activated by oncogenes which are refractory to common therapies *in vitro* and in animal models
- AT13387 is active in B-Raf mutant melanoma models and is superior to DTIC in combination experiments
- Combining AT13387 in lung cancer with paclitaxel for 19 days of treatment was synergistic and resulted in a greater than a 60 day delay in tumour progression in mouse models of survival
- AT13387 displays pharmacodynamic activity when dosed via the oral route in a melanoma model

