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Significance of Long Term Pharmacodynamic Actions of the HSP90 Inhibitor AT13387

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INTRODUCTION

Heat Shock Protein 90 (HSP90) is a member of a family of molecular chaperone proteins which direct the folding of polypeptides into functional configurations affecting stabilisation and activation. Many of these oncogenic client proteins regulate tumour cell growth, survival and apoptosis. Inhibition of HSP90 with AT13387, a small molecule inhibitor discovered using fragment-based drug discovery, has been shown to result in client protein degradation, suppression of cytoplasmic signalling, cell cycle arrest and apoptosis. We investigated the kinetics of the direct effects on client protein levels and compared them to the kinetics of the biochemical and functional consequences of client protein loss (e.g. HSP70 induction, Akt pathway status, PARP cleavage) to understand the significance of these different events.

CELLULAR DATA

AT13387, along with other HSP90 inhibitors was profiled in a panel of cell lines reported to have an activated Akt pathway through different mechanisms and results are shown in Table 1.

Table 1. AT13387 Inhibits the Proliferation of Cell Lines With Activated Akt Pathways

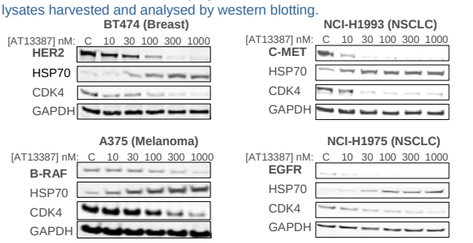
		IC50 (nM)				
Cell Line	**Source**	**Paclitaxel**	**AT13387**	**SNX-2112**	**BIIB021**	**17-AAG**
A375 [a] **(B-RAF mut)**	Melanoma	2.9	18	23	89	26
MV4-11 [b] **(Flt3-ITD)**	AML	3.3	12	11	52	31
NCI-H1975 [a] **(EGFR mut)**	NSCLC	3.8	22	36	210	ND
SKBr3 [a] **(HER-2+++)**	Breast	3.6	55	24	100	18
SK-MEL-28 [a] **(B-RAF mut)**	Melanoma	2.4	48	56	ND	40
SKOV-3 [a] **(HER-2+++)**	Ovarianc	6.3	48	67	260	64

Proliferation assays of ≥3 doubling times for each cell line were used. Relative cell numbers were determined using Alamar Blue™. Suppliers: [a] ATCC [b] DSMZ [c] ECACC. Mean IC50s shown, n=≥2. Final DMSO concentration of 0.1%.

The effects of AT13387 on HSP90 client protein levels were then investigated by western blotting and are shown in Figure 1.

Figure 1. Client Protein Knockdown in Breast, Melanoma and Lung Cancer Cell Lines

Cells were incubated with varying concentrations of AT13387 for 18 hours, lysates harvested and analysed by western blotting.



AT13387 knocks down known oncogenic protein clients of HSP90 (HER2, C-MET, B-RAF and EGFR), induces HSP70 and knocks down protein client CDK4 in all cases.

The proliferation assays show the effects of HSP90 inhibitors when compounds are continuously present on cells. We wanted to compare the duration of effects on cellular signalling when the cells were exposed to compound for a short period of time as would better reflect the case in the *in vivo* setting. We therefore investigated the signalling following compound wash off in the A375 B-RAF mutant melanoma cell line and results are shown in Figure 2.

Figure 2. *In vitro* Duration of HSP70 Induction, Client Knockdown and PI3K/Akt and Raf/Mek/Erk Pathway Signalling Following 24h Compound Exposure to published HSP90 Inhibitors

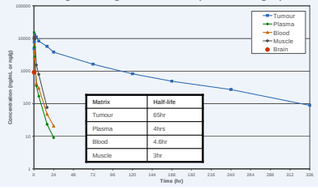
A375 B-RAF mutant melanoma cells were treated with 1μM HSP90 inhibitor for 24h, floating and adherent cells washed with PBS and culture medium replaced. Samples were harvested at the indicated times post wash. Final DMSO concentration of 0.1%.

All the inhibitors tested induced HSP70, reduced levels of the client protein Akt and phosphorylation of S6 and ERK1/2 following 24h exposure as can be seen at the 1h post wash time-point. AT13387 maintained these effects for 48h following compound removal compared to 24h or less for other inhibitors.

PHARMACOKINETIC/DYNAMIC PROFILING

Figure 3. Tumour Levels of AT13387 in HCT 116 Tumour Bearing Mice.

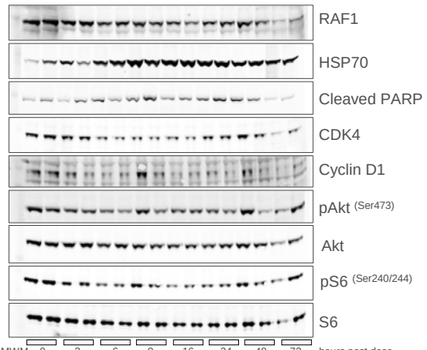
Pharmacokinetic profiling of AT13387 in plasma, blood, brain, muscle and tumour after a single 60mg free base equivalent/kg i.p dose



Pharmacokinetic profiling shows AT13387 retention in the tumour only, indicating that normal tissues have just a short exposure to compound. This suggests an opportunity for a greater therapeutic window. We investigated if this translated into sustained pharmacodynamic biomarker effects and the results are shown in Figure 4.

Figure 4. Pharmacodynamic Effects Following a Single Dose of AT13387

AT13387 formulated in 17.5% hydroxypropyl-beta-cyclodextrin was administered once via the i.p. route to male Nu/Nu Balb/c mice bearing A375 xenografts at the dose levels of 90mg free base equivalent/kg of body weight. Tumour samples were removed at the indicated times and analysed by western blotting.



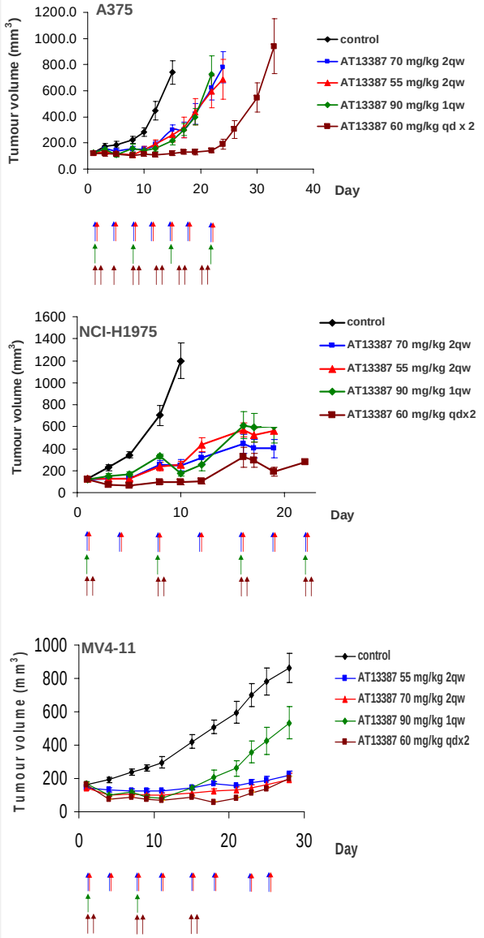
Pharmacodynamic studies show a single dose of AT13387 resulted in loss of the client proteins RAF1 and Akt for 72h or more. An increase in HSP70 protein levels was also observed to last at least 72h with a concomitant increase in cleaved PARP protein levels indicative of apoptosis. The earliest effects observed were reduced levels of phosphorylated Akt and S6 following treatment. This indicates that the effect of HSP90 inhibition on phosphorylation of key proteins in the Akt pathway is not simply a consequence of destabilisation of Akt and loss of Akt protein. It likely also includes either an action upstream of Akt or destabilization of a complex supported by HSP90 and including Akt activating kinases.

These *in vitro* (Figure 2) and pharmacokinetic results (Figure 3) suggest that these sustained effects on biomarkers following a single dose (Figure 4) could be due to a combination of tumour specific retention of the compound and a sustained inhibition of HSP90 by AT13387.

EFFICACY

Figure 5. Efficacy of AT13387 in Xenograft Models

Tumour cells were injected s.c. into the flank of male nude mice. A treatment group consisted of 6-8 animals. AT13387 was formulated in 17.5% hydroxypropyl-beta-cyclodextrin and dosed at a dose volume of 10 ml/kg i.p. Results were plotted as mean ± SEM



A single dose of AT13387 resulted in significant tumour growth delay in a xenograft models of melanoma, NSCLC and AML.

A comparison of the binding potency, tumour half-life, duration of biomarker response and reported efficacy on a once weekly dosing schedule previously published for a number of HSP90 inhibitors in development is summarised in Table 2. AT13387 HSP90 binding potency was measured by ITC.

Table 2. HSP90 Inhibitors in Development

Compound	**Binding Potency**	**Tumour Retention** T1/2 (mouse xenograft)	**Biomarker duration from single dose**	**Efficacy reported on 1x weekly schedule**
AT13387 Astex	0.6nM	65hr	≥72hr	YES
NVP-AUY922 Novartis/Vernalis	1.7nM[1]	30hr[2]	24-48hr[2] Her-2 72-96h[2] HSP70	YES[2]
XL888 Exelixis	1.6nM[3]	Not reported	Not reported	YES[3]
IPI-504 Infinity	9nM[4] (reducing conditions)	~16hr[5]	~24 - 48 hr[4]	YES
SNX-2112/5422 Pfizer/Serenex	200nM[5]/30nM[6]	~10 -15 hr[5]	24hr[6]	NO
MPC-100 Myriad Pharma	140nM[7]	48hr[7]	Not reported	NO
CU-0305 Curis	100nM[8]	15-20hr[8]	48hr[8]	NO
BIIB021 Biogen-Idec	600nM[9]/1.7nM[10]	<6hr	<24hr[10]	NO

CONCLUSIONS

HSP90 is an attractive oncology target and several inhibitors have been developed including natural products (ansamycins), purines and synthetic small molecules and different dosing schedules have been exploited for each.

We have used an *in vitro* system to demonstrate that AT13387 benefits from having the longest sustained inhibitory effect on client proteins and biomarkers of HSP90 inhibition.

AT13387 tumour specific retention combined with long duration of biomarker knockdown, allows dosing once weekly, minimising effects on normal tissues whilst maintaining good efficacy.

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