Poster: A217

Comparison of Long Term Pharmacodynamic Actions of the Synthetic Small Molecule HSP90 Inhibitor AT13387 in Multiple Xenograft Models

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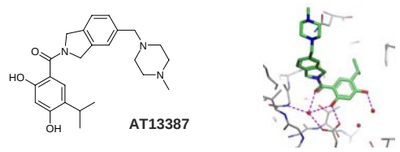
INTRODUCTION

AT13387 is a novel small molecule inhibitor of HSP90, a member of a family of molecular chaperones. Previously we highlighted an association between the high affinity binding of HSP90 inhibitors to the N-terminal ATPase domain of HSP90 and the duration of target inhibition in tumour cell lines *in vitro*. One potential benefit of this long duration of action is that it might allow for less frequent dosing thus minimising systemic exposure and the potential for side effects. Here we have expanded the investigation to a wider number of tumour cell lines and to xenograft models *in vivo*. These studies demonstrate that AT13387 can suppress the levels of HSP90 client proteins for 7 days or more following a brief treatment *in vitro*. This is significantly longer than all other HSP90 inhibitors tested. AT13387 also has an extended pharmacodynamic action in xenograft tumours in nude mice. Client proteins were suppressed for up to 96hr following a single dose. Again this is significantly longer than observed or reported for other HSP90 inhibitors.
These data provide further support for the potential benefit of long acting HSP90 inhibitors as a way of maintaining anti-tumour effects whilst minimising potential for undesirable effects associated with systemic exposure.

Figure 1. Structure of AT13387 and key interactions with protein



AT13387

AT13387 was discovered using a fragment–based approach the details of which are described in the accompanying poster # A211 by Murray *et al*.

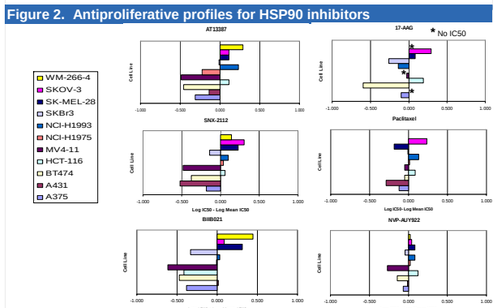
Table 1. Antiproliferative activity of AT13387

Origin	Cell line	IC_{50} (nM)
Colon Carcinoma	HCT116[c]	58
	HT-29[c]	80
	SW620[c]	220
Lung Carcinoma	A549[c]	22
	NCI-H1975[a]	14
	NCI-H1703[a]	58
	NCI-H1993[a]	63
Epidermoid Carcinoma	A431[b]	27
Breast Carcinoma	MCF-7[c]	64
	MDA-MB-231[c]	260
	MDA-MB-468[a]	26
	SK-BR3[a]	64
	T47D[c]	30
	BT474[a]	23
Multiple Myeloma	U266[b]	70
	RPMI 8226[a]	70
Pancreatic Cells	PANC1[a]	61
Glioblastoma	U87MG[a]	410
Prostate Carcinoma	DU145[a]	95
	PC3[a]	140
	LNCaP[c]	78
	22Rv1[b]	46
Uterine Sarcoma	MES-SA[c]	67
	MES-SA/Dx5[c]	47
Ovarian	SKOV3[a]	44
Leukemia	HL60[c]	23
	K562[c]	49
	MV4-11[b]	12
Melanoma	A375[a]	18
	WM-266-4[a]	72
	SkMel 28[a]	46

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. Final DMSO concentration of 0.1%.

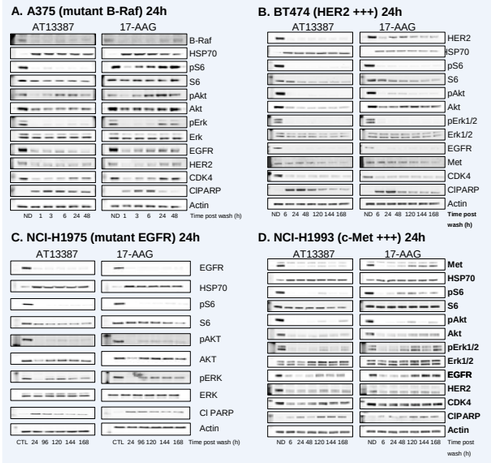
RESULTS

Figure 2. Antiproliferative profiles for HSP90 inhibitors



AT13387 inhibited the proliferation of a wide range of tumour cell lines with IC_{50}S in the range of 12 – 410nM [Table 1]. EGFR-family dependent lines were particularly sensitive to the agent e.g. NCI-H1975 (14nM), BT474 (23nM), A431 (27nM).
In general, the relative sensitivity of different cell lines was similar for a range of different HSP90 inhibitors [Figure 2]. For AT13387, SNX-2112, BIIB-021 and 17-AAG the cell lines MV4-11, BT-474, and A375 were the most sensitive whilst WM-266-4, SKOV-3 and SK-MEL-28 were the least sensitive. The pattern of sensitivity of cell lines to NVP-AUY-922 was slightly different however, in that there was a much smaller range between the most and least sensitive lines.

Figure 3. Suppression of clients *in vitro* by AT13387 and 17-AAG



Cells were treated with 1μM of AT13387 or 17-AAG for 24hr before washing to remove the compound. Levels of client proteins and phospho-signalling were monitored by immunoblotting at different times following the wash procedure

Figure 4. Suppression of client proteins in A375 cells *in vitro*

A375 (melanoma) cells were treated with 1μM of each HSP90 inhibitor for 24 hr before washing to remove the compound. Levels of client proteins and phospho-signalling were monitored by immunoblotting at different times following the wash procedure

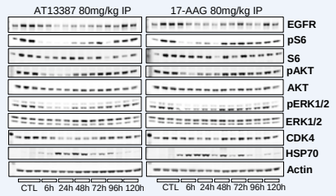
Treatment of several different tumour cell lines for 24hr *in vitro* with AT13387 led to suppression of client proteins and phospho-signalling for up to 7 days after removal of the inhibitor [Figures 3]. The duration of action of other HSP90 inhibitors was found to be significantly shorter. For 17-AAG [Figure 3] and SNX-2112 [Figure 4] most client proteins returned within 24 to 48hr, whilst for BIIB-021 [Figure 4] the duration of action was much shorter.
Reducing the period of treatment with the HSP90 inhibitors from 24hr to 7hr did not change the duration of action of AT13387. Client proteins in NCI-H1975 cells remained suppressed for 7 days [Figure 5]. For 17-AAG and SNX-2112 it appeared that the shorter treatment period reduced the extent of client protein suppression but the duration of action remained at 24 – 48hr.

Figure 5. Suppression of client proteins in NCI-H1975 cells *in vitro*

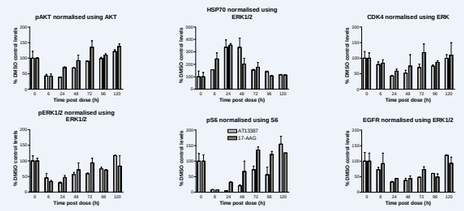
NCI-H1975 NSCLC cells were treated with 1μM of each HSP90 inhibitor for 7 hr before washing to remove the compound. Levels of client proteins and phospho-signalling were monitored by immunoblotting at different times following the wash procedure

Figure 6. Pharmacodynamics in NCI-H1975 (NSCLC) xenografts

A) NCI-H1975 (mutant EGFR) western blots



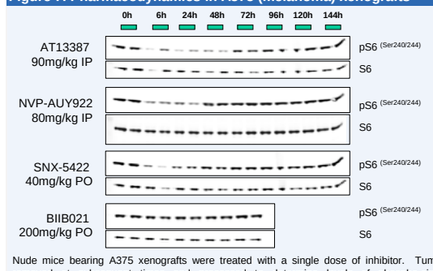
B) Semi-quantitative analysis of western blots



Nude mice bearing NCI-H1975 xenografts were treated with a single dose of either AT13387 or 17-AAG. Tumours were removed at subsequent times and processed to determine levels of client proteins and phospho-signalling by immunoblotting (A). Semi-quantitative analysis of the immunoblots is shown in (B).

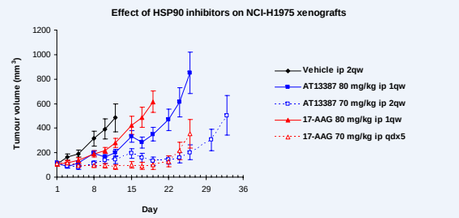
Following a single dose of AT13387 to mice bearing NCI-H1975 xenografts, levels of the client protein EGFR and phospho S6 were suppressed for 96hrs before returning to normal at 120hr after the treatment [Figure 6]. For other clients, levels were reduced at 48hr and had started to return to normal by 72hrs. The effects of 17AAG were similar to AT13387 in the NCI-H1975 xenografts [Figure 6] except levels of clients and phospho-signalling tended to return to normal earlier than for AT13387. This is particularly clear for AKT and phospho S6 which for 17-AAG had reached their minimum levels at 24hr but had started to recover by 48hr. In A375 xenografts a single 90mg/kg dose of AT13387 suppressed client proteins and phospho-signalling for 120hr [Figure 7], longer than observed with other HSP90 inhibitors.

Figure 7. Pharmacodynamics in A375 (melanoma) xenografts



Nude mice bearing A375 xenografts were treated with a single dose of inhibitor. Tumours were removed at subsequent times and processed to determine levels of phospho-signalling by immunoblotting.

Figure 8. 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 administered at 10 ml/kg i.p. 17-AAG was formulated in 3.2% Cremophor, 5.7% ethanol and 4.4% propylene glycol and administered at 30 ml/kg i.p. Results were plotted as mean ± SEM.

Nude mice bearing NCI-H1975 xenografts were used to investigate whether the longer duration of action exhibited by AT13387 had any beneficial effects in terms of the anti-tumour efficacy. AT13387 and 17-AAG were administered to mice at doses equating to the maximum tolerated dose on a given schedule. Figure 8 indicates that tumour growth can be completely inhibited by 70 mg/kg 17-AAG given on a daily x5 schedule over 3 weeks. Equivalent tumour growth inhibition could be achieved with 70mg/kg AT13387 given less frequently on a twice a week schedule over 3 weeks. The beneficial effects of the longer duration of action of AT13387 were further apparent from studies in which each agent was dosed 1x weekly at 80mg/kg. On this schedule 17-AAG lost much of its anti-tumour activity whilst AT13387, although less effective than when dose twice weekly, retained a significant anti-tumour activity.

CONCLUSIONS

AT13387 is a potent HSP90 inhibitor which *in vitro* and *in vivo* shows sustained suppression of client proteins and phospho-signalling.

Structurally different inhibitors share a similar profile of activity against a panel of tumour cell lines.

Agents in the class can be differentiated on the basis of their duration of action. *In vitro*, AT13387 has the longest duration of action and BIIB-021 the shortest.

The suppression of client proteins for 7 days following a brief treatment *in vitro* is the longest duration of action reported to date for agents in this class.

In xenografts, client proteins require 120hr to return to normal following treatment with AT13387.

Longer suppression of client proteins allows less frequent dosing with AT13387 without compromising the efficacy.

Presented at the EORTC-NCI-AACR Meeting in Boston on Monday November 16th 2009. This poster can be downloaded from the Astex website at www.astex-therapeutics.com