3069

Heat Shock Protein 90 Inhibitor in Patients With Refractory Solid Tumors: A Phase I Pharmacokinetic And Pharmacodynamic Study Of AT13387

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INTRODUCTION

- AT13387 is a highly potent Heat Shock Protein 90 (HSP90) inhibitor (Kd 0.71 nM). HSP90 is required for the functional stabilization of numerous oncogenic client proteins such as HER-2, ER, c-Met, EGFR, AKT, C-KIT, VEGF-R and B-RAF.
- *In vitro* preclinical experiments have shown that AT13387 can bind to the N-terminal ATP domain on HSP90, resulting in up regulation of HSP70, and down-regulation of key client proteins, causing growth arrest and apoptosis.
- *In vivo* experiments in murine xenograft models demonstrated that AT13387 causes prolonged knockdown of client proteins and has a half-life of 65-78 hours in the tumor xenografts.
- Due to the prolonged pharmacodynamic effect of AT13387, two dosing schedules are being explored clinically; twice weekly or once weekly administration, three weeks of every four.
- The results of the ongoing Phase I dose escalation study, AT13387/0001, in which patients have been treated with the twice weekly dosing schedule are presented.
- We present preliminary data demonstrating that we achieve inhibition of HSP90 in tumor that results in an increase in the apoptosis marker, cleaved caspase 3.

OBJECTIVES

Primary

- To identify the MTD of AT13387 when administered both once and twice weekly for three weeks out of every four.

Secondary

- To characterise the safety and tolerability of AT13387 including the identification of DLTs.
- To define the pharmacokinetics of AT13387 in plasma and urine.
- To demonstrate the pharmacodynamic activity of AT13387 in plasma, circulating peripheral blood mononuclear cells (PBMC) and in tumor using PET scans and biopsies.

METHODS

Study Design

- Phase I study in which AT13387 is administered IV for 1 hr, either once and twice weekly for three weeks out of every four; patients with histologically confirmed metastatic solid tumors, including lymphoma, who are refractory to standard therapy.
- Dose escalation is performed in accordance with the modified Fibonacci principle.
- DLTs were defined as;
  - Neutropenia <0.5 x 10^9/L for > 5 days;
  - Neutropenia <1 x 10^9/L with fever; Thrombocytopenia <25 x 10^9/L accompanied by bleeding or thrombocytopenia <10 x 10^9/L;
  - Any Grade 3 or 4 non-hematological toxicity which is not a consequence of tumor progression (other than nausea, vomiting or diarrhea in the absence of appropriate prophylaxis);
  - More than one individual dose omission during the first cycle of treatment due to the appearance of drug related toxicity where, in the investigator's opinion, it is likely that administration of AT13387 is causally linked with the toxicity or observed effect.

DOSE ESCALATION AND SAFETY

Table 1: Dose Escalation Scheme

Dose Level *(mg/m²/day)*	Number of Patients Treated	Number of Cycles Received (median)	Dose Limiting Toxicities and Adverse Events
10	4	1 – 3 *(1)*	None
20	3	1 – 6 *(2)*	None
40	3	1 – 3 *(3)*	None
80	5	1 – 8 *(2)*	None Grade 2 diarrhea
120 *	6	1 – 4 *(2)*	Diarrhea & fatigue (Grade 1 and 2) Reversible visual changes (Grade 1) Hypotension during dosing (Grade 2) Dry skin/mouth (Grade 1)

* Excludes patients enrolled in the expansion cohort

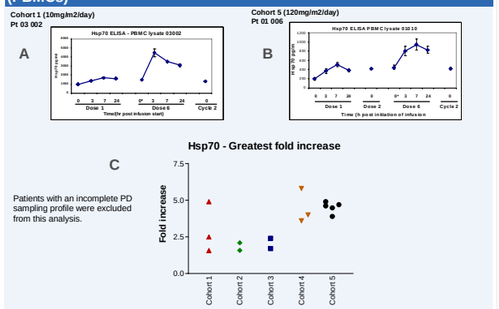
Table 2: Patient Demographics

Demographics	Median 57 years (range 28 – 77) Female 15: Male 11
Diagnoses	Glioblastoma Multiforme 1 (4%) Colon Cancer 3 (12%) Breast cancer 1 (4%) Prostate Cancer 2 (8%) Carcinoma of the thyroid 1 (4%) Squamous cell carcinoma of esophagus 1 (4%) Metastatic uveal melanoma 6 (24%) Carcinoma of the pituitary gland 1 (4%) NSCLC 4 (16%) Synovial sarcoma 1 (4%) Small cell bladder 1 (4%) Rectal cancer 3 (12%) Pancreatic cancer 1 (4%)

- To date a total of 26 patients have received AT13387 on the twice weekly schedule for this study. These are included in the patient demographics in Table 2.
- The safety data presented in Table 1 is for the patients in cohorts 1-5 (21 patients) and does not include information from the patients in the expansion cohort.
- No deaths have been reported within 30 days of receiving treatment with AT13387.
- Nine serious adverse reactions have been reported, of which two were suspected unexpected serious adverse reactions; decreased LVEF (outcome resolved) and retinal changes (outcome resolved).
- A variety of mild or moderate drug-related reversible toxicities have been reported, with gastrointestinal toxicity, especially transient diarrhea, and fatigue being most commonly reported.
- Reversible Grade 1 visual changes, usually occurring at Day 11 or later, were observed at the highest dose. These included blurred vision, flashes and delayed light/dark accommodation, although only 1 patient was withdrawn as a result of these changes.
- Some patients experienced hypotension and light-headedness during the infusion.
- In conclusion, no DLTs were recorded in Cohorts 1-5. Dose escalation beyond Cohort 5 (120 mg/m²/dose) was not considered suitable due to an accumulation of the low grade toxicities described above.
- A dose of 120 mg/m²/dose was defined as the MTD for the twice weekly dosing schedule.

PHARMACODYNAMICS

Figure 1: Induction of HSP70 in Peripheral Blood Mononuclear Cells (PBMCs)



Blood samples were taken prior to and 3, 7 and 24h following administration of AT13387 on days 1 and 18 of cycle 1. Additional samples were taken prior to dosing on day 4 and prior to dosing on day 1 of cycle 2. PBMCs were prepared and analysed for HSP70 induction by ELISA.

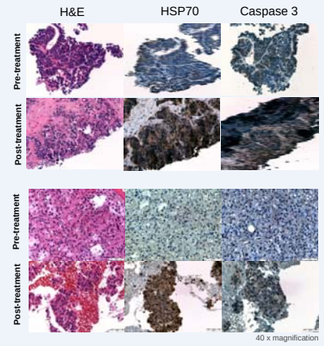
- HSP70 induction is a sensitive marker of HSP90 inhibition and has been demonstrated to be upregulated in both PBMCs and tumor tissue following dosing with AT13387 in pre-clinical models.
- Figure 1 shows that we observed HSP70 induction to some extent in all patients that received drug on the study and had a complete PD profile.
- Figures 1A and 1B show individual profiles for 2 patients, one from cohort 1 and one from cohort 5. The pattern of induction was similar in both cases with a transient increase in HSP70 following infusion, peaking at 7 hr.
- The magnitude of the increase was greater on Day 18 than on Day 1 and in later cohorts the trough sample levels of HSP70 were not returned to baseline prior to receiving the next infusion.
- Figure 1C summarises the data across all cohorts presented as the single maximal fold increase in HSP70 for each individual patient. At cohort 4 and above there is a greater fold increase in HSP70 than at lower doses. This suggests a greater pharmacodynamic activity at doses above 80 mg/m².

Figure 2: Client Protein Knockdown in PBMCs

PBMCs were prepared and analysed by western blotting for a number of putative HSP90 client proteins. Representative blots are shown for the two patients whose HSP70 ELISA data are described above.

- Client protein knockdown in PBMCs was demonstrated in several patients across the cohorts (Figure 2).
- In the examples above Akt, CDK4, Raf-1 and Lck levels were all reduced following infusion of AT13387, with levels recovering prior to administration of the next dose.
- The results were not as consistent as those for HSP70 and reflective of the shorter plasma half life of the compound perhaps being insufficient to drive the exposures required for extended client protein knockdown.
- These data were consistent with the plasma PK (Figure 4).
- The preclinical data suggested that extended exposures may be achieved in tumor tissue, and paired tumor biopsies were mandated in the dose expansion cohort to enable this hypothesis to be tested.

Figure 3: Pharmacodynamics in Tumor Biopsies

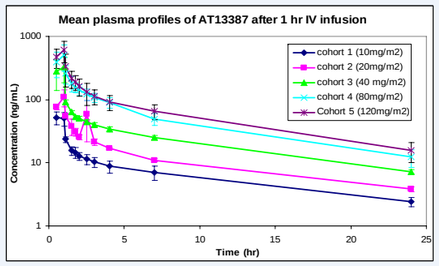


- Patient diagnosis – (A) Small Cell Bladder Carcinoma, (B) Prostate.
- Post-treatment biopsies were taken on day 19 of cycle 1, 24 hr after administration of AT13387.
- Tissue samples were fixed in 10% neutral buffered formalin for 24h prior to paraffin embedding. Slides were prepared and stained using hematoxylin and eosin to identify areas of tumor tissue or HSP70 and caspase 3 to monitor inhibition of HSP90 and induction of apoptosis respectively.

- HSP90 inhibition as indicated by an induction of HSP70 was clearly observed in tumor tissue at 24 hr following infusion with AT13387 (Figure 3).
- This was associated with an increase in caspase 3, indicative of apoptosis within the tumor.
- These data suggest that we are achieving sufficient AT13387 levels in tumor to cause inhibition of HSP90.

PHARMACOKINETICS

Figure 4: Plasma Time Concentration Profile of AT13387 by Cohort



- The mean pharmacokinetics for patients in the dose escalation cohorts are presented in Figure 4. The $t_{1/2}$ mean cohort values ranged from 6.5 to 9.1 hr. There was no notable accumulation or reduction in exposure between Day 1 and Day 18 of Cycle 1.
- Plasma clearance of AT13387 was independent of dose and showed a mean value of 26.3 ml/min/kg (SD 6.4 ml/min/kg).
- Measurement of the concentration of AT13387 excreted in urine showed that renal elimination is of minor importance in the total clearance of the drug. Less than 5% of the administered dose was recovered in the urine 48 hours post-dose.

DISEASE RESPONSE

- Patients with measurable disease had their tumor dimensions calculated according to RECIST at screening and every two months during the first 6 months of treatment.
- Stable disease for at least six months was observed in 2/21 (10%) of patients.
  - Patient 01-003 who had thyroid cancer and was treated with 20 mg/m² per dose and patient 01-007 who had metastatic uveal melanoma and was treated with 80 mg/m²
- Stable disease for 2-6 months was observed in 5/21 (24%) of patients.

CONCLUSIONS

Safety

- AT13387 is well tolerated between 10-120mg/m². The most commonly reported AEs were gastrointestinal toxicity, especially transient diarrhea and fatigue, which have been previously reported for geldanamycin HSP90 inhibitors, and reversible visual symptoms.
- The maximum tolerated dose of AT13387 for a twice weekly dosing schedule (three weeks out of four) was defined as 120 mg/m² per dose.

Pharmacokinetics/Pharmacodynamics

- Inhibition of HSP90, as shown by induction of HSP70 in a dose-dependent manner, was observed in PBMCs from patients treated with AT13387 across all cohorts.
- Similarly, upregulation of HSP70 and caspase 3 was observed in post-dose biopsy samples collected from patients in the expansion cohort on the twice weekly schedule.
- The PD data demonstrate that we are achieving pharmacologically active levels of drug in both the plasma and in tumor, and suggest that consistent with preclinical data we can exert a prolonged tumor PD effect in comparison to the plasma half life.
- The $t_{1/2}$ mean cohort values ranged from 6.5 to 9.1 hr. Maximum $t_{1/2}$ observed for any individual profile was 14hrs. There was no notable accumulation or reduction in exposure between Day 1 and Day 18 of Cycle 1.
- Plasma clearance of AT13387 is independent of dose and showed a mean value of 26.3 ml/min/kg (SD 6.4 ml/min/kg). Excretion in urine is minimal.

Response

- Stable disease for at least six months has been observed in 2 patients and in a further 5 patients for beyond 2 cycles of treatment.

Ongoing work

- Completion of the twice weekly dose expansion phase with associated tumor PD sampling (biopsy and PET imaging).
- Tumor biopsies will be assessed for client protein knockdown.
- The MTD for a once weekly dosing schedule will be determined.

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