

A Dose Escalation and Pharmacodynamic Study of AT9283 in Patients with Refractory Solid Tumours

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Ruth Plummer¹, Hilary Calvert¹, Hendrik-Tobias Arkenau², Vicky Lock³, Matt Squires³, Donna-Michelle Smith³, Samantha Lewis³, Ian Judson²
¹The Northern Centre for Cancer Treatment, Newcastle upon Tyne, UK. ²The Royal Marsden Hospital, London, UK. ³Astex Therapeutics Ltd., Cambridge, UK.

BACKGROUND

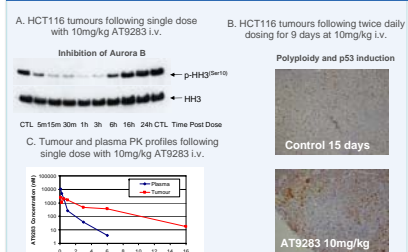
- Aurora kinases are key regulators of mitosis having roles in centrosome function, mitotic spindle formation, chromosome segregation and cytokinesis.
- Aurora A is thought to be involved in centrosome separation, maturation and bipolar spindle assembly through recruitment and phosphorylation of microtubule associated proteins. It also phosphorylates p53 targeting the phosphoprotein for degradation and thereby bypassing the G2/M checkpoint.
- Aurora B is a 'chromosome passenger protein', its localization changing throughout mitosis. During prophase and metaphase it is concentrated along the inner centromeres and at anaphase moves to the central spindle, mediating chromosome segregation and cytokinesis. It associates with specific proteins, such as survivin forming 'chromosome passenger' complexes and phosphorylates a number of targets, including histone H3.
- Over-expression of the Aurora kinases A and B have been linked to genetic instability and cancer, due to dysregulation of cell division.

INTRODUCTION

- AT9283 is a small molecule inhibitor of several serine/threonine and tyrosine kinases with an IC50 < 10 nm against the Aurora kinases A and B, Tyk2, JAK2, RSK2, Ret, Mer, Yes and GSK3 beta.
- The preliminary results of a phase I and pharmacodynamic study of AT9283 administered as a continuous intravenous infusion over 72 hours every three weeks to patients with refractory solid tumours are presented here. Inclusion and exclusion criteria were standard. Dose escalation was performed according to a standard 3+3 design.

PRECLINICAL DATA

Figure 1: Preclinical Pharmacokinetic/Pharmacodynamic Studies with AT9283



- HCT116 tumour bearing mice received either a single dose of AT9283 at 10mg/kg i.v. (Fig 1A and C) or twice daily doses at 10mg/kg (Fig 1B). Tumours were removed at the indicated times following dosing (A and B) and prepared for western blotting and immunohistochemistry respectively. Tumour and plasma concentrations were determined by LC-MS/MS (C).
- Efficacious doses of AT9283 induced a transient knockdown of Aurora B markers (pH3) for ~6h, consistent with the levels of compound seen in the tumour.
- Polyploidy and p53 induction was observed in tumours following several days dosing.

PATIENT DEMOGRAPHICS

- A total of 33 patients have been treated, median age 63 years (range 33 – 77 years), Male:Female 25:8.
- The most common diagnosis was colorectal cancer although patients with breast, ovarian, NSCLC, upper gastrointestinal, GIST and thyroid cancer have also been treated with AT9283 as part of the study.

Table 1: Dose Escalation Scheme

Dose Level (mg/m ² /day)	Number of Patients Treated	Number of Cycles Received (Median)	Dose Limiting Toxicities (DLT)
1 (1.5)	3	2 – 7 (2)	None
2 (3)	3	1 – 4 (4)	None
3 (6)	3	1 – 2 (2)	None
4 (12)	6	1 – 6 (2)	Febrile Neutropenia (3)
5 (9)	18	1 – 6 Ongoing (TBC)	Hickmann Line Infection in the presence of Grade 3 neutropenia

DOSE ESCALATION

- No evidence of DLT was observed until cohort four (12 mg/m² per day) where three of six patients experienced febrile neutropenia during their first cycle of treatment.
- The administered dose was reduced in cohort five (9 mg/m² per day) where only one patient of 18 treated experienced a DLT during their first cycle of treatment.
- 9 mg/m² per day was identified as the maximum tolerated dose (MTD).

RESULTS

- Treatment has been well tolerated with toxicities being predominantly CTC v3.0 Grade 1 or 2 in severity.
- The only dose limiting toxicities observed to date have been febrile neutropenia.
- To date the maximum number of cycles of treatment received is 7.
- Four patients have received at least 6 cycles of treatment with a best response to treatment of stable disease (NSCLC, Colorectal carcinoma [3]).

SERIOUS ADVERSE EVENTS

The following Serious Adverse Events were reported as being at least possibly related to treatment with AT9283:

- Febrile neutropenia (x3)
- Central line infection
- Vomiting (Grade 3)/ Infection (Grade 3)/ Neutropenia (Grade 2)

PHARMACODYNAMICS

Table 2: Summary Table

Dose mg/m ² /day	ELISA		IHC			
	M30 increase	M65 increase	pH3 inhibition	p53 stabilisation	PCNA reduction	Ki67 reduction
1.5	0/3	0/3	2/3	2/3	0/3	1/3
3	0/3	0/3	2/3	2/3	1/3	0/3
6	2/3	2/3	3/3	3/3	0/3	1/3
12	3/4	4/4	3/4	1/4	3/4	0/4

- Skin punch biopsies were taken at 0h and 48h prior to and during the infusion of AT9283 in cycle 1. Immunohistochemistry was performed on paraffin embedded sections.
- Serum samples were obtained at 0h, 24h, 48h 72h and 8 days after commencement of infusion during cycles 1 and 2. M65 and M30 ELISA assays were performed to detect cytokeratin and its caspase cleaved form as an indirect marker of tumour apoptosis.
- Table 2 summarises the numbers of patients per cohort that exhibited the changes in biological markers anticipated from pre-clinical studies following administration of AT9283.

Figure 2: Immunohistochemistry for PCNA Prior to and During an Infusion of AT9283

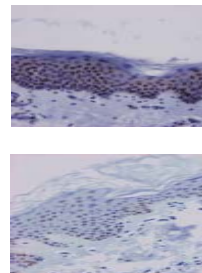
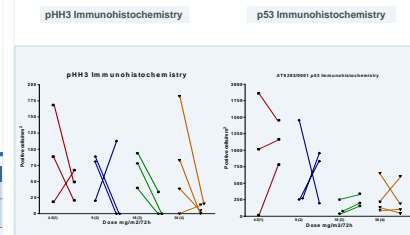
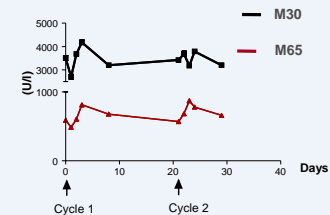


Figure 3: Aurora Inhibitory Effects are Observed Across the Dose Range



- Pharmacodynamic effects were observed at all dose levels

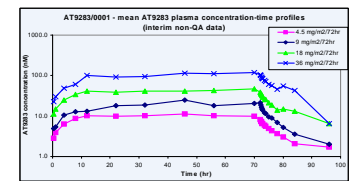
Figure 4: M30/M65 ELISA Data from 2 Patients at 6mg/m²/day



- Typical profile of the apoptotic markers M65 and M30 in serum.
- The levels of both M30 and M65 increased during the infusion of AT9283 (Cycles one and two).
- Serum levels peak at 48-72h after starting the intravenous infusion.

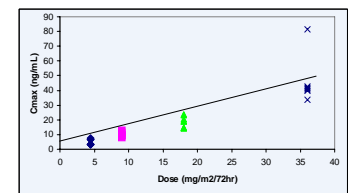
PHARMACOKINETICS

Figure 5: Plasma Concentration-time Profile of AT9283



- Steady state plasma concentrations were achieved at all dose levels
- Plasma elimination was biphasic with only modest inpatient variation

Figure 6: The Relationship Between Cmax and Administered Dose



CONCLUSION

- The MTD of AT9283 when administered as a 72 hour continuous intravenous infusion is 9 mg/m²/day in patients with solid tumours
- The dose limiting toxicity is febrile neutropenia and treatment results in significant disease stabilisation in a small proportion of patients.
- Treatment was otherwise well tolerated with low levels of fatigue and gastrointestinal toxicity reported.
- Pharmacodynamic evidence of aurora kinase A and aurora kinase B inhibition was observed during the infusion at all dose levels studied.

