

Biomarker Identification for AT9283: A Multitargeted Kinase Inhibitor with Aurora A and B Activities from Pre-clinical Models to Clinical Trials

John Lyons, Jayne Curry, Matthias Reule, Victoria Lock, Murray Yule, Neil Thompson and Matt Squires
Astex 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, JAK2 and Abl kinase.
- Biomarkers were tested *in vitro*, *in vivo* and then in patient samples to confirm the mechanism of action of AT9283 during pre-clinical and early clinical development.
- Biomarkers tested were p53 stability, Ser10 phosphorylation on Histone H3, Ki67, ploidy and cytochrome degradation (M30/M65 ELISA).

PRECLINICAL DATA

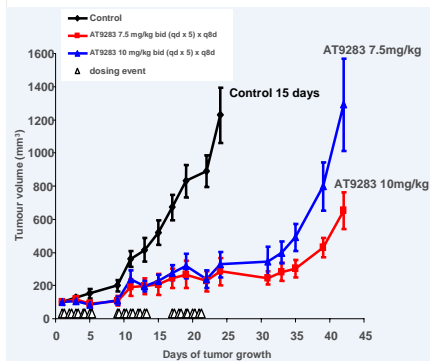


Figure 1: Effect of AT9283 on Growth of a Human Xenograft in Mice after IP Administration

AT9283 COMPOUND PROFILE

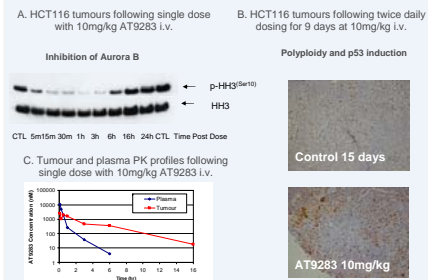
Figure 2: X ray Crystal Structure of AT9283 complexed to Aurora A Kinase



Table 1: *In vitro* Activity of AT9283 against Aurora Kinases A and B

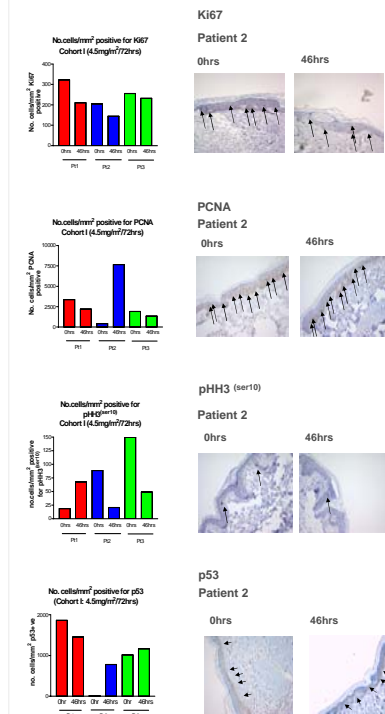
Enzyme	Inhibition
Aurora A	52% at 3 nM
Aurora B	58% at 3 nM

Figure 3: Pre-clinical Pharmacokinetic/Pharmacodynamic Studies with AT9283



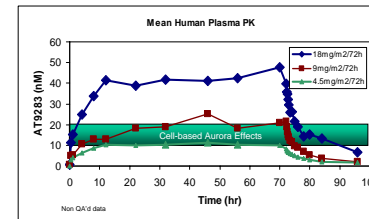
- HCT116 tumour bearing mice received either a single dose of AT9283 at 10mg/kg i.v. (Fig 2A and C) or twice daily doses at 10mg/kg (Fig 1B). Tumours were removed at the indicated times following dosing (A and B) and prepared for western blotting and immunohistochemistry respectively.
- Tumour and plasma concentrations were determined by LC-MS/MS (C).
- Efficacious doses of AT9283 induced a transient knockdown of Aurora B markers (pHH3) for ~6h, consistent with the levels of compound seen in the tumour.
- Ploidy and p53 induction was observed in tumours following several days dosing.

Figure 4: Example of Patient Biomarker Changes on Administration of AT9283 at a Dose of 4.5mg/m²/72h



- Staining is robust with very little inter-patient variability is noted
- Biomarkers Inhibition of Ser10 pHH3 is noted in 2/3 patients in cohort 1 and p53 stabilisation is observed

Figure 5: Comparison of PD Responses Expected to Modulate Aurora Activity with Plasma Profiles Obtained From AT9283/0001



- At a dose of 18 mg/m²/72hr, levels of AT9283 in human plasma are maintained well above those required for modulation of Aurora in cell based assays.
- PPB was measured at approx. 80% and is not accounted for here.

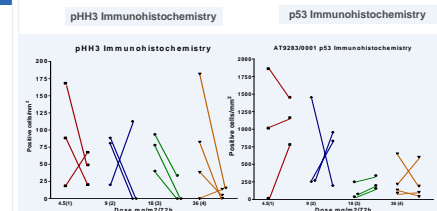
PHARMACODYNAMICS

Table 2: Summary Table of Biomarker Data in Patient Samples

Dose mg/m ² /72h	ELISA		IHC			
	M30 increase	M65 increase	pHH3 inhibition	p53 stabilisation	PCNA reduction	Ki67 reduction
4.5	0/3	0/3	2/3	2/3	0/3	1/3
9	0/3	0/3	2/3	2/3	1/3	0/3
18	2/3	2/3	3/3	3/3	0/3	1/3
36	3/4	4/4	3/4	1/4	3/4	0/4

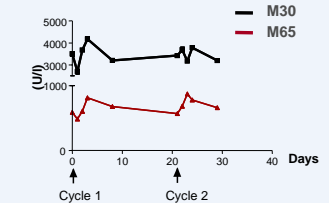
- Skin punch biopsies were taken at 0h and 48h prior to and during the infusion of AT9283 in cycle 1. Immunohistochemistry was performed on paraffin embedded sections.
- Serum samples were obtained at 0h, 24h, 48h 72h and 8 days after commencement of infusion during cycles 1 and 2. M65 and M30 ELISA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

Figure 6: Aurora Inhibitory Effects are Observed Across the Dose Range



- Pharmacodynamic effects were observed at all dose levels.

Figure 7: 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.

CONCLUSION

- Series of robust markers developed and applied to surrogate tissue (skin) in clinical study
- At doses >4.5mg/M² a robust and consistent biochemical effect is observed on the target kinases (Auroras A and B)
- At doses >9mg/M² a robust biological consequence of this inhibitory effect is observed in inhibition of cell proliferation (Ki67 and PCNA) and induction of apoptosis (M30/M65 ELISA)
- Biomarkers were tested *in vitro*, *in vivo* and then in patient samples to confirm the mechanism of action of AT9283 during pre-clinical and early clinical development
- Biomarkers tested were p53 stability, Ser10 phosphorylation on Histone H3, Ki67, PCNA and cytochrome degradation (M30/M65 ELISA)
- Tracking PK/PD relationships in pre-clinical and clinical studies has confirmed its MOA and confirmed biologically effective doses in patients

