

A Phase I and Pharmacodynamic Trial of AT9283, an Aurora Kinase Inhibitor in Patients with Refractory Leukemia

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INTRODUCTION

- AT9283 is a small molecule inhibitor of several serine/threonine and tyrosine kinases including the Aurora kinases, JAK2 and 3, Flt3 and Abl (Table1).
- Overactivity of several of these kinases either via the wt or mutant forms has been implicated in the etiology of a variety of hematological malignancies. *In vitro* studies indicate that AML cell lines are highly sensitive to inhibition of these signalling pathways by AT9283 (Figure 1).
- The preliminary results of a phase I study of AT9283 administered as a continuous intravenous infusion over 72 hours to patients with pre-treated acute leukemias, chronic myeloid leukemia, high-risk myelodysplastic syndromes or myelofibrosis are presented here. Dose escalation was performed according to a standard "3+3" design.

Table 1: AT9283 In Vitro Kinase Inhibition

Aurora A	Aurora B	JAK2	Flt3	TSH3	QSOX	HSP90	MSH1	V52P	PK3	DESY1	c-Met	V560
50nM	50nM	1.2	110	4	10000	20-100	20-100	20-100	575nM	70nM	110	20-100
50nM	50nM	1.2	110	4	10000	20-100	20-100	150nM	30nM	30nM	110	20-100

Table 2: Growth Inhibitory Effect of AT9283 in AML Cell Lines

Cell Line	Key Molecular Defect	AT9283 IC50 (nM)
MV4-11	FLT3-ITD	18
MOLM13	FLT3-ITD	32
Kasumi-1	c-KIT ^{WT2K}	18
CMK	JAK3 ^{WT2V}	66
HEL	JAK2 ^{WT1F}	150
MOLM-16	None Characterised	220
MUTZ-2	None Characterised	12
ML2	K-Ras	Polyploidy @ 30
THP-1	N-Ras12/61	Polyploidy @ 30

Figure 1: AT9283 Mechanism of Action in MV4-11 Cells



- Human AML cell lines were exposed to AT9283 for 72h. Cell viability was determined using an Alamar Blue™ assay.
- AT9283 potently inhibited the proliferation of a range of AML cell lines with a variety of mutations in transmembrane signalling pathways.
- AT9283 inhibits Flt3 (pERK), JAK (pSTAT5) and Aurora (pHH3) signalling in MV4-11 cells.
- In cases where the driving mutation is in a kinase or pathway inhibited directly by AT9283 the dominant Aurora phenotype of polyploidy is no longer observed suggesting a contribution of the additional kinase inhibitory activities of AT9283 to the overall effect.

Table 3: Patient Demographics

Age	Median 58 years (range 22 – 86) Male: Female 1:1
Diagnoses	AML 23 (78%) AMML 1 (3%) MDS 1 (3%) Granulocytic sarcoma 1 (3%) CML 2 (7%) ALL 1 (3%) Myelofibrosis 1 (3%)
Number of Prior Lines of Therapy (Median 3)	One 5 (17%) Two 10 (33%) Three 12 (40%) Four 2 (7%) Five 1 (3%)
Previous Allograft	3 (10%)

Notes: To date 30 patients have been treated on seven different dose levels

Table 4: Dose Escalation Scheme

Dose Level (mg/m ² /day)	Number of Patients Treated	Number of Cycles Received (Median)	Dose Limiting Toxicities (DLT)
1 (3)	3	1 – 3 (1)	None
2 (6)	3	1 – 2 (1)	None
3 (12)	7	1 – 3 (2)	Tumour Lysis Syndrome/ Pulmonary Insufficiency Supraventricular arrhythmia (Considered to be a single event)
4 (24)	3	1 – 2 (2)	None
5 (48)	4	1 – 2 (1)	None
6 (72)	3	2 – 6 (3)	None
7 (108)*	4	1 – 2 (1)	Hypertension (1) Elevated Liver Function tests (1) Myocardial infarction (1)
8 (162)	3	1 (1)	Elevated Liver Function tests (1)

DOSE ESCALATION

- No evidence of DLT was observed until cohort three where one patient developed Grade 4 Tumour Lysis Syndrome requiring short term dialysis. This dose level was expanded to a total of seven patients and no further DLTs were observed.
- Dose escalation was reduced to 50% of the previous dose following the appearance of mucositis (Grade 2) at dose level five.
- Two DLTs and 3 deaths (multiorgan failure, MI, GI hemorrhage) were observed amongst the first three patients treated at dose level 6, and expansion of dose level 7 is ongoing to define 72 hour potential MTD*.

Figure 2: Reduction in Marrow Blasts in Seven Patients with Relapsed/Refractory AML Following Treatment with AT9283

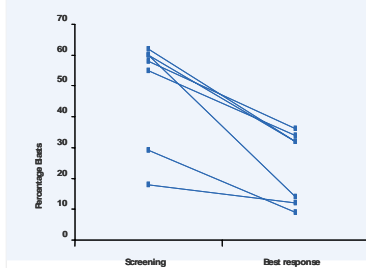
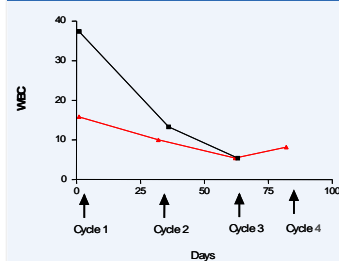


Figure 3: Reduction in WBC in Two Patients with Accelerated CML who had Failed Previous Therapy with Imatinib and Dasatinib

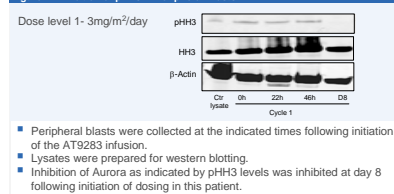


TOXICITY

- Treatment at doses greater than 6 mg/m² per day was associated with reversible myelosuppression and alopecia.
- The most common SAEs reported to date have been infectious complications of neutropenia including septicemia and pneumonia.
- Several patients experienced Grade 2 mucositis which in one case was associated with dehydration and delirium.
- One patient treated at 12 mg/m² per day developed tumour lysis necessitating temporary dialysis. Later during the first cycle of therapy this patient developed septicemia with subsequent multi-organ failure and later died from pneumonia.
- One patient with a history of hypertension experienced a Grade 3 elevation in blood pressure during her first infusion of AT9283.
- Elevations in liver function enzymes and creatine kinase (non cardiac in origin) have been reported in a dose dependent manner.

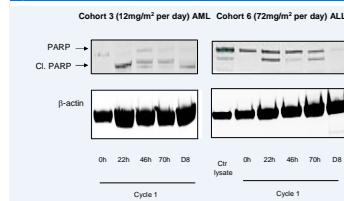
PHARMACODYNAMICS

Figure 4: Inhibition of pHH3 in Peripheral Blasts



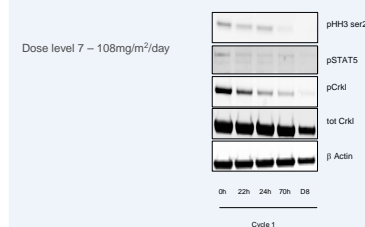
- Peripheral blasts were collected at the indicated times following initiation of the AT9283 infusion.
- Lysates were prepared for western blotting.
- Inhibition of Aurora as indicated by pHH3 levels was inhibited at day 8 following initiation of dosing in this patient.

Figure 5: Induction of Apoptosis in Peripheral Blasts



- Induction of apoptosis as measured by the increase in the caspase cleaved form of PARP was observed across multiple dose levels.

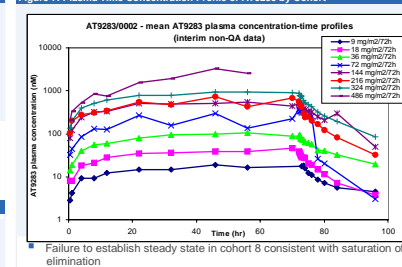
Figure 6: Multiple Activities of AT9283 Observed in Peripheral Blasts



- Inhibition of Aurora (pHH3), JAK(pSTAT5) and Abl (Crkl) observed in peripheral blasts following dosing with AT9283.

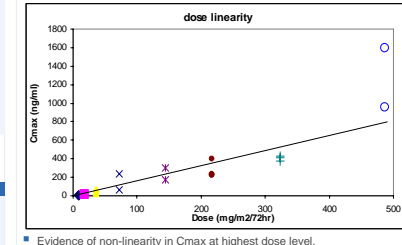
PHARMACOKINETICS

Figure 7: Plasma Time Concentration Profile of AT9283 by Cohort



Failure to establish steady state in cohort 8 consistent with saturation of elimination

Figure 8: Cmax versus Administered Dose



Evidence of non-linearity in Cmax at highest dose level.

CONCLUSIONS

- AT9283 exhibits preliminary evidence of activity in patients with relapsed/refractory AML and accelerated CML with a predictable and reversible toxicity profile.
- Confirmation that a dose of 108 mg/m² per day is the MTD for a 72-hour continuous infusion schedule is ongoing.
- The mechanism of this antileukemic effect remains under investigation although the ability of AT9283 to inhibit multiple signalling pathways may mean that the drug induces different biological effects according to the importance of specific signalling pathways in the survival of specific leukemias.

