

# AT9283, a Potent Inhibitor of BCR-Abl T315I, is Active in CML Models

M S Squires, M Reule, J Curry, K Mallett, M Yule, R Tanaka\*, N T Thompson, J F Lyons, S Kimura\*

Astex Therapeutics Ltd., 436 Cambridge Science Park, Milton Road, Cambridge, CB4 0QA, UK. \*Department of Transfusion Medicine and Cell Therapy, Kyoto University Hospital, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto, 606-8507, Japan.

## INTRODUCTION

- AT9283 is a potent inhibitor of several protein kinases, including BCR-Abl, in the low nanomolar range.
- CML is caused by a consistent genetic abnormality, termed the Philadelphia chromosome, that results from a reciprocal (9;22) translocation leading to the expression of the BCR-Abl fusion protein.
- Although the introduction of kinase inhibitors such as imatinib has revolutionised treatment of CML by targeting Abl activity, reactivation of the kinase via several different point mutations remains problematic.
- We describe here the characterisation of the anti-tumour effects of AT9283 in models of BCR-abl dependent disease.
- AT9283 has potent anti-proliferative activity in a panel of Ba/F3 and human cell lines expressing the BCR-Abl fusion protein or its mutant forms including T315I.
- Treatment of several *in vivo* models of imatinib resistant CML resulted in significant growth inhibition and, in several cases, regression of the tumour. In some instances mice remained tumour free at 90 days following initial administration of compound, 72 days after the final administration.

## In vitro of AT9283 Activity vs BCR-Abl

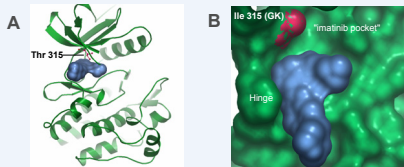


Figure 1: Model of AT9283 bound within active site of BCR-Abl (A) or BCR-Abl T315I (B)

- Figure 1A shows AT9283 bound within the active site of BCR-Abl with Threonine 315 highlighted in red. AT9283 does not make a hydrogen bond interaction with Threonine 315 in the same way other kinase inhibitors in the class do.

- Figure 1B demonstrates that because the pocket behind the Threonine 315 gatekeeper is not occupied by AT9283 there is no clash with this residue upon mutation to Isoleucine.

- Hence AT9283 maintains activity vs the mutant forms of BCR-Abl (Table 1)

## RESULTS

Table 1: AT9283 In Vitro Activity profile

Protein Kinase	AT9283 IC <sub>50</sub> (nM)
BCR-Abl (T315I)	4
JAK2	1.2
JAK3	1.1
Aurora A	52% @ 3nM
Aurora B	58% @ 3nM

Table 1: In vitro activity profile of AT9283

Ba/F3	AT9283 IC <sub>50</sub> (nM)
WT p190	16
WT p210	13
Y253F	16
T315I	11
T315A	10
Q252H	21
M351T	18
M294V	18
H396P	18
G250E	12
F317V	14
F317L	15
E255K	13

Table 2: Activity of AT9283 in a panel of BCR-Abl Ba/F3 cell lines.

- Ba/F3 cells were engineered to stably express wild-type (WT) or mutant forms of BCR Abl
- The above panel were exposed to AT9283 for 72h. Cell viability was determined using an Alamar Blue™ assay.

Cell Line	BCR Abl Status	Additional Characteristics	IC <sub>50</sub> (nM)
BV173	+		5.5
KU812	+		26
MYL	+		21
KT-1	+		81
KBM-5	+		84
MEG-01	+		31
K562	+	Polyplidy @ 30	
HL60	-	Polyplidy @ 30	
KBM5-STIR	+	(T315I)	16
K562/D1-9	+	PgP Overexpressor	>1000
K562/Bcl-2	+	Bcl2 Overexpressor	50% @ 300
K562/NIR	+	BCR-Abl overexpressor	64% @ 300
K562/Bcl-xL	+	Bcl-xL Overexpressor	41% @ 300
BV173/hBim	+	Bim Knockdown	12

Table 3: Activity of AT9283 in a panel of Ph+ human CML cell lines.

- Human CML cell lines were exposed to AT9283 for 72h. Cell viability was determined using an Alamar Blue™ assay

## RESULTS

- AT9283 inhibits BCR-Abl (T315I) at 4nM in an *in vitro* assay (Table 1).

- Proliferation of a panel of Ba/F3 cell lines harbouring either wild type or imatinib resistant, mutant forms of the BCR-Abl kinase were inhibited with an IC<sub>50</sub> of 10-21nM (Table 2).

- AT9283 inhibited Ba/F3 cells expressing a range of mutant forms of BCR-Abl including those highlighted in blue which represent the most problematic resistant mutants.

- AT9283 is effective at inhibiting survival of BCR-Abl (T315I)-dependent cell lines. Human cells harbouring this mutation are resistant to all current CML therapies.

- Similar data was obtained in human CML cell lines that are either sensitive or have an acquired resistance to imatinib therapy (Table 3).

- In certain cases, as indicated in Table 3, the dominant phenotype observed is that of polyploidy resulting from Aurora B inhibition. It is likely that we observe both Aurora and BCR-Abl inhibition in these cell lines and the pre-dominant phenotype observed depends upon the specific genetic background of the cell in question.

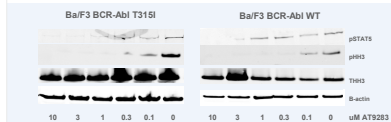


Figure 2: Mechanism of action of AT9283 in Ba/F3 BCR-Abl cells

Figure 3: Mechanism of action of AT9283 in K562 CML cells

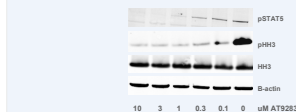


Figure 3: Mechanism of action of AT9283 in K562 CML cells

- Ba/F3 wild type BCR-Abl or T315I mutant cells were treated with AT9283 for 4 hours. Samples were prepared for western blotting.

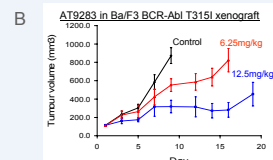
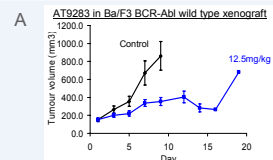
- Following exposure to AT9283 downstream substrates of Aurora B (p-Histone H3 (pH3)) and BCR-Abl (pSTAT5) were inhibited.

- Similar data were obtained in the human CML cell line K562 which expresses the wild type version of the BCR-Abl fusion protein.

- These data suggest that both the Aurora and BCR-Abl inhibitory effects of AT9283 manifest themselves in CML cell lines and that either or both activities could result in the efficacy of the compound in these CML models. Dogma suggests that in a BCR-Abl-dependent system it is this kinase that is primarily responsible for the phosphorylation of STAT5. However JAK2 can also perform this function and AT9283 is a potent Jak inhibitor.

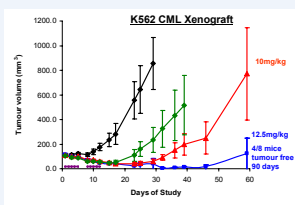
- The interplay and contribution of these three activities of AT9283 in a BCR-Abl background could be a powerful means of tackling CML and warrants further investigation.

## RESULTS



Dosed twice daily i.p. for 5 days followed by 2 days break. Repeated twice

Figure 4: AT9283 is efficacious in Ba/F3 BCR-Abl xenograft models

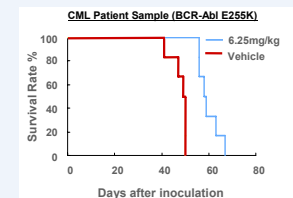


Dosed twice daily i.p. for 5 days followed by 2 days break. Repeated twice

Figure 5: AT9283 is efficacious in K562 human CML xenograft model

- In each of the studies shown tumour-bearing nude mice were dosed via the i.p route twice daily for 5 days in 7, repeated for 2 cycles.
- The volume of the tumour was calculated as an ellipsoid volume every 2 days.
- Figures 4A and B show that AT9283 is efficacious in subcutaneous xenograft models with Ba/F3 cells transfected with either wild type BCR-abl kinase or the T315I mutant form.
- Figure 5 shows that AT9283 induced prolonged inhibition of tumour growth and regression in a human CML xenograft model, K562 cells.
- At 12.5mg/kg regressions were observed and 50% of the mice in this dose group remained tumour free out to 90 days, 78 days after administration of the final dose of AT9283.

## RESULTS



Dosed twice daily i.p. for 5 days followed by 2 days break. Repeated four times

Figure 6: AT9283 is efficacious in a primary cell BCR-Abl (E255K) xenograft model

- Male NOD/SCID mice were sub-lethally irradiated and inoculated intravenously with primary leukaemic cells harbouring BCR-Abl E255K
- Kaplan Meijer survival curves show that treatment with 6.25mg/kg AT9283 twice daily resulted in a significant survival advantage (p=0.008) over vehicle treated animals of 17 days.

## CONCLUSIONS

- AT9283 is a multi-targeted kinase inhibitor with activity against Aurora A and B, BCR-Abl, including many of the identified mutant forms and JAK2.
- AT9283 inhibits survival of engineered cell lines expressing BCR-Abl or its mutant forms as well as human leukaemia cells harbouring the same mutations.
- These antiproliferative effects are correlated with inhibition of BCR-Abl and Aurora in CML cell lines. The relative contributions of these two activities of AT9283, along with the outcome of inhibition of additional targets such as JAK remain the subject of further investigation.
- AT9283 exhibited potent inhibitory effects in animal models with either Ba/F3 cells, human leukaemic cell lines or primary human CML samples.
- These data suggest that AT9283 has the potential to benefit CML patient populations that prove refractory to standard therapy by the nature of the activities described above.
- These data support further clinical investigation of AT9283 in patients with treatment resistant CML.

Presented at the AACR Annual Meeting, April 12-16, 2008, San Diego, CA

