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AT7519, A Potent CDK Inhibitor, is Active in Leukaemia Models and Primary CLL Patient Samples

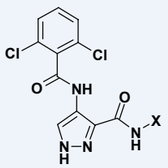
MS Squires[1], RE Feltell[1], V Lock[1], D Smith[1], EJ Lewis[1], J Higgins[1], M Yule[1], NT Thompson[1], L Cooke[2], K Della Croce[2], W Qi[2], JF Lyons[1], D Mahadevan[2].

[1]Astex Therapeutics Ltd., 436 Cambridge Science Park, Milton Road, Cambridge, CB4 0QA, UK. [2]Arizona Cancer Center, 1515N Campbell Avenue, Tuscon, Arizona, 85724.

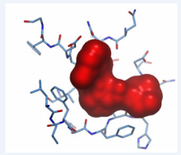
INTRODUCTION

- AT7519 is a selective Cyclin Dependent Kinase (CDK) inhibitor developed using Astex's fragment based medicinal chemistry approach.
- AT7519 is a potent inhibitor of cyclin dependent kinases 1, 2, 4, 5 and 9 currently in early phase clinical studies.
- We describe here preclinical characterisation of the mechanism of action of the compound in both leukaemia cell lines and Chronic Lymphocytic Leukaemia (CLL) patient samples.
- Both cell lines and primary patient samples undergo rapid apoptosis upon treatment with AT7519 following depletion of key anti-apoptotic proteins such as Mcl-1. This mechanism is consistent with the transcriptional inhibitory effects of the compound attributed, at least in part, to its activity vs CDK9

COMPOUND PROFILE



Where X = group to pick up lipophilic interactions and introduce aqueous solubility



AT7519 bound within ATP binding site of CDK2

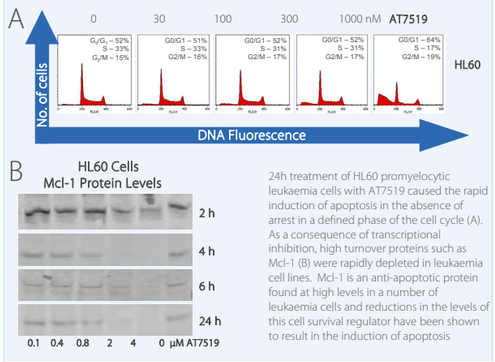
Figure 1: AT7519 Compound Structure

Table 1: *In Vitro* Kinase Inhibition

Protein Kinase	AT7519 IC_{50} (nM)	Protein Kinase	AT7519 IC_{50} (nM)
CDK1/Cyclin B	**190**	EGFR	>10000
CDK2/Cyclin A	**44**	FGFR3	>10000
CDK2/ Cyclin E	510	IR	>10000
CDK4/ Cyclin D1	67	Jnk2	>10000
CDK6/ Cyclin D3	660	MAPK 1	>10000
CDK5/ p35	18	MEK1	>10000
CDK7/ Cyclin H	2800	met	>10000
CDK9/ Cyclin T1	<100	P38	>10000
GSK3 beta	98	p70S6K	>10000
Aurora A	>10000	PDGFR	>10000
c-abl	>10000	PDK1	>10000
cSrc	>10000	VEGFR 1	>10000
Chk1	>10000	PKBbeta	>10000

Table 2: Cell Based Activity in a 72 hour Proliferation Assay

Tissue	Cell Line	AT7519 IC_{50} (nM)
Colon Carcinoma	HCT116	54
	HT29	170
Ovarian Cancer	A2780	350
	SK-OV-3	400
Lung Carcinoma	A549	380
Breast Carcinoma	MCF-7	40
	BT-20	320
	MDA-MB-468	340
	SK-BR3	140
Leukaemia	HL60	90
	K562	40
	MOLT4	310
Lymphoma	GRANTA-519	160
	JEKO-1	70
Fibroblast	MRC 5	980
	MRC 5 (Non-Proliferating)	>10000



24h treatment of HL60 promyelocytic leukaemia cells with AT7519 caused the rapid induction of apoptosis in the absence of arrest in a defined phase of the cell cycle (A). As a consequence of transcriptional inhibition, high turnover proteins such as Mcl-1 (B) were rapidly depleted in leukaemia cell lines. Mcl-1 is an anti-apoptotic protein found at high levels in a number of leukaemia cells and reductions in the levels of this cell survival regulator have been shown to result in the induction of apoptosis

Figure 2: Mechanism of Action in Leukaemia Cell Lines

HL60 - AT7519 dosed i.p. daily for 5 days, repeated twice

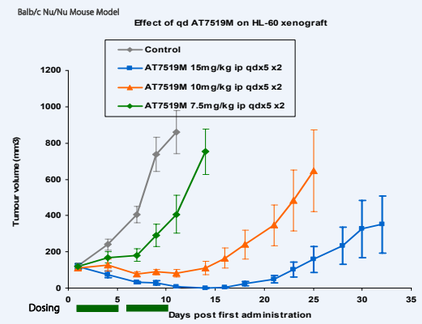


Figure 3: Effect of AT7519 on HL60 Xenograft Growth

HL60 Single 10mg/kg dose i.p.

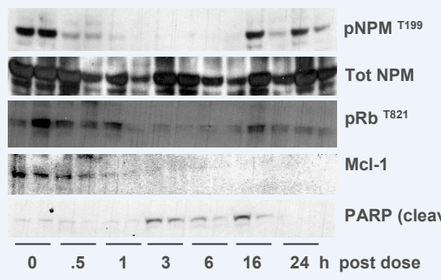


Figure 4: Effect of AT7519 on Xenograft Growth

RESULTS

- AT7519 was dosed via the i.p route to HL60 tumour bearing mice once a day for 5 days followed by a 2 day break (A). Two dosing cycles resulted in dose dependent tumour growth inhibition with cytoreduction observed at the 10 and 15mg/kg/day doses.
- Complete regressions were achieved in this experiment in 4/8 mice at 15mg/kg and 2/7 at 10mg/kg. The efficacy observed in this model is consistent with inhibition of markers of CDK activity and knockdown of Mcl-1 protein levels observed in a pharmacodynamic study performed in the same model (B). In this study tumour bearing mice received a single dose of AT7519 at 10mg/kg. Phosphorylation of NPM was inhibited out to 16h following dosing. A rapid and sustained reduction in Mcl-1 protein levels was associated with the induction of apoptosis indicated by the appearance over time of the cleaved form of PARP.

Table 3: AT7519 Inhibits Cell Survival in CLL Patient Samples

Patient	Disease Stage	72h Cytotoxicity IC_{50} (nM)
1	II	178
2	IV	356
3	0/I	108
4	0	312
5	0	180
6	IV	136
7	0	155
8	0/I	697
9	II	161
10	IV	132

RESULTS

AT7519 was cytotoxic to CLL cells following treatment for 72h and an MTT assay as assessment of cell viability. Similar activity was observed irrespective of disease stage.

Patient 3

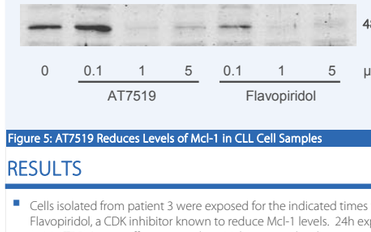


Figure 5: AT7519 Reduces Levels of Mcl-1 in CLL Cell Samples

RESULTS

- Cells isolated from patient 3 were exposed for the indicated times to either AT7519 or Flavopiridol, a CDK inhibitor known to reduce Mcl-1 levels. 24h exposure of CLL cells to 1uM AT7519 was sufficient to reduce Mcl-1 protein levels.
- The reduction of this important survival protein is consistent with the anti-transcriptional effects of the compound and the concentrations required to have a cytotoxic effect in this patient sample.

Table 4: AT7519 Inhibits Cell Survival in CLL Patient Samples

Exposure time	1hr	4hr	6hr	24hr	72hr
IC_{50} (nM)	9450	2066	660	226	161

- Cells isolated from patient 9 were treated for the indicated times and the proportion of surviving cells quantified (Table 4). There is a time dependent reduction in the concentration of AT7519 required to kill 50% of the CLL cell population.

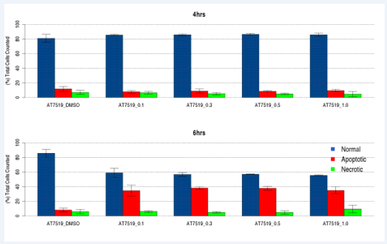


Figure 6: AT7519 Rapidly Induces Apoptosis in CLL Patient Samples

- Cells isolated from patient 9 were treated for the indicated times and the percentage of apoptotic, necrotic and viable cells quantified (Figure 6). There is a time and dose-dependent induction of apoptosis in the CLL cell population. Significant increases in apoptosis in AT7519-treated cells compared to vehicle controls was observed following 6h compound treatment at 100nM.

AT7519 1hr infusion 28mg/m² (Free Fraction)

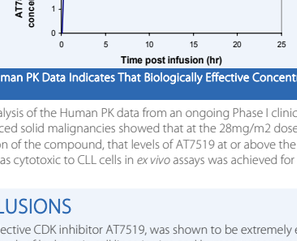


Figure 7: Human PK Data Indicates That Biologically Effective Concentrations Are Achieved

- An analysis of the Human PK data from an ongoing Phase I clinical study in patients with advanced solid malignancies showed that at the 28mg/m2 dose level, following a 1h infusion of the compound, that levels of AT7519 at or above the efficacious dose range that was cytotoxic to CLL cells in *ex vivo* assays was achieved for 8h following dosing.

CONCLUSIONS

- The selective CDK inhibitor AT7519, was shown to be extremely effective at inhibiting the growth of leukaemia cell lines *in vitro* and human tumour xenografts in mouse models. In these cell lines AT7519 caused rapid induction of apoptosis in the absence of arrest in a particular phase of the cell cycle. This induction of apoptosis is consistent with a reduction in the levels of anti-apoptotic proteins such as Mcl-1. The activity of AT7519 in CLL patient samples was confirmed and the mechanism of action shown to be consistent with the depletion of anti-apoptotic proteins via the transcriptional effects of the compound.
- CLL cells which rely on the expression of short half-life transcripts such as Mcl-1 for survival are particularly sensitive to this mechanism of action and AT7519 is cytotoxic to *ex-vivo* CLL cells at concentrations equivalent to the free plasma levels achievable in ongoing clinical studies. The data here supports further clinical investigation of the compound in B-Cell lymphoproliferative disorders where survival proteins play a pivotal role.

Disclosure Statement

This work and the development of this poster were supported by Astex Therapeutics Limited. D. Mahadevan acts as a consultant to, and is the recipient of a research grant from, Astex Therapeutics Ltd

Presented at the American Society for Hematology 49th Annual Meeting and Exposition, December 8-11, 2007, Atlanta, GA

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