

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

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## 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

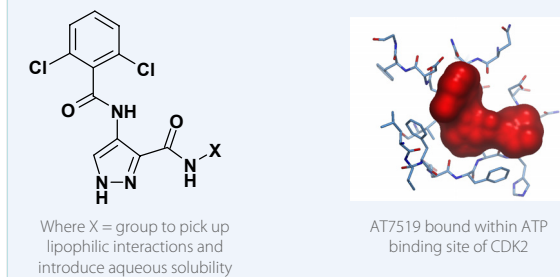


Figure 1: AT7519 Compound Structure

Table 1: In Vitro Kinase Inhibition

Protein Kinase	AT7519 IC <sub>50</sub> (nM)	Protein Kinase	AT7519 IC <sub>50</sub> (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 <sub>50</sub> (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
Lymphoma	GRANTA-519	160
	JEKO-1	70
Fibroblast	MRC 5	980
	MRC 5 (Non-Proliferating)	>10000

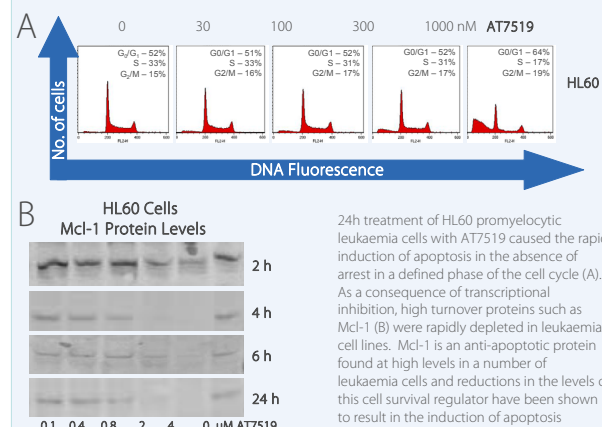


Figure 2: Mechanism of Action in Leukaemia Cell Lines

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

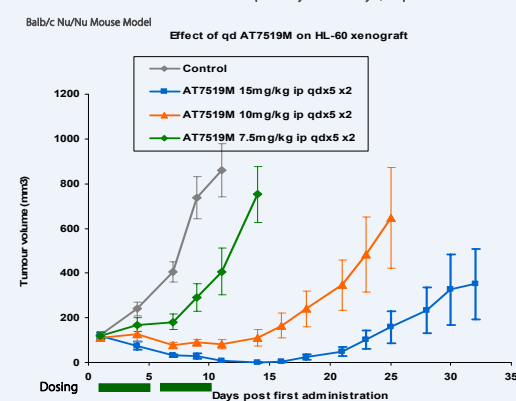


Figure 3: Effect of AT7519 on HL60 Xenograft Growth

HL60 Single 10mg/kg dose i.p.

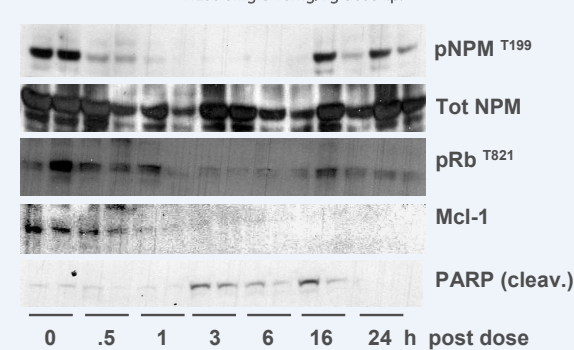


Figure 4: Effect of AT7519 on Xenograft Growth

## 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 <sub>50</sub> (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

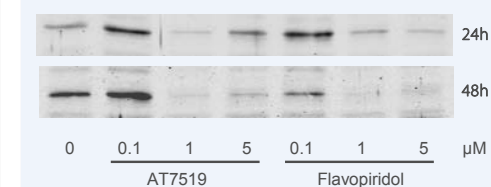


Figure 5: AT7519 Reduces Levels of Mcl-1 in CLL Cell Samples

## 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 1μM 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 <sub>50</sub> (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.

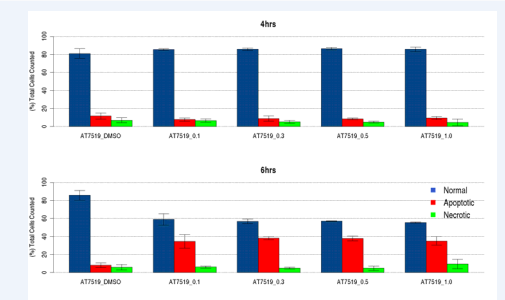


Figure 6: AT7519 Rapidly Induces Apoptosis in CLL Patient Samples

- 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/m<sup>2</sup> (Free Fraction)

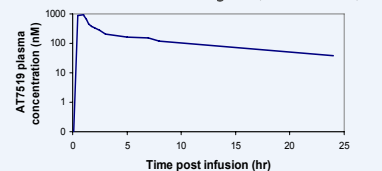


Figure 7: Human PK Data Indicates That Biologically Effective Concentrations Are Achieved

- 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/m<sup>2</sup> 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

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