

AT7519, a Potent Multi-targeted CDK Inhibitor, is Active in CLL Patient Samples Independent of Stage

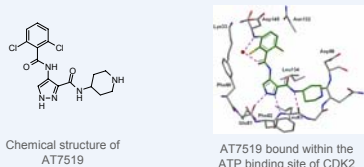
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INTRODUCTION

- Chronic lymphocytic leukemia (CLL) is characterized by a population of malignant B cells with heterogeneous biological features but representative of a predominant phenotype aberrant in proliferation and apoptosis.
- The targeting of the slow-to-moderate proliferating, apoptosis-resistant, CLL phenotype with a multitude of agents has not led to significant progression free and overall survival events.
- Hence, agents targeting both aberrant proliferation and anti-apoptosis are needed to effectively treat patients with CLL.
- 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 characterisation of the mechanism of action of AT7519 in CLL patient samples.
- CLL cells were isolated from 15 patients with disease of various stages.
- 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 and was shown to be independent of disease stage.

COMPOUND PROFILE



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Figure 1: AT7519 Compound Structure

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	<10	P38	>10000
GSK3 beta	98	p70S6K	>10000
Aurora A	>10000	PDGFR	>10000
c-abl	>10000	PDK1	>10000
cSrc	>10000	VEGFR 1	>10000
Chk1	>10000	PKB beta	>10000

Table 1: *in vitro* Kinase Inhibition

- AT7519 inhibits CDKs 1, 2, 4, 5 and 9 *in vitro* kinase assays. Apart from the CDKs the only other kinase inhibited of those tested was GSK3 beta (Table 1).

ACTIVITY OF AT7519 IN CLL PATIENT SAMPLES

Patient	Rai Stage	WBC Count	Cytogenetic Abnormalities	AT7519 IC ₅₀ (nM)
1	II	25.5	13q del	178
2	IV	7.4	normal	356
3	0/I	55	normal	108
4	0	14.2	normal	312
5	0	31	13q14	180
6	IV	42.8	normal	136
7	0	35.8	13q14	155
8	0/I	22.7	13q del	697
9	II	43.9	normal	161
10	IV	53.8	none	132
11	IV	153	normal	144
12	IV	77.1	normal	111
13	0	47.2	Trisomy 12; 13q del	110
14	IV	7.7	Trisomy 11	580
15	IV	12.5	13q del	160

Table 2: Cytotoxicity of AT7519 vs CLL Patient Samples Following 72h Exposure

AT7519 Exposure (h)	AT7519 IC ₅₀ (nM)
1	9500
4	2100
6	860
24	230
72	160

Table 3: Exposure versus Time Dependence of AT7519 Cytotoxic Effects



Figure 2: Exposure versus Time Dependence of AT7519 Cytotoxic Effects

- CLL cells were isolated from 15 patients with diseases of various stages.
- MTS cytotoxicity assays were performed following incubation of cell samples with AT7519 for the indicated times.
- AT7519 is cytotoxic to CLL cells derived from patients following 72h incubation (Table 2). AT7519 reduced cell viability with a mean IC₅₀ of 235 nM and a range from 108-697nM.
- Cells isolated from patient 9 were treated for the indicated times and the proportion of surviving cells quantified (Table 3). There is a time dependent reduction in the concentration of AT7519 required to kill 50% of the CLL cell population.
- Cells isolated from patient 9 were treated for the indicated times and the percentage of apoptotic, necrotic and viable cells quantified (Figure 2). 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.

MECHANISM OF ACTION OF AT7519 AND FLAVOPIRIDOL IN PATIENT SAMPLES

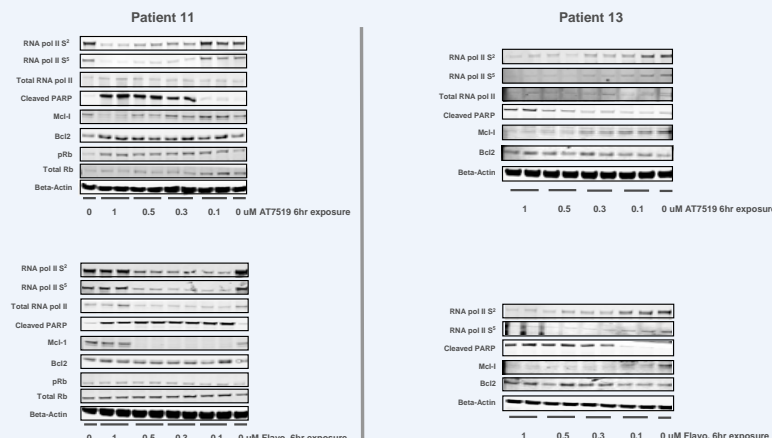


Figure 3: Mechanism of Action of AT7519 in CLL Patient Samples

- Cells isolated from patients 11 and 13 were exposed to AT7519 or the known transcriptional regulator Flavopiridol at the indicated concentrations (Figure 3).
- Western blots were performed on lysates derived from these samples and probed with antibodies for a variety of CDK substrates and apoptosis markers.
- Six hours exposure to >100nM AT7519 or Flavopiridol was sufficient to inhibit phosphorylation of the transcriptional regulator RNA polymerase II on sites phosphorylated by CDKs 7, 8 and 9. Consistent with this transcriptional inhibitory activity levels of the anti-apoptotic protein Mcl-1 was reduced in parallel with an increase in the apoptotic marker cleaved PARP.
- Substrates of the cell cycle CDKs including the Rb protein remained unaffected at this early timepoint suggesting the effects observed were independent of any cell cycle regulatory activity. In addition the levels of other anti-apoptotic proteins such as Bcl-2 remained unaffected.

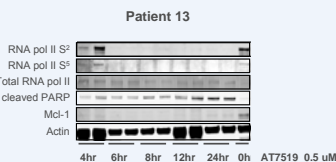


Figure 4: Timecourse of AT7519 Effects in CLL Patient Samples

- To investigate the timecourse of this transcriptional modulation samples isolated from patient 13 were exposed to 0.5μM AT7519 for the indicated times (Figure 4).

- These data show that the effects on transcriptional regulators and subsequent downstream transcripts require only a short term exposure to AT7519 at these concentrations. This is consistent with data presented in Table 3 and Figure 2 showing that short term exposure to AT7519 in the 500nM range is sufficient to have a cytotoxic effect.

- The reduction of the important survival protein Mcl-1 is consistent with the anti-transcriptional effects of the compound and the concentrations required to have a cytotoxic effect in this patient sample.

AT7519 PHARMAKOKINETICS

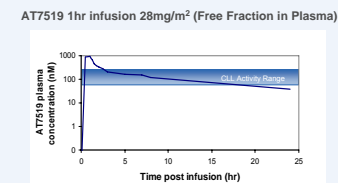


Figure 5: Human PK Data Indicates That Biologically Effective Concentrations Are Achieved

- 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, levels of AT7519 at or above the concentration range that was cytotoxic to CLL cells in *ex vivo* assays, were achieved for 16h following dosing.

CONCLUSIONS

- The selective CDK inhibitor, AT7519, was shown to be extremely effective at inhibiting the viability of CLL cells derived from patients with disease of various Rai stages of progression.
- AT7519 caused a rapid induction of apoptosis and this induction occurred in parallel with a reduction in the levels of anti-apoptotic proteins such as Mcl-1.
- The mechanism of action of this depletion of anti-apoptotic proteins was shown to be via the transcriptional activity of AT7519 and in comparison to flavopiridol, a pan-CDK inhibitor currently in Phase III/III development in CLL, was shown to be equipotent in these *ex-vivo* studies.
- CLL cells which rely on the expression of short half-life transcripts such as Mcl-1 for survival are particularly sensitive to the transcriptional inhibition caused by AT7519 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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This poster is available to download at www.astex-therapeutics.com

