

# A Comparison of the Pharmacological Profile of AT7519 in Solid Tumour and Haematological Cell Lines.

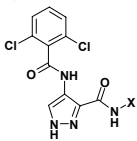
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## Introduction

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 solid tumour and leukaemia cell lines. In solid tumour cell lines treatment with AT7519 results in cell cycle arrest followed by induction of apoptosis. In contrast leukaemia cell lines undergo rapid apoptosis in the absence of arrest in a specific phase of the cell cycle by a mechanism consistent with the transcriptional inhibitory effects of the compound attributed, at least in part, to its activity vs CDK9.

## Figure 1. AT7519 Compound Profile

### Compound Structure



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

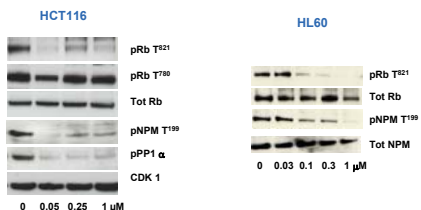
### In vitro kinase inhibition profile

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/MAT1	2600	met	>10000
CDK9/Cyclin T1	<100	P38	>10000
GSK3 beta	98	p70S6K	>10000
Aurora A	>10000	PDGFR	>10000
c-abl	>10000	PKD1	>10000
cSrc	>10000	VEGFR 1	>10000
Chk 1	>10000	PKBbeta	>10000

### Cell Based Activity in a 72h Proliferation Assay

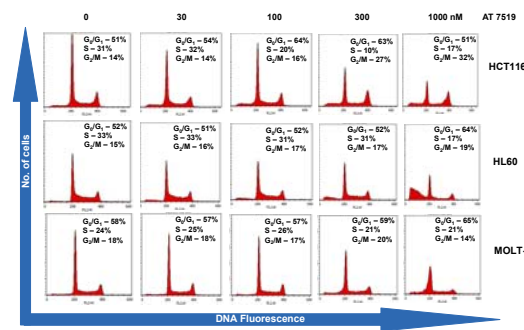
Tissue	Cell Line	AT7519 IC <sub>50</sub> (nM)
Colon Carcinoma	HCT116	54
	HT29	170
	A2780	350
Ovarian Carcinoma	SK-OV-3	400
	A549	380
Lung Carcinoma	MCF-7	40
	BT-20	320
Breast Carcinoma	MDA-MB-468	340
	SK-BR3	140
Leukaemia	HL60	90
	K562	40
	MOLT4	310
Lymphoma	GRANTA-S19	160
	JEKO-1	70
Fibroblast	MRC 5	980
	MRC 5 (Non Prolif)	>10000

### Inhibition of CDK 1 and 2 in Tumour Cell Lines



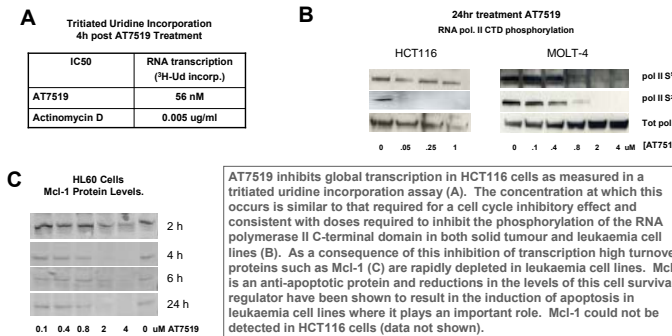
Treatment of Cells for 24 hours inhibited phosphorylation of the CDK-1 substrate protein phosphatase 1 α (PP1α) and CDK-2 substrates Retinoblastoma protein (Rb) and nucleophosmin (NPM) at phosphorylation sites specific for the indicated kinases. The biochemical inhibition and the inhibition of proliferation observed in the same cell line occur at similar concentrations.

## Figure 2. Cell Cycle Modulation in Solid Tumour and Leukaemia Cell Lines

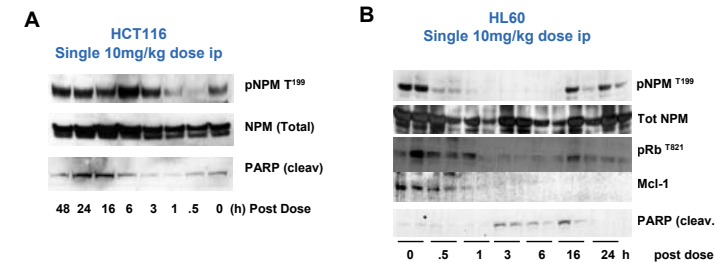


24h treatment with AT7519 causes a G<sub>2</sub>/M arrest followed by apoptosis in HCT116 cells whereas the predominant effect in the leukaemia cell lines MOLT4 and HL60 is the induction of apoptosis in the absence of arrest in a defined phase of the cell cycle.

## Figure 3. AT7519 Inhibits Transcriptional Activity in Tumour Cell Lines

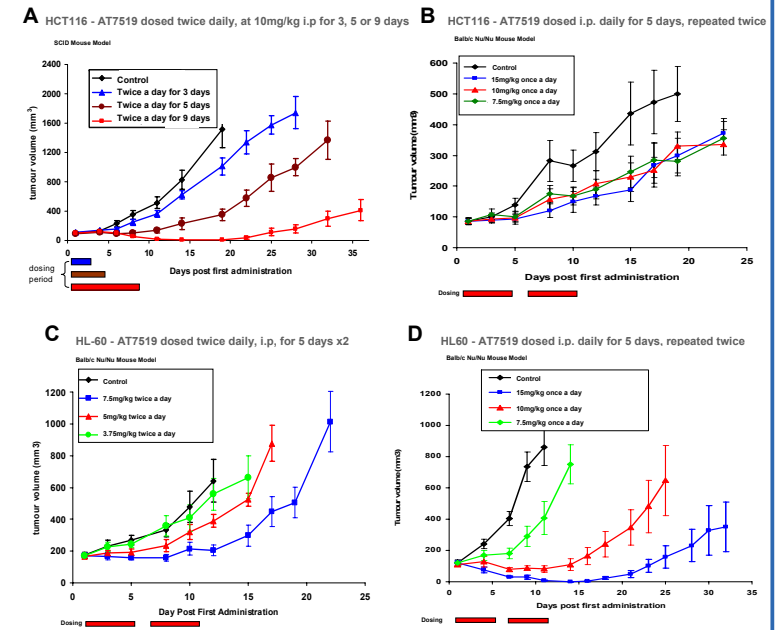


## Figure 4. Modulation of PD endpoints by AT7519 in Xenograft Tumours



Xenograft tumour lysates were monitored for phosphorylation of NPM and Rb, for the levels of Mcl-1 and cleavage of the caspase substrate PARP following a single 10mg/kg dose of AT7519 administered via the i.p. route. The rapid inhibition of phosphorylation of the CDK substrates pNPM and pRb shows that these effects are a direct consequence of compound treatment rather than cell cycle arrest. 3h knockdown of the CDK2 substrate pNPM was sufficient to induce apoptosis within HCT116 tumours (A). In HL60 xenografts the CDK substrates were also inhibited rapidly upon compound dosing (B). A rapid and sustained knockdown of the anti-apoptotic protein Mcl-1 was also observed in the leukaemia line. Mcl-1 depletion correlated with increased levels of the cleaved form of PARP, indicative of induction of apoptosis within the tumour.

## Figure 5. Effect of AT7519 on Xenograft Growth



AT7519 was dosed i.p. to HCT116 tumour bearing mice twice a day for either 3, 5 or 9 days (A). Inhibition of tumour growth was observed following only 5 days dosing. 9 Days of dosing was sufficient to cause cytoreduction and sustained inhibition to around 20 days following the first dose before any regrowth was observed. In the solid tumour cell line poorer efficacy was observed on a once per day dosing schedule (B). In contrast a single daily dose in the leukaemia cell line, HL60 (D) was sufficient to give excellent efficacy 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 was superior to that achieved when the same total dose of AT7519 was given on a twice daily schedule (C).

## Conclusion

The selective CDK inhibitor AT7519, was shown to be extremely effective at inhibiting the growth of tumour cell lines *in vitro* and human tumour xenografts in mouse models. In solid tumour cell lines AT7519 inhibits proliferation and causes a specific cell cycle arrest. This cell cycle arrest was shown to be consistent with the inhibition of substrates of CDKs 1 and 2 followed by apoptosis upon exposure to the compound for between 16 and 24 hours. In leukaemia cell lines AT7519 causes 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. We show here that these distinct pharmacological profiles *in vitro* correspond result in differences in the optimal compound administration schedules for efficacy in an *in vivo* model. In solid tumour cell lines which undergo cell cycle arrest upon AT7519 treatment twice daily dosing achieving longer coverage is more effective than single daily dosing with the same total amount of compound. The opposite is observed for leukaemia cell lines in which maximal efficacy is achieved with higher doses given once daily as opposed the same amount of compound spread across two doses.

CDK9 has been shown to play a role in the regulation of transcription via phosphorylation of RNA polymerase II. Data shown here confirms that AT7519 inhibits transcription at doses at which we observed inhibition of CDK9 in cells. The consequence of this transcriptional inhibition depends upon the phenotypic background of the tumour cell in question. Cell types, including many leukaemias, 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 the data here supports further clinical investigation of the compound in B-Cell lymphoproliferative disorders where survival proteins play a pivotal role.