AT7519, a Selective Small Molecule Inhibitor of Cyclin Dependent Kinases: Pharmacodynamic Biomarker Activity in a Phase I Study.

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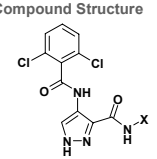
Introduction

AT7519 is a selective Cyclin Dependent Kinase (CDK) inhibitor developed using Astex's fragment based medicinal chemistry approach. This compound is currently in early phase clinical development. We describe here preclinical validation of pharmacodynamic (PD) biomarkers that are being utilised as exploratory end points in the clinical studies. The development of such biomarkers has become increasingly important with the advent of novel molecularly targeted therapies to aid clinical development.

Figure 1. AT7519 Compound Profile

Compound Structure



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

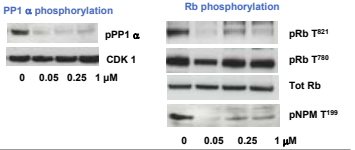
In vitro kinase inhibition profile

Protein Kinase	AT7519 IC_{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/MAT1	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
Chk 1	>10000	PKBbeta	>10000

Cell Based Activity

Tissue	Cell Line	AT7519 IC_{50} (nM)
Colon Carcinoma	HCT116	54
	HT29	170
Ovarian Carcinoma	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
Fibroblast	MRC 5	980
	MRC 5 (Non Prolif)	>10000

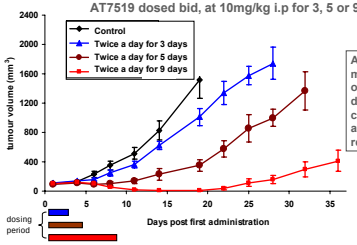
Inhibition of *CDK 1 and 2* in HCT116 cells



Treatment of HCT116 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. This inhibition occurs at the same concentrations as the inhibition of proliferation observed in the same cell line.

Figure 2. Effect of AT7519 on HCT116 Xenografts Growth

AT7519 dosed bid, at 10mg/kg i.p for 3, 5 or 9 days

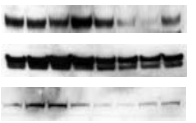


AT7519 was dosed i.p to HCT116 tumour bearing mice twice a day for either 3, 5 or 9 days. Inhibition of tumour growth was observed following only 5 days dosing. 9 Days dosing was sufficient to cause cytoreduction that was extended out to around 20 days following the first dose before any regrowth was observed.

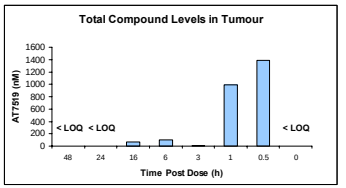
Figure 3. Modulation of PD endpoints by AT7519 in Xenograft Tumours

A

Single 10mg/kg dose ip



B



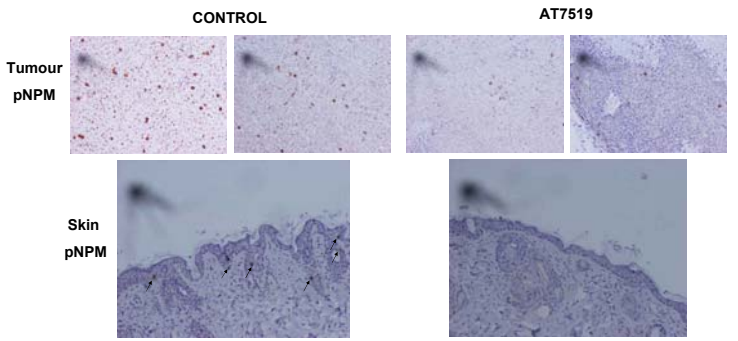
< LOQ – Below Limit Of Quantitation

HCT116 xenograft tumour lysates were monitored for phosphorylation of NPM and cleavage of the caspase substrate PARP following a single dose of AT7519 administered at 10mg/kg i.p. (A). The compound levels within each individual tumour was quantified (B), and shown to correlate with inhibition of pNPM induction. The data shown represents one mouse per timepoint and are representative of 3 individual mice per treatment group. The rapid inhibition of pNPM shows that these effects are a direct consequence of compound treatment rather than cell cycle arrest. 3h knockdown of the CDK2 marker was sufficient to induce apoptosis within the tumour and repeated knockdown in this fashion gives efficacy in the xenograft model.

Figure 4. Modulation of PD Endpoints by AT7519 in Xenograft Tumours and Skin Samples.

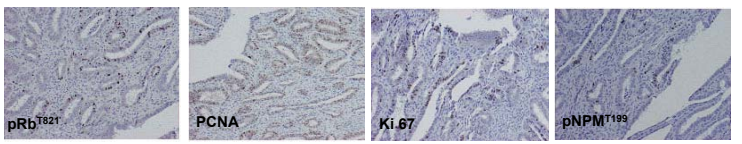
A. AT7519 inhibits phosphorylation of CDK2 substrates in tumour and skin samples taken from tumour-bearing mice.

AT7519 7.5mg/kg bid x3 i.p. – Samples taken 1h following final dose



B. Markers of CDK Inhibition and Proliferation can be Monitored in Human Skin Samples

Human Skin Sections



Phospho-NPM was inhibited in both tumour sections and skin samples taken from HCT116 tumour bearing mice dosed with AT7519 (A). Control human skin (transverse sections) were stained for the proliferation markers Ki67 or PCNA and for the CDK substrates phospho-Rb and phospho-NPM to test the application of the markers in human samples (B). Clear and robust staining was obtained indicating that these markers can be monitored in human skin samples taken during clinical development.

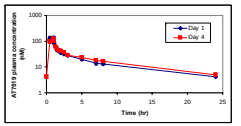
Monitoring PD Endpoints in a Phase I Dose escalation trial in Patients with Advanced Solid Malignancies

The study is a Phase I, open label study in patients with advanced solid malignancies. Treatment cohorts are being dosed in escalating order to determine the safety and tolerability of AT7519 in this first in man trial.

Pre and post-dose samples are being taken for pharmacodynamic biomarker analysis as follows:

1. Skin punch biopsy for analysis by immunohistochemistry as a surrogate tissue (Post dose sample taken 1-2 h following dosing.
2. Serum samples for analysis of cleaved cytokeratin 18 (Post dose sample taken 1-2h following dosing.

Figure 5. Mean PK data from patients in cohort 1 on day 1 and 4 of dosing

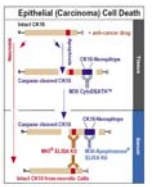


Cohort 1

- PK profiles are very consistent between patients and within patients on repeated dosing
- Sustained compound levels at this first, low dose, are below those that would be expected to achieve biomarker modulation in this early cohort

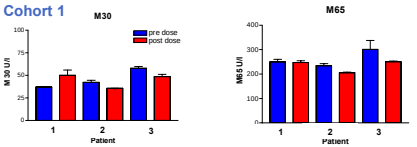
Figure 6. Monitoring of PD Endpoints in Clinical Samples

A. Assay of Cytokeratin-18 from serum samples



Source : Axxora UK Ltd.

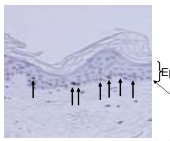
Cohort 1



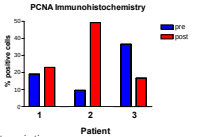
- Assay is robust with little inter-patient variability
- The lack of effect observed at this low dose was expected in this first treatment cohort.

B. PCNA staining in skin biopsy samples

Staining Example



Cohort 1



- Staining is robust with little intra-patient variation
- Inter-patient variation is significant
- The lack of effect observed at this low dose was expected in this first treatment cohort.

Conclusion

The selective CDK inhibitor AT7519, was shown to be highly efficacious in xenograft models and was used in the studies described to help develop biomarker methodologies that would be applicable to the clinical development of this and other cell cycle inhibitors. Pharmacodynamic markers of the compounds' action, identified in cell based studies, were applied to xenograft and skin samples taken from tumour bearing mice. Immunohistochemistry showed that for certain markers down-regulation of a marker in the tumour correlated with down-regulation in the proliferating layer of the skin. Considered together these data represented a strategy for monitoring compound activity in skin samples taken from patients during early phase clinical development. Samples are currently being collected from an ongoing Phase I clinical trial aimed at characterising a number of assay systems and markers in the clinical setting in order to monitor pharmacodynamic activity of AT7519 and to suggest assays that may be useful in future clinical development.

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