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Identification of a Predicted Biologically Effective Dose of AT7519, a Cyclin Dependent Kinase Inhibitor, in a Phase I Study

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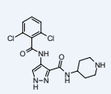
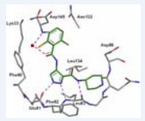
INTRODUCTION

- AT7519[1] is a potent inhibitor of several cyclin dependent kinases (CDKs).
- CDK inhibition has been shown to induce cell cycle arrest followed by apoptosis in numerous pre-clinical models[2].
- A dose escalation study was performed in patients with refractory solid tumours using a 1h intravenous infusion on days 1 through 5 of a 21 day cycle.
- Trial endpoints included pharmacodynamic (PD) and pharmacokinetic (PK) sampling.
- Pharmacodynamic endpoints were monitored in skin samples as a surrogate proliferative tissue and in serum samples.
- Both biochemical (direct inhibitory activity of CDKs) and functional (phenotypic consequence of CDK inhibition) markers were assessed.
- We describe here how these markers were used to monitor CDK inhibitory activity during the dose escalation phase and how a certain magnitude or duration of CDK inhibition was required prior to observation of a downstream functional consequence that allows the definition of a predicted biologically effective dose.

COMPOUND PROFILE



Chemical structure of AT7519[1]



AT7519 bound within the ATP binding site of CDK2

Figure 1: AT7519 Compound Structure

- Chemical structure of AT7519 and AT7519 bound within the active site of CDK2 (Figure 1).

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
Leukaemia	HL60	90
	K562	40
	MOLT4	310
Lymphoma	GRANTA-519	160
	JEKO-1	70
Fibroblast	MRC 5	980
	MRC 5 (Non Prolif)	>10000

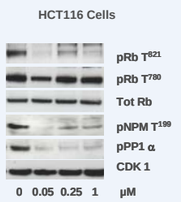


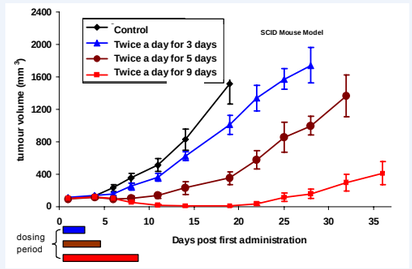
Figure 2: AT7519 Cell Based Activity

- A cell proliferation assay in a panel of human tumour cell lines resulted in IC_{50} values of between 40 and 400nM following 72h exposure (Figure 2).
- Non-proliferating fibroblast cells (MRC-5) were unaffected in this assay.
- Treatment of cells for 24 hours inhibited phosphorylation of the CDK-1 substrate, protein phosphatase 1 α (PP1α) and CDK2 substrates, retinoblastoma protein (Rb) and nucleophosmin (NPM), at phosphorylation sites specific for the indicated kinases.

PHARMACODYNAMICS

HCT116 - AT7519 dosed twice daily, at 10mg/kg i.p for 3, 5 or 9 days

A



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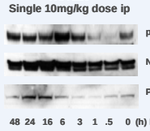

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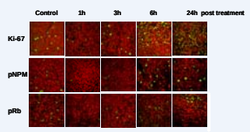


Figure 3: Efficacy and PD Modulation in Xenograft Models

- AT7519 was dosed i.p to HCT116 tumour bearing mice twice a day for either 3, 5 or 9 days (Figure 3A).
- Inhibition of tumour growth was observed following 5 days dosing or longer. Nine days of dosing was sufficient to cause cytoreduction and sustained inhibition to around 20 days following the first dose before any regrowth was observed.
- HCT116 tumour bearing mice were administered a single dose of AT7519 at 10mg/kg via the i.p. route (Figure 3B and C). Tumours were removed at the indicated time points following dosing and prepared for western blotting (B) or immunofluorescent staining (C).
- Both methods of measuring pharmacodynamic markers of AT7519 activity showed rapid inhibition of phosphorylation of the CDK substrates NPM and Rb associated with a functional response in terms of reduced proliferation (Ki67) and increased apoptosis (cleaved PARP).

PHASE I CLINICAL STUDY

- A dose escalation study was performed in patients with refractory solid tumours using a 1h intravenous infusion on days 1 through 5 of a 21 day cycle.
- Inclusion and exclusion criteria were standard.
- Dose escalation was performed according to a standard "3 + 3 design".

DOSE ESCALATION

Table 1: Dose Escalation Scheme

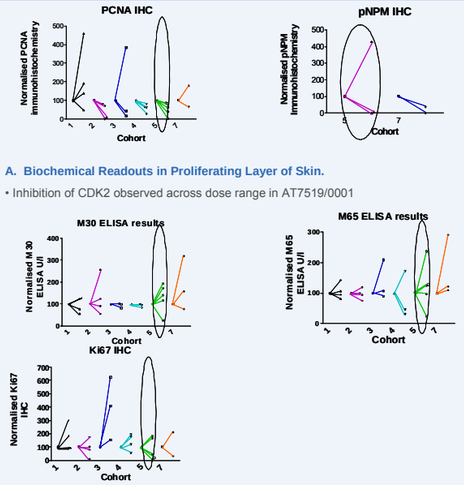
Dose Level *(mg/m²/day)*	Number of Patients Treated	Number of Cycles Received (Median)	Dose Limiting Toxicities (DLT)
1 *(1.8)*	4	2 – 12 *(6)*	None
2 *(3.6)*	4	1 – 8 *(2)*	None
3 *(7.2)*	3	2 – 4 *(2)*	None
4 *(14.4)*	5	1 – 5 *(2)*	Allergic Bronchospasm
5 *(28.8)*	8	1 – 8 *(2)*	None
6 *(40)*	1	1 *(1)*	Hypotension and ST segment elevation
7 *(34)*	3	*1-2 (1)*	QTc prolongation

- No evidence of DLT was observed until cohort four (14.4 mg/m^2 per day) where one patient experienced bronchospasm during their first and second cycles of treatment. No DLTs were observed in cohort five (28.8 mg/m^2 per day).
- Only one patient was treated in cohort six because of the onset of hypotension and ST segment changes. Thereafter an intermediate dose of 34 mg/m^2 per day was explored in cohort seven.
- Grade 5 QTc prolongation was observed in cohort seven and recruitment was suspended pending ECG review.
- The study was discontinued following evidence of a dose-related increase in QTc on day 5 of treatment. Similar toxicity has not been reported from studies of alternative administration schedules.

PHARMACODYNAMIC SAMPLING

- Skin biopsies were taken prior to commencement of AT7519 infusion and 1-2h post administration of study medication on day 3 of cycle 1. Immunohistochemistry was performed to assess inhibition of CDKs and the cell cycle (PCNA and pNPM) and downstream biological consequences (Ki67).
- Serum sampling was performed pre-dose and 1-2h post administration of AT7519 on day 5 of cycle 1. Induction of cytokeratin (M65) and its caspase cleaved form (M30) was assessed by ELISA as an indirect measure of apoptosis.

PHARMACODYNAMICS



A. Biochemical Readouts in Proliferating Layer of Skin.

• Inhibition of CDK2 observed across dose range in AT7519/0001

B. Biological readouts in skin and serum

• Biological readouts positive at 28.8mg/m2 (Cohort 5) and above

• Biological readout requires sufficient knockdown of CDK2 (magnitude or duration).

Figure 4: Biomarker Changes in Surrogate Tissue Across Dose Levels

Table 2: Biomarker Changes in Surrogate Tissue Across Dose Levels

Dose mg/m^2/ day	ELISA		IHC		
	M30 increase	M65 increase	Ki67 reduction	PCNA reduction	pNPM reduction
1.8 (1)	1/3	1/4	2/4	2/4	ND
3.6 (2)	2/4	2/4	2/4	3/4	ND
7.2 (3)	0/2	2/3	0/3	1/2	ND
14.4 (4)	0/3	1/3	2/4	1/3	ND
28.8 (5)	**4/5**	**3/5**	**3/5**	**4/5**	**2/3**
34 (7)	2/3	3/3	1/2	1/2	2/2

- Inhibition of CDKs, as determined by the biochemical readout (decrease in PCNA and pNPM), was observed across the does range (Figure 4). At 28.8mg/m^2 biological consequences of this inhibitory effect were observed in the form of inhibition of proliferation (decrease in Ki67) and induction of the apoptotic markers M30 and M65 in serum (Figure 4 and Table 2).

PHARMACOKINETICS

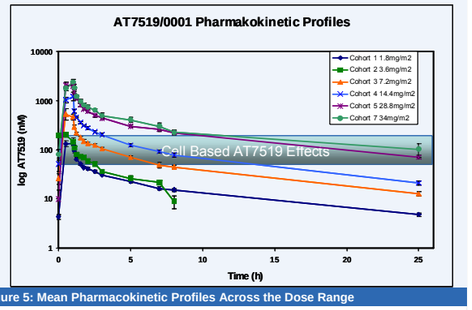


Figure 5: Mean Pharmacokinetic Profiles Across the Dose Range

- AT7519 undergoes multiphasic elimination with a long terminal half-life and only modest inter-patient variation.
- Based on extrapolation from preclinical models, even in the lowest dose cohorts, plasma levels of AT7519 are reached that should modulate CDKs.
- It is only from a dose of 28.8mg/m^2 (cohort 5) that these levels are sustained beyond 12h.

CONCLUSIONS

- This study demonstrates that it is possible to monitor pharmacodynamic markers of CDK inhibition in surrogate tissue (skin) and serum samples from patients receiving AT7519 in a clinical study.
- AT7519 levels in the plasma that provided sufficient exposure in tissues to inhibit CDK activity were achieved at the lowest dose.
- However these effects were insufficient to result in a decrease in proliferative markers or an increase in apoptotic markers. Inhibition of proliferation and induction of apoptosis was observed at doses resulting in >12h exposure to effective concentrations of AT7519 in the plasma.
- These data suggest it is possible to determine a predicted biologically effective dose from an early phase clinical trial utilising a combination of assays in surrogate tissue.

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References

[1] P G Wyatt *et al.* J Med. Chem.51, 4986-99, 2008

[2] M S Squires *et al.* Mol. Cancer Ther. 8 (2), 324-32, 2009

Poster can be downloaded from www.astex-therapeutics.com. Presented at the 100th Annual AACR meeting 18th-22nd April 2009, Denver, CO.