

# 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<sup>1</sup> 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<sup>2</sup>.
- 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



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 <sub>50</sub> (nM)
Colon Carcinoma	HCT116	54
	HT29	170
Ovarian Carcinoma	A2780	350
	SK-OV-3	400
Lung Carcinoma	A549	390
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	990
	MRC-5 (Non Prolif)	>10000

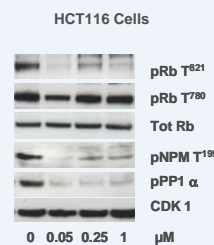


Figure 2: AT7519 Cell Based Activity

- A cell proliferation assay in a panel of human tumour cell lines resulted in IC<sub>50</sub> 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

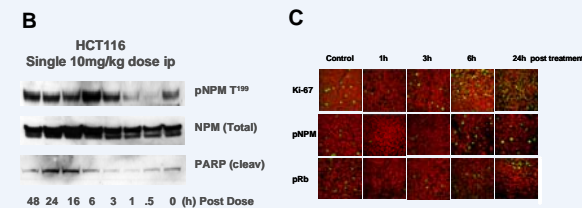
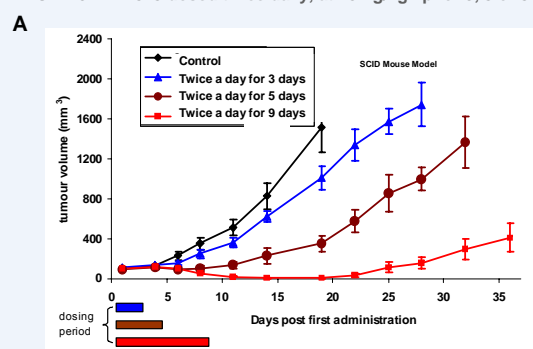


Figure 3: Efficacy and PD Modulation in Xenograft Models

- 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

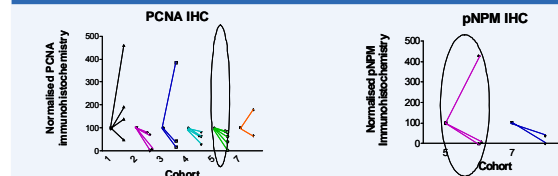
Dose Level (mg/m <sup>2</sup> /day)	Number of Patients Treated	Number of Cycles Received (Median)	Dose Limiting Toxicities (DLT)
1 (1.8)	4	2-12 (6)	None
2 (3.6)	4	1-8 (2)	None
3 (7.2)	3	2-4 (2)	None
4 (14.4)	5	1-5 (2)	Allergic Bronchospasm
5 (28.8)	8	1-8 (2)	None
6 (40)	1	1 (1)	Hypotension and ST segment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

- No evidence of DLT was observed until cohort four (14.4 mg/m<sup>2</sup> 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<sup>2</sup> 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<sup>2</sup> 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

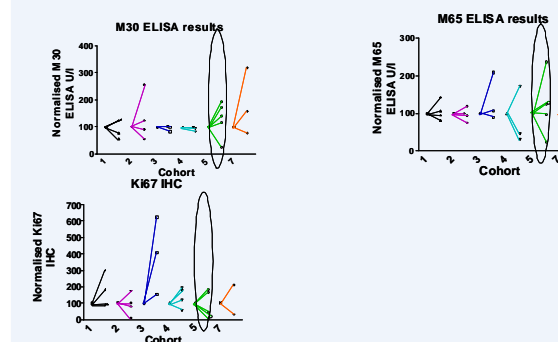
- Skin biopsies were taken prior to commencement of AT7519 infusion and 1-2h post administration of study medication on day 3 of cycle 1. Immunohistochemistry was performed to assess inhibition of CDKs and the cell cycle (PCNA and pNPM) and downstream biological consequences (Ki67).
- Serum sampling 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/m<sup>2</sup> (Cohort 5) and above
- Biological readout requires sufficient knockdown of CDK2 (magnitude or duration).

Figure 4: Biomarker Changes in Surrogate Tissue Across Dose Levels

Dose mg/m <sup>2</sup> /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 dose range (Figure 4). At 28.8mg/m<sup>2</sup> 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

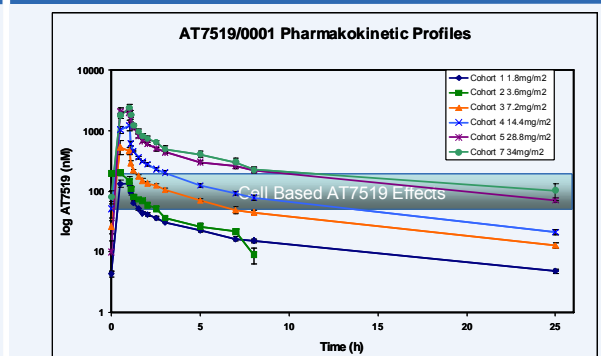


Figure 5: Mean Pharmacokinetic Profiles Across the Dose Range

- 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<sup>2</sup> (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.



References  
<sup>1</sup> P G Wyatt et al. J Med. Chem. 51, 4986-99, 2008  
<sup>2</sup> M S Squires et al. Mol. Cancer Ther. 8 (2), 324-32, 2009