

A Dose Escalation, Pharmacokinetic and Pharmacodynamic Study of AT7519, a Cyclin-Dependent Kinase Inhibitor in Patients with Refractory Solid Tumours

3533

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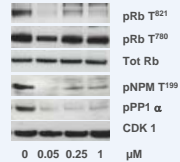
INTRODUCTION

Uncontrolled proliferation is characteristic of cancer cells and can often be attributed to an alteration in the regulatory mechanisms controlling the cell cycle. Deregulation of cyclin dependent kinase (CDK) activity is frequently seen in human cancers, for example the overexpression of D-cyclins and loss of the inhibitory co-factor (INK) proteins or Rb. The key roles of CDKs in the different stages of the cell cycle mean that their inhibition should limit uncontrolled proliferation in cancer cells. AT7519 is a small molecule inhibitor of multiple CDKs, including 1, 2, 4, 5 and 9. The results of a phase I and pharmacodynamic study of AT7519 administered as a one hour intravenous infusion on days one, two, three, four and five every three weeks to patients with refractory solid tumours is presented here. Inclusion and exclusion criteria were standard. Dose escalation was performed according to a standard "3 + 3 design".

Table 1: Antiproliferative Activity of AT7519 in Human Tumour Cell Lines

Tissue	Cell Line	AT7519 IC ₅₀ (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
	MOL14	310
Lymphoma	GRANTA-519	160
	JEKO-1	70
Fibroblast	MRC 5	880
	MRC 5 (Non Prolif)	>10000

Figure 1: Inhibition of CDKs 1 and 2 in HCT116 Colon Carcinoma Cells



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 nucleoposmin (NPM) at phosphorylation sites specific for the indicated kinases.

Table 2: Patient Demographics

Age	Median 64 years (range 39 – 81)	
Sex	12 (43% Female)	
Diagnosis		6
	Colorectal cancer	5
	Non-Small Cell Lung Cancer	4
	Adenocarcinoma of the pancreas	2
	Adenocarcinoma of the breast	5
	Gastroesophageal cancer	1
	Hepatocellular cancer	1
	Mesothelioma	1
	Adenocarcinoma of the parotid	1
	GIST	1
	Melanoma	2
Previous lines of chemotherapy	One	4
	Two	4
	Three	6
	Four	9
	Five	1
	Six	2 (median 4)
Other previous anticancer Therapy (excluding surgery)	Chemoembolisation	2
	Radiotherapy	13

Table 3: Dose Escalation Scheme

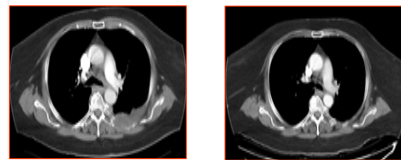
Dose Level (mg/m ² /day)	Number of Patients Treated	Number of Cycles Received (Median)	Dose Limiting Toxicities (DLT)
1 (1.8)	4	2 – 12 (6)	None
2 (3.6)	4	1 – 8 (2)	None
3 (7.2)	3	2 – 4 (2)	None
4 (14.4)	5	1 – 5 (2)	Allergic Bronchospasm
5 (28.8)	8	1 – 8 (2)	None
6 (40)	1	1 (1)	Hypotension and ST segment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

DOSE ESCALATION

- No evidence of DLT was observed until cohort four (14.4 mg/m² 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/m² 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/m² per day was explored in cohort seven.
- Grade 4 QTc prolongation was observed in cohort seven and recruitment was suspended pending ECG review.

EFFICACY

Figure 2: Disappearance of Pleural Deposit in a Patient with Pretreated NSCLC after Four Cycles of Treatment with AT7519

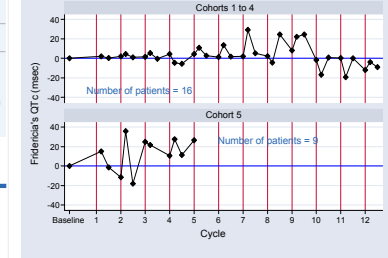


Four patients completed at least six cycles of treatment (adenocarcinoma of the lung, adenocarcinoma of the pancreas (2) and HER2 overexpressing adenocarcinoma of the breast). The best response to treatment in all of these cases was stable disease although one patient experienced a mixed response with a maximum reduction in the sum of unidimensional measurements of approximately 80%.

TOXICITY

Patients treated in cohorts five and seven developed Grade 2 thrombocytopenia and early Grade 3/4 neutropenia and lymphopenia. Fatigue and stomatitis were reported in a dose dependent manner but were not dose limiting. 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.

Figure 3: Evidence of QTc Prolongation on Day 5 in Patients Treated in Cohorts 5, 6 and 7



Definitive assessment of QTc risk will require further investigation in a larger patient population

PHARMACODYNAMICS

Figure 4: Biomarker Changes in Surrogate Tissue Across Dose Levels

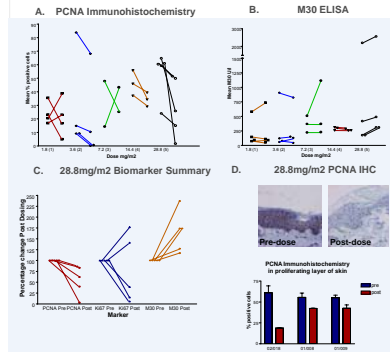


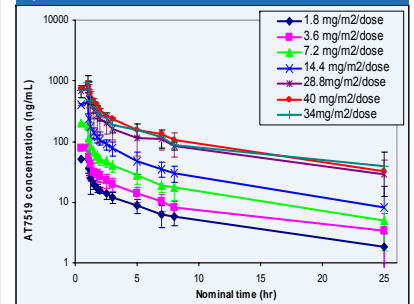
Table 4: Biomarker Changes in Surrogate Tissue Across Dose Levels

Dose mg/m ² /day	M30 increase	M65 increase	PCNA reduction	Ki67 Reduction
1.8 (1)	1/4	1/4	2/4	1/4
3.6 (2)	1/4	2/4	3/4	0/4
7.2 (3)	0/2	1/2	1/2	0/2
14.4 (4)	0/3	1/3	1/3	0/3
28.8 (5)	5/5	3/5	4/5	4/5
34 (6)	1/2	2/2	2/3	3/3

- 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 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.
- Inhibition of CDKs, as determined by the biochemical readout (PCNA), was observed across the dose range (Fig 6A). At 28.8mg/m² biological consequences of this inhibitory effect was observed in the form of inhibition of proliferation (Ki67) and induction of the apoptotic marker M30 in serum (Fig 6 B-D and Table 4).

PHARMACOKINETICS

Figure 5: Plasma Concentration-time Profiles Across all Administered Doses



- AT7519 undergoes multiphasic elimination with a long terminal half-life and only modest inter-patient variation

CONCLUSIONS

- This study was unable to identify a maximum tolerated dose of AT7519 when administered as a one hour intravenous infusion on days one, two, three, four and five every three weeks because of concerns over a possible dose-dependent increase in QTc which resulted in premature study closure. Similar concerns have not arisen during alternative administration schedules of AT7519.
- Preliminary evidence of anticancer activity was observed.
- Pharmacodynamic evidence of CDK/cell cycle inhibition (PCNA) was observed across the dose range. Anti-proliferative activity and tumour apoptosis (cytokeratin 18 cleavage) were consistently observed in the majority of patients treated at a dose of 28.8 mg/m² per day.

