A phase I pharmacokinetic and pharmacodynamic study of AT7519, a cyclin-dependent kinase inhibitor in patients with refractory solid tumors


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Background: AT7519 is an inhibitor of multiple cyclin-dependent kinases (CDKs). Based on potent antitumor activity in preclinical models, a first-in-human clinical trial in refractory solid tumors investigated its safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD).

Patients and methods: AT7519 was administered in a ’3 + 3’ dose-escalation scheme on 5 consecutive days every 3 weeks to patients with advanced, refractory solid tumors. Samples to monitor AT7519 PK and PD were obtained.

Results: Twenty-eight patients were treated at seven dose levels (1.8–40 mg/m²/day). At 40 mg/m²/day, one patient developed hypotension and ST segment elevation. At 34 mg/m²/day, dose-limiting toxic effects (DLTs) were QTc prolongation with one death (grade 5), fatigue (grade 4) and mucositis (grade 3). Electrocardiogram review suggested a dose-dependent increase in QTc and recruitment was discontinued without establishing a maximum tolerated dose. Four patients exhibited stable disease for >6 months and one had a prolonged partial response. Inhibition of markers of CDK activity was observed across the dose range and manifested in antiproliferative activity at a dose of 28 mg/m².

Conclusion: AT7519 elicited clinical and PD activity resulting from CDK inhibition at doses below the appearance of DLT of QTc prolongation.

Key words: AT7519, cyclin-dependent kinases, pharmacodynamics, pharmacokinetics, refractory solid tumors

Introduction

Cyclin-dependent kinases (CDKs), and their regulatory cyclin partners, play a central role in eukaryotic cell growth, division and death. This key role in cell cycle progression, and their deregulation in a number of human cancers, makes them attractive therapeutic targets in oncology [1]. The eukaryotic cell cycle is a series of tightly integrated events divided into four phases comprising two gaps [gap 1 (G1) and gap 2 (G2)], DNA synthesis (S) and mitosis (M). Correct progression through the various phases of the cell cycle has been shown to be critically dependent upon a structurally related family of CDKs (serine-threonine kinases) and their activating cyclin partners. For example, CDK2/cyclin E, CDK4/cyclin D and CDK6/cyclin D complexes primarily regulate progression from G1 to S phase of the cell cycle, CDK2/cyclin A and CDK1/cyclin A complexes function during the S phase and control progression to G2 and CDK1/cyclin B complex initiates mitosis. Failure of these control mechanisms can lead to cell cycle arrest and/or cellular apoptosis [1] or alternatively to aberrant cellular proliferation, as manifested in cancer [2, 3].

CDK5 is known to play a role in neuronal and secretory functions [4], while CDK7 and 9 are involved in transcriptional regulation, independent of the cell cycle. CDK7 and 9 act via phosphorylation of the COOH-terminal domain of RNA polymerase II and promote initiation and elongation of nascent messenger RNA transcripts [5]. Inhibition of transcriptional CDKs is expected to produce anticancer activity or augment apoptotic responses because the transcripts most sensitive to CDK inhibition include those with short half-lives, which encode cell cycle regulators, mitotic kinases, nuclear factor-kB-responsive gene transcripts and apoptosis regulators such as MCL-1 and X-linked inhibitor of apoptosis [6, 7]. The central role of CDKs in cell cycle control and regulation of transcription has led to the development of a number of small-molecule inhibitors (SMIs) of CDKs as potential anticancer drugs [8, 9]. Flavopiridol, a natural product-derived inhibitor of multiple CDKs is in late-phase development for

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the treatment of chronic lymphocytic leukemia and several second-generation agents are progressing through early-phase clinical trials [10]. A series of CDK ATP site SMIs was developed by Astex Therapeutics Limited (Cambridge, UK) using fragment-based medicinal chemistry approaches. A compound from this series, AT7519, is currently in early-phase clinical development [11, 12]. In vitro protein kinase activity demonstrated concentration that causes 50% inhibition of growth for AT7519 of selective inhibition of CDK1 (220 nM), CDK2 (44 nM), CDK4 (67 nM), CDK5 (11 nM) and CDK9 (<100 nM) [12, 13] and its role in targeting the respective CDKs in human malignancy is highlighted (Figure 1). Furthermore, AT7519 demonstrated potent antiproliferative effects in preclinical models where it inhibited phosphorylation of downstream substrates of various CDKs [7, 12]. Exploration of a series of administration schedules in vivo identified repeated daily dosing as providing an optimum therapeutic index which lead to the introduction of a daily times five administration schedule in this first dose-finding study.

**methods**

**patient population**

The study was conducted at two sites (United States and United Kingdom) with appropriate regulatory and institutional review board approval and patients providing informed consent. Patients were 218 years with advanced solid malignancies refractory to standard treatments. The main inclusion criteria were life expectancy ≥12 weeks; Karnofsky performance status ≥70; women of nonchildbearing potential (postmenopausal or history of bilateral oophorectomy or hysterectomy) or not pregnant or willing to use contraception; left ventricular ejection fraction >45% by echocardiogram or multiple gated acquisition scan. Exclusion criteria included chemotherapy or radiotherapy within 4 weeks of study start; inadequate hematologic (neutrophils ≤1.5 × 10^9 per liter, platelets ≤100 × 10^9 per liter, hemoglobin ≤9 g/dl), hepatic (serum bilirubin higher than upper limit of normal (ULN) of reference range or alanine aminotransferase or aspartate aminotransferase or alkaline phosphatase >2.5 ULN of reference range or >5 times ULN of reference range with liver metastases) or renal function (serum creatinine higher than ULN of reference range or creatinine clearance of <50 ml/min or >2+ proteinuria on two consecutive dipsticks within 24 h); history of ischemic heart disease or myocardial infarction within 3 months of the study; severe or uncontrolled systemic conditions or current unstable or uncompensated respiratory or cardiac conditions.

**study objectives**

This was a phase 1 open-label, dose-escalation study whose primary objective was to identify a safe dose of AT7519, on a daily times five schedule for exploration in future studies. Other objectives included to determine pharmacokinetics (PK) of AT7519 and to assess antitumor activity using RECIST (version 1.1) [14]. Dose-limiting toxicities (DLTs) were assessed only during the first cycle and were febrile neutropenia, grade 4 neutropenia for >4 days, grade 3 or 4 thrombocytopenia, grade 3 or 4 non-hematologic toxic effects and neuropathy higher than grade 1. Additional exploratory objectives were to demonstrate pharmacodynamic (PD) activity of AT7519 by establishing its effects on relevant biological end points in skin epidermis and in serum.

**drug administration**

AT7519 was administered as an intravenous infusion over 1 h (1.8, 3.6, 7.2, 14.4, 28.8, 34, 40.0 mg/m^2/day) once daily for 5 days repeated every 3 weeks until study withdrawal. No intrapatient dose escalation was permitted. Patients continued on AT7519 in the absence of disease progression (clinical benefit) or toxicity, which could not be managed by dose reduction.

**study parameters**

Screening and baseline assessments included obtaining relevant history and details of previous cancer treatment. Safety was monitored at baseline and throughout the study by adverse event (AE) reporting, physical examination including full neurological assessment, resting 12-lead electrocardiogram (ECG), vital signs, clinical chemistry, hematology and

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**Figure 1.** The resolution of the left upper lobe mass after treatment with AT7519 at 1.8 mg/m^2 daily for 5 days every 3 weeks (baseline, after two cycles, after four cycles and after six cycles).
urinalysis. An additional detailed analysis of available ECG data was carried out to investigate the effects of AT7519 on heart rate and QTc interval following an unexpected death of one subject after 4 days of treatment at 34.0 mg/m²/day.

ECG analysis
Existing paper ECGs were digitalized and the RR, PR, QRS, QT intervals and QRS axis, were read in a blinded fashion by an independent observer. In order to assess the relevance of the standard QT correction factors, Bazett [15], Frederica [16] or Framingham’s [17], the relevant QTc data were regressed against RR (using screening and preinfusion data from day 1 of each cycle) to estimate the (within-patient) slope of the QTc–RR relationship. In addition, a study-specific correction factor was determined using the approach described by Van de Water et al. [18] in which only preinfusion data were used to estimate the (within-patient) slope of the QTc–RR relationship. As the mean slopes of the Frederica, Framingham and study-specific correction factors were all similar and were not statistically different from zero, the study-specific correction factor was used for the primary interpretation of the QT data. Comparisons were made between, during and at the end of the cycle for the first four cycles of treatment of all cohorts.

PK analysis
At each dose level, sequential PK sampling was carried out on study days 1 and 8 during cycles 1 and 3. Schedule of blood sampling for PK analysis (times relative to the end of a 1-h infusion) was as follows: before (~60 min before infusion) and during infusion and postinfusion ~30 min (midpoint of infusion), 0 min (immediately before end of infusion), 5 min, 15 min, 30 min, 45 min, 60 min, 90 min, 2 h, 4 h, 6 h, 8 ± 1 h, (12 ± 1 h), and 24 ± 1 h and on day 8.

At each dose level, sequential PK sampling was carried out on study days 1 and 4 during cycles 1 and 3 only. A validated liquid chromatography–tandem mass spectrometric (LC-MS/MS) assay method is used to determine AT7519 concentrations for all human PK evaluations. Plasma samples for AT7519 analysis were first mixed with Tris buffer, pH 11.5, and an aliquot of internal standard solution (AT7518). The diluted plasma sample was then mixed with 10 volumes of methyl t-butyl ether (MTBE) in order to separate AT7519 from plasma proteins and other material that could interfere with the MS/MS assay. The MTBE extract was evaporated to dryness and the residue obtained was dissolved in methanol : water (50 : 50, by vol) in order to prepare the samples for liquid chromatography on a C18 reverse-phase LC column. The mass spectrometric detection of AT7519 and AT7518 in the LC column utilized TurboIonSpray in the positive ion mode with Multiple Reaction Monitoring. The following MS/MS ion transitions were used to specifically measure AT7519 and the internal standard AT7518: AT7519 m/z 382.1 → m/z 281.9, AT7518 m/z 366.1 → m/z 136.1. The validated LC-MS/MS method as described above achieved a lower limit of quantification for AT7519 in human plasma of 1 ng/ml. PK parameters were calculated using WinNonLin® noncompartmental analysis.

PD analysis
For the purposes of this study, proliferating cell nuclear antigen (PCNA), which forms part of a cyclin complex whose assembly is regulated by CDK phosphorylation, and nucleophosmin (NPM), which is involved in spindle regulation and a direct CDK2 substrate, were monitored as markers of CDK inhibition. Skin punch biopsies were obtained predose on day 1 and within 1–2 h postdose of AT7519 on day 3 during cycle 1. Skin punch biopsies were formalin fixed, paraffin embedded and 4-μm sections generated using a microtome (Leica, Wetzlar, Germany). Sections were deparaffinized and rehydrated and antigen unmasking was carried out by heating sections in 10 mM sodium citrate buffer (pH 6) at 98°C for 20 min. Sections were stained using antibodies specific for Ki67 (Invitrogen, Carlsbad, CA) or CDK substrates PCNA (Invitrogen) and phospho-nucleophosmin (pNPM) (Cell Signaling Technology, Beverly, MA). Primary antibodies were detected using the SuperPicture™ system (Invitrogen). One section was also stained with hematoxylin and eosin to aid histological interpretation. Positive cells in the proliferating layer of the skin section were scored as a percentage of the total cell population by microscopy (Olympus BX51, with Olympus DP50 camera; Olympus, Southend on Sea, UK).

Serum samples were obtained predose on day 1 and within 1–2 h postdose of AT7519 on study day 5 (cycle 1). These samples were analyzed for intact (M65) and cleaved (M30) forms of cytokeratin 18 by commercially available enzyme-linked immunosorbent assay (Periva, Bromma, Sweden).

results
patient demographics and dose escalation
A total of 55 complete cycles of AT7519 were administered to 28 patients in this study. The median age was 64 years (39–81 years) with 12 (43%) females. The most common tumor types enrolled were colorectal cancer and non-small-cell lung cancer (Table 1). A total of seven dose levels (1.8–40.0 mg/m²/day) were explored (Table 2) in a 3 × 3 design. There were no DLTs in the first three cohorts but there was an episode of allergic bronchospasm in one patient administered 14.4 mg/m²/day, which occurred during the second cycle of treatment and its temporal association led this to be categorized as a serious adverse event (SAE). No DLTs were experienced in the 28.8-

Table 1. Summary of patient demographics

<table>
<thead>
<tr>
<th>Age Median 64 years, range (39–81)</th>
<th>Number of patients</th>
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<tbody>
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<tr>
<td>Colorectal cancer</td>
<td>12 (43%)</td>
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<td>Non-small-cell lung cancer</td>
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<tr>
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<tr>
<td>Radiotherapy</td>
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mg/m²/day group so the dose was escalated to 40 mg/m²/day. However, the first patient to receive this dose experienced reversible hypotension and nonspecific ST elevation on ECG. As a result, no additional patients were treated at this dose level. A dose of 34.0 mg/m²/day was selected and three patients were treated. One patient experienced two AEs, which were considered to be DLTs. The patient developed progressive fatigue (grade 3) on days 1–4 and had a grade 5 SAE on day 5 consistent with a cardiac event, possibly a sudden-onset ventricular arrhythmia. A postmortem examination showed no evidence of pulmonary embolus or a gastrointestinal hemorrhage. The patient’s heart was structurally normal with no evidence of significant coronary artery disease or an acute myocardial infarction. The only significant postmortem finding was bronchopneumonia due to metastatic esophageal carcinoma with a contributing condition of pulmonary emphysema. Following this fatal event, a detailed review of all available ECG results was carried out. The results were suggestive that increasing doses of AT7519 were associated with a dose-dependent increase in QT and QTc. As a result, the study was terminated without establishing a maximum tolerated dose (MTD).

### ECG findings

A total of 241 ECGs from 24 patients were analyzed. Inspection of the RR plots indicated that for most cycles there was a decrease in the mean RR interval following the administration of AT7519. Changes in RR from the cycle baseline were also more frequently negative than positive, especially in the higher dose cohorts. Changes in the uncorrected QT interval from the study baseline show no marked tendency for any changes, which in association with an apparent decrease in RR interval was suggestive of an increase in the QTc interval. Regression analysis for the study-specific QTc following treatment with AT7519 revealed a strong positive tendency, which was statistically significant for two of the first four cycles of AT7519. Extrapolation of the study-specific QTc from the single patient treated at 40 mg/m²/day at the time point showing maximum change was consistent with a treatment-related QTc prolongation of as much as 50 ms. Similarly, if the MTD had been identified as 28.8 mg/m²/day, assuming a linear dose–response relationship, the estimated QTc at this level (and time point) would be 38 ms higher than that with the initial 1.8-mg/m²/day dose consistent with a dose-dependent increase in QTc.

### Pharmacokinetics

The PK of AT7519 was reproducible with modest inter- and intrapatient variability (Figure 2A). Total plasma clearance for AT7519 was low (<30% of hepatic blood flow) and its volume of distribution at steady state was large (>2 l/kg) achieving prolonged systemic exposure following a 1-h infusion. The mean cohort plasma half-life values ranged from 7 to 10 h with the maximum individual half-life observed being 25 h. There was a dose-proportional increase in exposure as measured by peak drug concentration (C\text{max}) and area under the plasma concentration versus time curve (AUC\text{0-tau}) (Figure 2B and C). There was no evidence of accumulation, as measured by C\text{max} and AUC\text{0-tau} between day 1 and 4 of cycles 1 and 3 or between dosing cycles 1 and 3.

### Pharmacodynamics

Skin punch biopsies were analyzed for direct markers of CDK inhibition and consequent biological effects in the form of inhibition of proliferation. For the purposes of this study, PCNA, which forms part of a cyclin complex whose assembly is...
regulated by CDK phosphorylation, and NPM, which is involved in spindle regulation and a direct CDK2 substrate, were monitored as markers of CDK inhibition. Reduction in PCNA levels and pNPM inhibition were observed at all doses above 1.8 mg/m²/day (Figure 3A and B). These data confirm that even at these lower doses, sufficient levels of AT7519 were achieved in the skin to inhibit CDK activity. The biological consequence of the CDK inhibition was monitored by staining the same punch biopsies for the cell proliferation marker Ki67 and by analyzing serum samples for intact (M65) and serum-cleaved (M30) forms of cytokeratin 18 as a marker of tumor cell apoptosis. A consistent decrease in Ki67 levels and increase in both the M30 and M65 forms of cytokeratin were observed in the majority of samples at >28.8 mg/m²/day (Figure 3C–E). These data indicate that although tissue levels of AT7519 may be sufficient to inhibit CDK activity at doses <28.8 mg/m²/day, only at ≥28.8 mg/m²/day are tissue levels sufficient to inhibit cell proliferation and induce apoptosis. Therefore, the 28.8-mg/m²/day dose may be considered the minimal biologically effective dose.

efficacy
Tumor assessments were carried out every second cycle using RECIST. In cohort 1 at 1.8 mg/m²/day, a heavily pretreated patient with metastatic non-small-cell adenocarcinoma of the lung had a PR with an 80% reduction in the sum of measurable tumor diameters (Figure 1). She completed 12 cycles of treatment and developed skin (9 months) and subsequently brain metastases (12 months). The PR within the lungs continued despite progression in skin and brain (Figure 1) with a progression-free survival of 13 months. A second patient treated at 1.8 mg/m²/day with gemcitabine-refractory metastatic pancreatic ductal adenocarcinoma completed nine cycles with a best response of stable disease. The patient discontinued treatment as a consequence of deteriorating performance status but lived another 5 months without any further therapeutic intervention. At the 3.6-mg/m²/day dose, a patient with gemcitabine-resistant pancreatic ductal adenocarcinoma completed eight cycles and lived >3 months after discontinuation with no further therapy. At the 14.4-mg/m²/day dose, a patient with metastatic breast cancer with stable brain metastasis completed eight cycles and lived another 10 months without any further treatment. Finally, a patient with c-Kit-positive gastrointestinal stromal tumor refractory to imatinib and sunitinib with rapidly progressing disease treated at 28.8 mg/m²/day completed four cycles but withdrew from study due to personal reasons and lived >13 months without any further treatment.

discussion
AT7519 is an inhibitor of CDK1, 2, 4, 5 and 9 with potent preclinical antitumor activity, a favorable tolerability profile...
and robust target inhibition. A first-in-human phase I study was conducted to determine the safety and tolerability of AT7519 in patients with refractory solid tumors. A total of seven dose levels were explored (Table 2). There was a dose-dependent increase in exposure with AT7519 measured by \( C_{\text{max}} \) and AUC\(_{0-tau} \) with a mean plasma half-life ranging from 7 to 10 h with a maximum half-life of 25 h (Figure 2B). Although there was no suggestion in preclinical safety pharmacology of AT7519 having any effect on cardiac repolarization, evidence of QTc prolongation was seen at a dose of 34 mg/m\(^2\)/day. ECG reviews were consistent with AT7519 inducing a dose-dependent increase in QTc and the study was discontinued without establishing an MTD. A second phase I study of refractory solid tumor patients with an alternative dose schedule (twice-weekly dosing for 2 weeks in three patients) showed no QTc prolongation at the MTD of 25 mg/m\(^2\)/day (clinicaltrials.gov/ct2/show/NCT00390117?term=AT7519&rank=1).

Fatigue was the other DLT. The most common treatment-emergent AEs were gastrointestinal in nature. Significant hematological toxicity was only observed at doses of >34 mg/m\(^2\)/day where transient neutropenia was observed with a blood cell count nadir occurring 24 h following the initial dose. The timing and duration of this event was unusual with a gradual recovery of normal neutrophil counts occurring during succeeding days despite continued treatment. The mechanism of this effect is unknown but similar events have been observed following treatment with other CDK inhibitors. As anticipated, lymphopenia was common at higher dose levels with circulating counts following a pattern similar to that of neutrophils. Although the mechanism of this effect was not investigated, it may be a consequence of a reduction in cellular levels of key antiapoptotic proteins such as Mcl-1 due to DNA transcriptional inhibition.

Five patients completed at least eight cycles and one patient completed four cycles of therapy with a best response of a PR (Figure 1). After discontinuation of AT7519, these five patients lived an average of 6.6 months (2–13 months) with no further therapy. The pharmacological activity of AT7519 was demonstrated in skin epidermis where reduced PCNA levels and NPM phosphorylation showed that CDK activity was inhibited at all doses >1.8 mg/m\(^2\). Changes in expression of Ki67 were only visible at doses ≥28.8 mg/m\(^2\), where the majority of patients showed a reduction in the postdose skin biopsy. Increases in the M30 and M65 forms of cytokeratin 18 were similarly evident in the majority of patients receiving doses of ≥28.8 mg/m\(^2\), suggesting that AT7519 was inducing tumor cell apoptosis (Figure 3). Further studies of AT7519 are warranted as alternative administration schedules do not
appear to increase the QTc interval. In particular, disease types that may be impacted by reduction in rapidly turned over transcripts may be of particular interest for further investigation.

acknowledgements

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disclosure

The authors declare no conflict of interest. The study was sponsored, monitored and funded by Astex Therapeutics Limited. MSS and VL are employees of Astex Therapeutics Limited.

references