A phase I dose escalation study of AT9283, a small molecule inhibitor of aurora kinases, in patients with advanced solid malignancies


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Background: AT9283 is an inhibitor of aurora kinases A and B with antitumor activity in preclinical models. This a First in Human phase I study assessed the safety, tolerability, pharmacokinetic and pharmacodynamic properties and preliminary efficacy of AT9283.

Patients and methods: Patients with advanced tumors received AT9283 as a continuous central venous infusion over 3 days in cohorts of three to six patients starting at 1.5 mg/m²/day (equivalent to 4.5 mg/m²/72 h). The oral bioavailability of AT9283 was assessed in a cohort of seven patients. Pharmacodynamic analysis of biomarkers included phosphorylation of histone H3 on serine 10, proliferating cell nuclear antigen, Ki67, M30 and M65 in skin and plasma.

Results: Forty patients were included in all analyses. AT9283 was generally well tolerated with main toxic effects of reversible dose-related myelosuppression, gastrointestinal disturbance, fatigue and alopecia. The dose-limiting toxicity of AT9283 was grade 3 febrile neutropenia in two patients at 36 mg/m²/72 h and the maximum tolerated dose (MTD) was established at 27 mg/m²/72 h. Systemic exposure was dose proportional. The mean oral bioavailability of a 0.9 mg/m² dose was 29.4% (range 11.2%–36.7%). Pharmacodynamic analyses indicated antiproliferative and apoptotic activity of AT9283. Four patients with esophageal, non-small-cell lung cancer (n = 2) and colorectal cancer demonstrated RECIST stable disease ≥6 months.

Conclusion: AT9283 was well tolerated up to the MTD of 27 mg/m²/72 h. AT9283 is currently assessed in phase II trials.

Key words: advanced solid malignancies, AT9283, aurora kinase inhibitor, phase I

introduction

The aurora family consists of three related serine/threonine kinases that share a high degree of homology in their catalytic domains and have been implicated as key regulators of both mitosis and meiosis [1]. Overexpression of aurora kinase A (AKA) has shown to override the spindle checkpoint and leads to aneuploidy and transformation [2–4]. AKA also has effects on cell survival via its interactions with the p53 tumor suppressor gene allowing cells to escape the G1 postmitotic checkpoint. AKA has been shown to phosphorylate p53 at Ser315, leading to its ubiquitination by Mdm2 and proteolysis. Inhibition of AKA results in reduced phosphorylation of p53 at Ser315 and, hence, its greater stability and as a consequence in cell cycle arrest at G2-M [5].

Aurora kinase B (AKB) is overexpressed in a variety of tumors and elevated levels of AKB correlate with advanced stages of many cancers [6, 7]. Cells overexpressing AKB form more aggressive tumors and develop metastases [8]. Aurora kinase C (AKC) functions as a protein supporting the role of AKB in mitotic cells and is largely found in testes where it plays a role in meiosis [1]. Preclinical studies of aurora kinase inhibitors (AKis) have shown promising antitumor efficacy in human cell lines and xenografts, leading to the development of a variety of new compounds [9, 10].

AT9283, a synthetic small heterocyclic molecule, is a potent inhibitor of serine/threonine kinases including the AKA and AKB, JAK2 and 3 and mutant V-abl Abelson murine leukemia viral oncogene homolog 1 [11]. In solid and leukemic tumor cell lines, AT9283 inhibited tumor growth and survival [12]. Preclinical validation of biomarkers encompassed in vitro studies on tumor cell lines demonstrating that exposure to
AT9283 for one complete cell cycle committed an entire population of p53 checkpoint-compromised cells (HCT116) to multinucleation and death, whereas treatment of p53 checkpoint-competent cells (HMEC, A549) for a similar length of time led to a reversible arrest of cells with 4N DNA. Further studies in synchronized cell populations suggested that exposure to AT9283 during mitosis was critical for optimal cytotoxicity and was necessary to optimize the activity of AKi for p53 checkpoint-compromised solid tumor lines. A continuous i.v. infusion for 72 h was selected for clinical exploration based on in vivo studies in human xenograft models.

The primary objective of this first in human (FIH) study was to determine the safety and tolerability of AT9283, administered as a continuous 3-day infusion every 21 days in patients with advanced solid malignancies. Secondary objectives were to establish the maximum tolerated dose (MTD), to define dose-limiting toxicities (DLTs), to determine pharmacokinetics, to perform pharmacodynamics and to evaluate preliminary antitumor activity.

**patients and methods**

**eligibility criteria**

Eligible patients were ≥18 years, with histological and/or cytological confirmation of advanced solid malignancy, refractory or resistant to standard therapies or for which no standard therapy existed. Patients had Eastern Cooperative Oncology Group performance status (ECOG PS) ≤2, life expectancy ≥12 weeks, negative pregnancy test or surgical sterility or evidence of severe unstable hemoglobin £ 5 times the upper limit of normal (ULN) or alanine aminotransferase or aspartate aminotransferase or alkaline phosphatase ≥2.5 times the ULN (or ≥5 times the ULN with liver metastases); abnormal coagulation; impaired renal function, i.e. creatinine ≤2.5 times the ULN or creatinine clearance of ≤50 ml/min determined by Cockcroft–Gault; unresolved Common Terminology Criteria for Adverse Events (CTCAE) toxicity ≥2 from previous therapies including alopecia; evidence of severe unstable respiratory or cardiac conditions; active or symptomatic brain metastases; myocardial infarction within 3 months of study entry; pregnant or breast-feeding and active infections.

Approvals from the relevant ethical committees were obtained and written informed consent was obtained from patients before study entry. The study was conducted to Good Clinical Practice in accordance with the Declaration of Helsinki and its amendments.

**study design**

This was a two center, open-label phase I dose escalation study. The primary aim was to assess the safety and tolerability of AT9283. Secondary aims were to establish a dose for phase II studies; determine the pharmacokinetic profile of i.v./oral administration; demonstrate pharmacodynamic activity in plasma, skin and tumor where appropriate and obtain preliminary antitumor activity. An exploratory objective was to define the oral bioavailability of the agent.

**drug administration**

AT9283 was administered as a 3-day continuous i.v. infusion via central venous access in dose-doubling cohorts of three to six patients starting at 1.5 mg/m²/day (4.5 mg/m²/72 h). Once the MTD was defined, the MTD cohort was expanded to further evaluate the safety and tolerability of that dose.

AT9283 was presented in vials as a sterile white to pink lyophilized solid for reconstitution for infusion. Each 20-ml vial contained 52 mg AT9283 free-base and 100 mg of citric acid. The solid was reconstituted with 5.1 ml of sterile 5% dextrose to give a concentration of 10 mg/ml AT9283 free-base in 100 mM citrate buffer at pH 4.0–5.0. For the i.v. infusion, the reconstituted solution was diluted further to a total of 100 ml using 5% dextrose for administration.

Oral administration was explored in an expanded cohort at the MTD level and an appropriate volume of the reconstituted solution equivalent to a dose of 0.9 mg/m² was administered 7 days before the i.v. administration schedule. Patients were allowed to receive AT9283 for as long as they derived clinical benefit with acceptable toxic effects.

**safety**

Patients underwent assessments [medical history, ECOG PS, adverse event and concomitant medication, vital signs, electrocardiogram (ECG), full blood count, coagulation, biochemistry and urinalysis] at baseline, on days 1–4 and days 8 and 15 of the 21-day cycle. Response evaluation was carried out after two cycles via RECIST 1.0 [13].

Toxic effects were assessed using the National Cancer Institute CTCAE Version 3.0. A DLT was defined as neutropenia <0.5 × 10⁹/l for ≥5 days or with fever, thrombocytopenia <25 × 10⁹/l, any non-hematological CTCAE grade 3/4 toxicity that was, in the opinion of the investigator, clinically significant. Only toxic effects during the first treatment cycle were part of DLT evaluation and at least three patients must have completed one complete cycle for a dose escalation decision to be made. If one of the first three patients in a cohort experienced a DLT during cycle 1, the cohort was expanded to six patients. The MTD was defined as the highest dose that could be given to six patients with no >1 patient experiencing a DLT. In case of a DLT, treatment was discontinued and supportive therapy administered where required. A delay of up to 14 days was permitted to allow for resolution of toxic effects to grade 0/1. If the toxicity had not resolved to grade 0/1 or baseline after a 14-day delay, the patient was withdrawn. If a patient withdrew or was withdrawn for reasons other than DLT before completing cycle 1, the patient was replaced.

**pharmacokinetics**

Blood for pharmacokinetics were collected at baseline and following commencement of the infusion at 30 min, 1, 4, 8, 12, 22, 32, 46, 56, 70, 72, 72.05, 72.25, 72.30, 72.45, 73, 73.30, 74, 75, 76, 78, 80, 84, 96 h and day 8, in cycles 1 and 2. Urine samples for pharmacokinetics were collected at baseline for 72 h (in 24 h periods) during infusion for cycles 1 and 2. With oral dosing at MTD, samples were taken at time 0 h, 25, 30, 45 min, 1, 1.5, 2, 4, 6, 8, 10, 12, 22 and 24 h at cycle 1. Pharmacokinetics were analyzed by non-compartmental analysis using WinNonlin version 4.1b (Pharsight Corp., Sunnyvale, CA). Pharmacokinetic parameters included: maximum plasma drug concentration [Cmax (ng/ml)], half-life (t½, hour), area under the plasma drug concentration versus time curve (AUC) up to infinite time [AUC0 to inf (min-ng/ml)], plasma clearance [CL (ml/min/kg)] and volume of distribution [Vd (l/kg)] and oral bioavailability.

**pharmacodynamics**

Blood samples for pharmacodynamic analysis were collected at time 0, 22, 46, 70 and 168 h, following the start of the infusion, in cycles 1 and 2. Skin samples for pharmacodynamic analysis by immunohistochemistry were collected at baseline and at 46 h, in cycle 1. The 46-h time point was chosen based on preclinical data and between 30 and 100 nM AT9283 were required to inhibit phosphorylation of histone H3 on serine 10 (pHH3ser10). These concentrations were required for ~ 48 h to establish a western blot signal for p53 stabilization [14]. We chose to use...
proliferating cell nuclear antigen (PCNA) and Ki67 as biomarkers of tumor proliferation based on preclinical confirmatory tests and previous experience in early clinical trials [15]. We analyzed the ratio of caspase-cleaved (M30) to total cytokeratin (CK) 18 (M65). CK18 is a serum biomarker for the determination of cell death of epithelial-derived tumors. Caspase-cleaved CK18 (M30) is released from apoptotic cells, while total CK18 (M65) is released by epithelial cells undergoing cell death by any cause (e.g. necrosis).

Pharmacodynamic samples were not collected for patients who were part of the oral cohort.

results

Between September 2006 and May 2009, 49 patients were enrolled [9 patients did not receive treatment, due to disease progression (n = 7) or difficulty with i.v. access (n = 2)] and baseline characteristics are listed in Table 1. The most common tumor types were colorectal (30%), non-small-cell lung cancer (NSCLC) (18%) and esophageal cancer (15%). Patients received between 1 and 12 cycles (median 2) with 70% receiving treatment of at least 2 cycles.

safety and tolerability

Dose escalation proceeded from 4.5 to 18 mg/m$^2$/72 h (dose levels 1–3; Table 2). At dose level 4 (36 mg/m$^2$/72 h), DLTs of febrile neutropenia were seen in two patients (nadir: cycle 1, day 3 + 5, respectively). Both patients recovered after withholding drug lasting 8 and 4 days, respectively. An intermediate dose of 27 mg/m$^2$/72 h was assessed in 18 patients and established as the MTD.

The majority of treatment-related toxic effects were grade 1–2 and included reversible dose-related myelosuppression, gastrointestinal disturbance, fatigue and alopecia (Table 2). Eight patients experienced adverse events resulting in treatment withdrawal, and a further five patients had adverse events that resulted in interruption or delay of treatment. Although five deaths were reported during the course of this study, none of these were related to study drug.

pharmacokinetics

Systemic exposure to AT9283 [$C_{\text{max}}$ (mean 4.66–42.9 ng/ml) and $\text{AUC}_{0-\text{inf}}$ (mean 278–2910 h ng/ml)] was dose proportional and comparable in cycles 1 and 2 of treatment. Both the plasma clearance of AT9283 (cohort mean 5.8–8.11 ml/min/kg) and the half-life of AT9283 in plasma following i.v. infusion were dose independent. The amount of AT9283 excreted unchanged in the urine was 30% and was comparable in both i.v. dosing cycles. Renal clearance rates were generally dose independent and ranged from (cohort mean) 76 to 180 ml/min. (Table 3, Figure 1). Pre-dosing with a single oral dose of AT9283 at 0.9 mg/m$^2$ did not affect exposure in subsequent i.v. infusion cycles. The mean oral bioavailability was 29.4%, ranging from 11.2% to 36.7%.

antitumor activity

No objective tumor responses were observed, however, four patients with oesophageal, colorectal and NSCLC [2] demonstrated stable disease lasting 8.3, 8.2, 6 and 5.6 months, respectively.

biomarker analysis

Pharmacodynamic activity of AT9283 was demonstrated by the modulation of pH3ser10, PCNA, Ki67 and M30/M65 in skin and plasma, respectively. Analysis of these biomarkers indicated antiproliferative activity and an increase in epithelial-derived cellular apoptosis following infusion of AT9283.

pHH3ser10, a substrate of AKB, and p53 which may be stabilized as a direct result of inhibition of AKA or as a consequence of cell cycle arrest, were proposed as direct pharmacodynamic biomarkers for the measurement of AT9283 activity. Both were analyzed directly from paired skin biopsies. Inhibition of pHH3ser10 in skin was observed in the majority of patients in cohorts 1 and 2 (Figure 2) and all patients in cohort 3. Concentrations of AT9283 achieved in these cohorts were between 10 and 40 nM in plasma (colony formation inhibition were achieved with concentrations between 10 and 20 nM in preclinical experiments). The results of patients treated in cohorts 4 and 5, the MTD, were more variable, although the majority of the patients still exhibited target inhibition. In cohorts 1–3, where all patients demonstrated inhibition of pHH3ser10, a concomitant increase in p53 was observed (Figure 2), whereas in cohorts 4 and 5, this relationship was less consistent, Figure 3.
No consistent patterns were observed in changes of either the PCNA (a marker of biochemical effects associated with inhibition of cell cycle progression) or Ki67 expression in the epidermis. An increase in M30 and M65 levels were observed during and following infusion of AT9283 in 16/30 and 17/30 of the patients where these analyses were carried out, respectively. Of all, eight patients displayed an increase in M30 and M65 levels during the infusion of AT9283, with M30 and M65 levels returning to pre-dose levels before subsequent infusions. A further three patients showed an increase of M30 and M65 levels during infusion, and before subsequent infusions, these levels were lower than the pre-dose level. M30 : M65 ratios provided information as to whether tumor cells undergo apoptosis or necrosis, Figure 4.

**discussion**

Increasing preclinical and clinical evidence support the notion of investigating AKis in patients with advanced malignancies. In this FIH study, we demonstrated that AT9283 was well tolerated with a manageable class-specific toxicity profile. The DLT of AT9283 was febrile neutropenia. Clinically relevant disease stabilization was demonstrated in various tumors, including esophageal, NSCLC and colorectal cancer. AT9283 was successfully administered as an orally available liquid to a number of seven patients at MTD to confirm its oral bioavailability.

As part of the biomarker analysis in surrogate tissue (skin samples/plasma), inhibition of aurora kinase activity were observed in skin biopsies even at the lowest dose levels and were consistent with plasma levels and activity observed in in vitro models. The inhibition of Ser10 phosphorylation in histone H3 confirmed findings from earlier preclinical data and from other AKi [14]. However, the magnitude or duration of this inhibitory effect appeared not to be sufficient to induce growth arrest in skin biopsies although this conclusion is based on normal skin biopsies rather than tumor tissue. In addition, since AT9283 has a multitargeted profile of kinase inhibition, it might be possible that at higher doses of AT9283, i.e. cohorts 4 and 5, additional kinase inhibitory activity may have interfered with

### Table 2. Number of patients experiencing hematological and non-hematological toxic effects including dose-limiting toxicities (grade 4 febrile neutropenia and neutropenia, cohort 4)

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Cohort 3</th>
<th>Cohort 4</th>
<th>Cohort 5</th>
<th>Expansion</th>
<th>Oral dose</th>
<th>Total</th>
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<tr>
<td>N = 3</td>
<td>1.5 mg/m²</td>
<td>3 mg/m²</td>
<td>6 mg/m²</td>
<td>9 mg/m²</td>
<td>9 mg/m²</td>
<td>9 mg/m²</td>
<td>0.9 mg/m²</td>
<td>Any</td>
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<tr>
<td>CTC grade</td>
<td>1/2</td>
<td>3/4</td>
<td>1/2</td>
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<td>1</td>
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<td>1</td>
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<td>0</td>
<td>0</td>
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<td>1</td>
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<td>0</td>
<td>0</td>
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<td>GI toxicity</td>
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<td>0</td>
<td>2</td>
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<td>0</td>
<td>0</td>
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<tr>
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<td>0</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Fatigue</td>
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<td>2</td>
<td>3</td>
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<td>3</td>
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<tr>
<td>Proteinuria</td>
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<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>5</td>
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</table>
| CTC, Common Toxicity Criteria; GI, gastrointestinal.

### Table 3. Cohort mean plasma pharmacokinetics parameters of AT9283 at cycle 1

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>No.</th>
<th>Cmax (ng)</th>
<th>t1/2 (h)</th>
<th>AUC0-∞ (h ml/ml)</th>
<th>CI (ml/min/kg)</th>
<th>Vz (l/kg)</th>
<th>CIr (ml/min)</th>
<th>ND</th>
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<tbody>
<tr>
<td>1.5</td>
<td>3</td>
<td>4.7</td>
<td>13.4</td>
<td>278</td>
<td>8.11</td>
<td>7.91</td>
<td>137</td>
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<tr>
<td>3</td>
<td>3</td>
<td>9.6</td>
<td>7.4</td>
<td>571</td>
<td>6.78</td>
<td>4.54</td>
<td>133</td>
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<tr>
<td>6</td>
<td>3</td>
<td>19.1</td>
<td>7.6</td>
<td>1200</td>
<td>6.45</td>
<td>4.10</td>
<td>174</td>
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</tr>
<tr>
<td>9b</td>
<td>7</td>
<td>25.4</td>
<td>7.8</td>
<td>1720b</td>
<td>6.91b</td>
<td>4.85b</td>
<td>180b</td>
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<tr>
<td>12</td>
<td>6</td>
<td>42.9</td>
<td>7.5</td>
<td>2910b</td>
<td>6.47a</td>
<td>3.44a</td>
<td>116a</td>
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<tr>
<td>9c</td>
<td>10</td>
<td>29.5</td>
<td>7.0a</td>
<td>1870</td>
<td>6.75a</td>
<td>3.9a</td>
<td>154</td>
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<tr>
<td>9d</td>
<td>5</td>
<td>33.7</td>
<td>9.18</td>
<td>2010</td>
<td>6.29</td>
<td>4.86</td>
<td>91</td>
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<tr>
<td>0.9 (oral)</td>
<td>7</td>
<td>3.75</td>
<td>7.8a</td>
<td>30.2a</td>
<td>28.8</td>
<td>14.06</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

a A full set of pharmacokinetic parameters was not obtained in all patients from some cohorts.
b Maximum tolerated dose (MTD) escalation cohort.
c MTD cohort expansion.
d 9 mg/m² i.v. + 0.9 mg oral dose group.
ND, not determined for oral dosing; CL, clearance; CLr, renal clearance.
readouts associated with inhibition observed in cohorts 1 to 3. An increase in M30 and M65 levels were observed during and following infusion of AT9283 in almost every patient on study indicating an increase in epithelial-derived cellular apoptosis, most likely occurring within the tumor. At this point, the biomarker analysis does not support a clear dose–response relationship and further biomarker analyses in other studies of AT9283 are ongoing.

To date, several early reports of AKIs have been reported [16–23] and there is emerging evidence that hematological toxic effects, in particular neutropenia, were specific class effects. In terms of clinical response, most reports showed stable disease as best response, with some patients having prolonged disease stabilization. The results of our and other studies raise several questions concerning the future development of AKIs in phase II–III trials including optimal dosing schedules and trial designs, potential combination with chemotherapies or molecular targeted agents and the development of appropriate biomarker.

Classical phase II end points such as response rates might not be appropriate for AKIs where prolonged disease stabilization was the best objective outcome in all reported phase I trials. At least for single-agent drugs where disease stabilization rather than tumor shrinkage can be expected, new concepts such as randomized discontinuation trials or the choice of time related end points, i.e. progression-free survival at 4 months, may be more appropriate end points. Another way of exploring the efficacy of AKIs could be through the design of randomized

Figure 1. Mean plasma concentration of AT9283 following 72-h infusion at 9 mg/m²/day (expansion cohort, cycles 1 and 2).

Figure 2. An example of skin biopsy staining for substrates of AT9283 at a dose level of 6 mg/m².
Interestingly, as demonstrated in the trial by Cohen et al. [17], a dose dense treatment schedule with granulocyte colony stimulating factor support may result in higher levels of drug exposure and subsequently in clinical response rates. Several successful potential drug combinations of AKis and cytotoxic agents have been reported in preclinical models. For example, synergistic effects between AKIs and taxanes were demonstrated in taxane-resistant ovarian, head and neck squamous cell and pancreatic cancer cell lines [24–26]. One of the proposed mechanisms associated with aurora kinase inhibition suggests that aurora kinase overexpression results in increased AKT1 and AKT2 signaling, a known mechanism of both taxane and platinum resistance [27]. Inhibition of aurora kinases could reverse this phenomenon by reconstituting sensitivity to taxanes. Data confirming this mechanism of resensitization have been observed with AKis in combination with etoposide in NSCLC and ovarian cancer and doxorubicin in prostate cancer. Clearly, a combination of AKis and chemotherapies are challenged by the side-effect profile of neutropenia and dosing schedules need to be carefully selected. New biomarkers predicting outcome of treatment with AKis are currently under investigation and one such biomarker is the tumor suppressor gene p53, a regulator of the postmitotic checkpoint. Preclinical studies have demonstrated that p53-deficient tumor cells including pancreatic, colon, NSCLC and oesophageal cancers were more sensitive to AKi compared with wild-type p53 tumor cells. A recent preclinical study of AT9283

**Figure 3.** Immunohistochemistry of PCNA, Ki67, pH3 and p53 pretreatment and post treatment (cohort 1: 1.5 mg/m², cohort 2: 3 mg/m², cohort 3: 6 mg/m², cohort 4: 9 mg/m² and cohort 5:12 mg/m²).

**Figure 4.** Examples of changes observed in M30/M65 profiles as a function of dosing of AT9283 in three selected patients over 72 h at the dose expansion cohort (patient 00107: profiles represent an M30 : M65 ratio of 0.155 at baseline with a maximum fold increase of 1.77, patient 02017: profiles represent an M30 : M65 ratio of 0.139 with a maximum fold increase in the ratio of 3.28 fold and patient 02019: profiles represent an M30 : M65 ratio of 0.383 with a maximum fold increase of 1.133).
confirms these findings showing that checkpoint-competent tumor cells, i.e. normal p53 status, returned to the regular cell cycle once AT9283 administration was withheld, whereas checkpoint-incompetent tumor cells, i.e. p53 deficient, underwent endoreduplication and apoptosis. Therefore, strategies where patients are selected based on their p53 functional status could potentially help in identifying patients most likely to benefit from therapy with drugs such as AT9283 [14].

In conclusion, AT9283 was well tolerated with febrile neutropenia being the DLT. Several trials of AT9283 are currently under way in solid and hematological malignancies (NCT01145989, NCT00985868 and NCT00522990) and future trials focusing on the optimal administration schedules, preferred combination partners and the development of adequate biomarker in selected tumor types are planned.

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disclosure

HTA, RP, LRM, DO, TAY, HC and IJ have no conflict of interest. MS, SL, VL, MY and JL are employees of Astex Therapeutics Ltd.

references