A Phase I Trial of AT9283 (a Selective Inhibitor of Aurora Kinases) in Children and Adolescents with Solid Tumors: A Cancer Research UK Study

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Abstract

Purpose: A phase I trial of AT9283 (a multitargeted inhibitor of Aurora kinases A and B) was conducted in children and adolescents with solid tumors, to identify maximum-tolerated dose (MTD), safety, efficacy, pharmacokinetics, and pharmacodynamic (PD) activity.

Experimental Design: AT9283 was administered as a 72-hour continuous intravenous infusion every 3 weeks. A rolling-six design, explored six dose levels (7, 9, 11.5, 14.5, 18.5, and 23 mg/m2/d). Pharmacokinetic and PD assessments, included inhibition of phospho-histone 3 (pHH3) in paired skin punch biopsies.

Results: Thirty-three patients were evaluable for toxicity. There were six dose-limiting toxicities and the MTD was 18.5 mg/m2/d. Most common drug-related toxicities were hematologic (neutropenia, anemia, and thrombocytopenia in 36.4%, 18.2%, and 21.2% of patients), which were grade ≥3 in 30.3%, 6.1%, and 3% of patients. Nonhematologic toxicities included fatigue, febrile neutropenia and ALT elevation. One patient with central nervous system–primitive neuroectodermal tumor (CNS-PNET) achieved a partial response after 16 cycles and 3 cases were stable for four or more cycles. Plasma concentrations were comparable with those in adults at the same dose level, clearance was similar although half-life was shorter (4.9 ± 1.5 hours, compared with 8.4 ± 3.7 hours in adults). Inhibition of Aurora kinase B was shown by reduction in pHH3 in 17 of 18 patients treated at ≥11.5 mg/m2/d.

Conclusion: AT9283 was well tolerated in children and adolescents with solid tumors with manageable hematologic toxicity. Target inhibition was demonstrated. Disease stabilization was documented in intracranial and extracranial pediatric solid tumors and a phase II dose determined. Clin Cancer Res; 21(2); 267–73. ©2014 AACR.

Introduction

Cancer is the commonest cause of death in children above 1 year of age (1), and there is an urgent need to develop new therapies to improve survival and reduce the burden of long-term toxicities.

Aurora kinases are a family of enzymes that are key regulators of mitosis. They comprise Aurora A (involved in centrosome separation and maturation and bipolar spindle assembly) and Aurora B (“chromosome passenger protein”; mediating chromosome segregation and cytokinesis and phosphorylation of a number of targets, including histone H3), which have been shown to act as oncogenic drivers in a number of human cancers (2, 3).

The preclinical rationale supporting the clinical development of Aurora kinase inhibitors in children with solid tumors is particularly strong because: (i) the target is dysregulated in a number of high-risk malignancies such as neuroblastoma, medulloblastoma, central nervous system primitive neuroectodermal tumor (CNS-PNET), and malignant glioma; (ii) there is a mechanistic justification of its indispensable role in MYC/MYCN-driven cancers such as neuroblastoma; (iii) there are in vitro and in vivo efficacy data, including in genetically engineered murine models; (iv) there are several drugs under development; and (v) pharmacodynamic (PD) biomarkers are available to demonstrate target inhibition in patients (4–10). AT9283 is a multitargeted inhibitor against Aurora A and B, JAK2 and ABL kinases, and has been tested in phase I/II trials in adults with solid and hematologic cancers (11–15).

This first-in-child phase I study of AT9283 in relapsed/refractory solid tumors was designed to incorporate PD and
Translational Relevance

Aurora kinases have been shown to be highly relevant targets for several high-risk pediatric solid tumors, such as neuroblastoma in which they play a critical role in stabilization of MYCN. We here report the first-in-child phase I trial of AT9283, the first dual inhibitor of Aurora A and B kinases tested in pediatrics. Pharmacodynamic (PD) biomarkers are rarely performed in pediatric trials but they are pivotal for successful drug development. In this trial, paired skin biopsies demonstrated inhibition of Aurora kinase B in the majority of patients treated above the 9 mg/m2/d dose level, hence providing proof-of-principle that PD biomarkers can be incorporated to pediatric phase I trials without causing significant risks to the patients.

Materials and Methods

Patient eligibility

Patients were included according to the following criteria: age >2 and <19 years, performance status Lansky ≥70% for those aged 1 to 12 years (>50% for children with CNS tumors and stable neurologic deficits) or WHO 0, 1, or 2 for those aged >12 years, life expectancy of at least 12 weeks, histologically proven solid tumor refractory to conventional treatment (relapsed/progressive typical diffuse pontine glioma allowed without histologic verification), adequate bone marrow function (Hemoglobin ≥ 9 g/dL, absolute neutrophil count ≥1,000/µL, and platelet count ≥100,000/µL unsupported) and biochemistry (creatinine kinase normal, ALT/AST <1.5 upper limit of normal, measured glomerular filtration rate [GFR] ≥60 mL/min/1.73 m2) and written informed consent.

Exclusion criteria were: radiotherapy, endocrine therapy, or chemotherapy within the previous 4 weeks, patients with CNS tumors on an unstable or increasing dose of corticosteroids, prior exposure to an Aurora kinase inhibitor, unrecovered toxicity from prior therapies, pregnant or lactating women, unrecovered major thoracic or abdominal surgery, high risk due to nonmalignant systemic disease, decreased cardiac function (shortening fraction ≤29% or left ventricular ejection fraction ≤50%), congenital heart disease or uncontrolled hypertension, autologous stem cell transplant within the previous 3 months or any previous allogeneic transplant. Patients of childbearing or child fathering potential had to agree to use a medically acceptable form of birth control, including abstinence, while on this study.

Informed consent was obtained from parents or guardians, and assent was obtained as appropriate at the time of study enrolment. The institutional review boards of each institution approved the study. The trial was sponsored by the Drug Development Office of Cancer Research UK, study number CR0708-11, EudraCT 2008-005542-23.

Study design

CR0708-11 was a multicentre, open-label, nonrandomized, dose-escalation pediatric phase I study. The primary objective was to evaluate the safety and tolerability of AT9283 by characterizing dose-limiting toxicities (DLT) and determining maximum-tolerated dose (MTD) in children and adolescents with relapsed and refractory solid tumors. Secondary objectives were to determine the pharmacokinetic profile, PD activity, and to assess preliminary evidence of activity of intravenous AT9283. The study used the rolling six design (17): 3 to 6 patients were enrolled at each dose level for the determination of the MTD. Dose was only escalated when ≤1 DLT was observed per cohort.

Adverse events (AE) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. DLTs were defined as almost certainly or probably drug-related grade ≥3 nonhematologic toxicity (excluding grade 3 nausea, vomiting, or diarrhea receiving suboptimal treatment), grade 3 fever without grade 3–4 neutropenia or reversible grade 3 transaminase elevations), grade 4 neutropenia lasting >7 days, grade 3 thrombocytopenia lasting >7 days, or any grade 4 thrombocytopenia. The DLT evaluation period was 21 days (one cycle).

Dose escalation, drug schedule, and assessments

First dose level was 7 mg/m2/d for 3 days, 80% of the adult solid phase I trial MTD (15). For each subsequent dose level, the dose was increased by up to a maximum of 30%. Body surface area was calculated according to the Mosteller formula.

AT9283 was supplied as Astex as a lyophilized solid for reconstitution in 20 mL vials that were stored at 15°C to 25°C. Vials were reconstituted to a total volume of 100 mL in 5% dextrose. AT9283 was administered i.v. continuously over 72 hours as three separate 24-hour infusions on days 1 to 3 of a 21-day cycle. Treatment was scheduled for 6 cycles, although extension could be considered for patients with stable or responding disease in which the benefit–risk balance was acceptable.

At screening, history, developmental status, performance status, physical examination, full blood count, biochemistry, urinalysis, electrocardiogram, echocardiogram, measured GFR, pregnancy test, and baseline imaging were obtained. Patients had clinic visits, physical examination, full blood count, and biochemistry weekly throughout the study treatment. Urinalysis, echocardiograms, and response assessments were performed every 2 cycles. An off study visit was performed 28 days after the last dose of AT9283. Disease evaluations included cross-sectional imaging according to RECIST 1.0 (18) and to the International Neuroblastoma Response Criteria (INRC; ref. 19) for those patients with neuroblastoma. Complete or partial responses (CR or PR) had to be confirmed by a repeat scan no less than 4 weeks after the criteria for response were first met. All stable disease (SD) responses had to meet SD criteria at least once and at least 6 weeks after study treatment was initiated.

Pharmacokinetic analysis

Blood samples were collected during screening and 4, 24, 48, 70, 73, 76, and 96 hours after the start of the infusion of cycle 1. Plasma was separated, immediately frozen at –20°C and then analyzed to determine the concentration of AT9283 using a previously developed the LC/MS method. Pharmacokinetic data were analyzed by noncompartmental methods using WinNonlin Version 5.3. Area under the plasma concentration–time curve (AUC0-t), maximum concentration achieved (Cmax), time to maximum concentration (Tmax), and elimination half-life (T1/2) for AT9283 were calculated.
Pharmacodynamic studies

**Immunohistochemical markers.** Skin biopsies for PD analysis by immunohistochemistry were collected at baseline and at 72 hours, in cycle 1. The 72-hour time point was chosen based on preclinical data. Sections (3–4 μm) from paraffin-embedded specimens were mounted on poly-L-lysine-coated glass slides. After rinsing with three changes of xylool for deparaffinization, the sections were incubated for 5 minutes in 3% H_{2}O_{2}, and then were rinsed with PBS. Specimens were incubated for 1 hour with the lyophilized monoclonal anti–phospho-histone 3 (pHH3; Cell Signalling Technology) at a dilution of 1:100; Ki67 (Zymed) at a 1:100 dilution and p53 (DAKO) at a 1:50 dilution. Immune complexes were subsequently treated with post primary block and then detected by the SuperPicTure Polymer Detection Kit (Invitrogen). Proliferating cell nuclear antigen detection was performed following the kit instructions (Invitrogen). Positive controls consisted of tissue specimen sections of breast carcinoma with known antigenic reactivity. A negative control was stained by omitting the primary antibody. The prepared specimens were stained with hematoxylin (Sigma), mounted, and evaluated using AnalySIS Software—Imaging Software (license no. A1534700). Images were captured under ×20 magnification, using the "touch count" function to count positively stained cells in the epidermal layer of the skin section.

**M30-M65 ELISA in plasma.** Blood samples for PD analysis were collected at time 0, 22, 46, 70, and 168 hours, following the start of the infusion, in cycles 1. The M30 apoptosense and M65 ELISA kits were both obtained from PEVIVA AB (Bromma), and these assays, previously validated, were performed under dedicated Good Clinical Laboratory Practice conditions. Background variation for M30 and M65 antigens was considered ±30% of the antigen level seen at the start of each treatment cycle as discussed previously. Caspase-cleaved CK18 (M30) is released from apoptotic cells, whereas total CK18 (M65) is released by epithelial cells undergoing cell death by any cause (e.g., necrosis). Any peaks or troughs seen in patient antigen levels falling outside this range were considered a direct result of treatment with the study drug: either tumor response or toxicity.

**Results**

**Patient characteristics**

Thirty-three patients were enrolled from October 2009 until December 2012. Twenty-two of the 33 patients (66.7%) were female. The median age of patients was 9 years (range, 3–18 years). Patient characteristics are provided in Table 1. All 33 patients enrolled received at least one administration of AT9283. Six-dose levels were explored: 7, 9, 11.5, 14.5, 18.5, and 23 mg/m²/d, and 103 cycles were delivered. Figure 1 depicts recruitment at all six-dose levels. Dose was escalated until it reached 23 mg/m²/d where the first 2 patients experienced a DLT. The last 2 patients were already in the screening period when the two DLTs were encountered: The first one received the dose below (18.5 mg/m²/d) and developed a DLT, so the final patient was dosed at the subsequent lower dose (14.5 mg/m²/d).

**Toxicities**

Six DLTs were observed: grade 4 neutropenia lasting ≥7 days in 3 patients (14.5, 18, and 23 mg/m²/d dose levels), grade 3 febrile neutropenia in 2 children (11.5 and 23 mg/m²/d) and grade 3 suspected bacterial infection in one case (18.5 mg/m²/d). All patients who experienced DLT recovered from them sufficiently to permit their continued treatment with AT9283 at a reduced dose level. The maximum administered dose of AT9283 was 23 mg/m²/d and the MTD was established at 18.5 mg/m²/d for pediatric solid tumors.

All 33 patients enrolled in the study were evaluated for safety. Thirty-two of the 33 patients (97.0%) who received treatment with AT9283 during the study presented with at least one treatment emergent AE, and 24 of 33 patients (72.7%) presented with at least one AE that was considered by the investigator to be related to AT9283. Twenty patients (60.6%) experienced at least one serious AE (SAE), 2 of whom had to withdraw from the study as an outcome of the SAE. Twelve patients (36.4%) had at least one CTCAE grade 4 or 5 AE, and 8 patients (24.2%) died during the study, two of unrelated SAEs (neurologic impairment and raised intracranial pressure) and 6 of disease progression. Drug-related toxicities are represented in Table 2. Most common related hematologic toxicities were neutropenia in 25 episodes per 12 patients, which were grade 3 to 4 in 18 episodes per 10 patients. Febrile neutropenia occurred in 5 episodes per 4 patients. Most common nonhematologic toxicities were fatigue (5 episodes/5 patients), rash (5 episodes/3 patients), vomiting (3 episodes/3 patients), ALT elevation (5 episodes/4 patients), and fever (3 episodes/3 patients).

**Antitumor activity**

A median of 2 cycles was administered (range, 1–30). Twenty-three patients were evaluable for response. Twenty-one patients had measurable disease according to RECIST v1.0. One patient with CNS-PNET experienced a confirmed PR according to RECIST v1.0 after 16 cycles of AT9283. At the time of this report, the patient has had 30 cycles and the response is sustained. Nine other patients (37.5% of patients evaluable for response) had stabilization of their disease after two courses, with three of these achieving SD for four or more cycles of AT9283 [1 patient with ependymoma (4 cycles), 1 with CNS-PNET (6 cycles), and 1 with alveolar soft part sarcoma (4 cycles)]. The 3 patients with neuroblastoma were also assessed with the International Neuroblastoma Response Criteria (19): 1 patient with MYCN-amplified disease experienced progressive disease, and 2 patients with MYCN-nonamplified disease achieved a mixed response and no response.

**Pharmacokinetics**

Plasma samples were taken for all 33 patients treated with AT9283 and were evaluable in 32 patients (97%). AT9283 plasma pharmacokinetic parameters are summarized in Table 3. Beyond the first dose level C_{max} and AUC seemed to plateau and a consistent relationship between plasma concentrations and dose was not observed. In pediatric patients, plasma concentrations were comparable with those seen in adults at the same dose level, clearance was similar although half-life was shorter (4.9 ± 1.5 hours, compared with 8.4 ± 3.7 hours in adults) as shown in Table 3. Volume of distribution in pediatric patients was similar to adult cases. Clearance was not predicted by body surface area. High intrapatient variability was observed (coefficient of variation in AUC>50% at 9 and 11.5 mg/m²/d dose levels). Figure 2
represents the AUC in the different dose levels. For comparison, values for AUC in the adult phase I were 1,730/C6 550 ng/mL at 9 mg/m2/d.

Pharmacodynamic biomarkers
PD activity of AT9283 was confirmed by inhibition of pHH3 Ser10 in skin in 6 of 7 patients treated at 14.5 mg/m2/d and all 12 patients treated at 11.5, 18, and 23 mg/m2/d. Figure 3 and Supplementary Material summarize immunohistochemical findings in skin biopsies. The results for the proliferation marker Ki67 in patients treated at 14.5 mg/m2/d and below were variable, although the majority of the patients still exhibited target inhibition measured by pHH3 inhibition. In those patients treated at 18.5 and 23 mg/m2/d all patients showed reduction in Ki67-positive cells, indicating an antiproliferative effect of AT9283 at high doses. p53 may be stabilized as a direct result of inhibition of Aurora kinase A or as a consequence of cell-cycle arrest. In skin biopsies, as surrogate for tumor tissue, results on p53-positive cells showed high variability and no direct dose-dependent effect was observed.

Of a total of 16 patients evaluated, 11 displayed an increase in M30 levels during the infusion of AT9283, the maximum increase was detected at different time points and it was not dose dependent. In most of the cases, M30 levels returned to predose levels before subsequent infusions. A further 5 patients showed no increase of M30 levels during infusion. M65 levels remained constant in most of the patients analyzed (Supplementary Figure). M30:M65 data do not support a clear dose–response relationship.

Discussion
This phase I is the first of a dual Aurora A/B kinase inhibitor in childhood cancer. AT9283 is a multitargeted inhibitor against Aurora A and B, JAK2 and ABL kinases and in this pediatric phase I study was well tolerated in a heavily pretreated population of children ages 3 to 18 years with expected DLTs of febrile neutropenia or grade 4 neutropenia. The toxicity profile was similar, but the MTD was significantly higher in the pediatric study (18.5 mg/m2/d) compared with the adult (9 mg/m2/d) solid tumor study using the same dosing schedule (15). Pharmacokinetic analysis revealed that plasma concentrations and clearance were similar to those in adults, with a shorter half-life and similar volume of distribution. AT9283 was given as a 72-hour infusion every 3 days.
weeks, which required an inpatient stay for these young patients, and to try and impact less on the child's quality of life in the future, portable continuous infusion pumps could be considered. Although oral Aurora kinase inhibitors have been developed, pediatric-friendly formulations in appropriate doses are not always available, and intravenous preparation and regimens may still play a role in young children.

This study included mandatory PD analyses pre- and post-study drug exposure that had been investigated in prior preclinical and adult clinical studies (11–13, 15, 20). These included skin punch biopsy samples as a surrogate tissue, and even with a pediatric population this PD analysis was successfully completed and demonstrated inhibition of Ser10 phosphorylation in histone H3 and inhibition of Aurora kinase activity even at the lowest

<table>
<thead>
<tr>
<th>Dose level, mg/m²/d</th>
<th>Number of patients</th>
<th>Cmax (ng/mL)</th>
<th>AUC0→t (ng/mL h)</th>
<th>Half-life (h)</th>
<th>CL (L/h)</th>
<th>Vss (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>5</td>
<td>7.0–19.0</td>
<td>639 ± 383</td>
<td>5.7 ± 0.4</td>
<td>39.7 ± 25.6</td>
<td>328 ± 180</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>14.7–68.2</td>
<td>1,817 ± 1,246</td>
<td>4.9 ± 1.5</td>
<td>20.4 ± 7.7</td>
<td>158 ± 74</td>
</tr>
<tr>
<td>11.5</td>
<td>6</td>
<td>14.5–68.8</td>
<td>2,102 ± 1,074</td>
<td>5.1 ± 1.2</td>
<td>22.3 ± 15.1</td>
<td>157 ± 122</td>
</tr>
<tr>
<td>14.5</td>
<td>7</td>
<td>14.8–60.6</td>
<td>2,267 ± 938</td>
<td>5.2 ± 2.0</td>
<td>21.0 ± 6.8</td>
<td>114 ± 72</td>
</tr>
<tr>
<td>18.5</td>
<td>7</td>
<td>17.3–80.9</td>
<td>1,946 ± 799</td>
<td>5.6 ± 1.5</td>
<td>37.8 ± 16.4</td>
<td>224 ± 79</td>
</tr>
<tr>
<td>23</td>
<td>2</td>
<td>631–89.0</td>
<td>3,436–5,300</td>
<td>4.0–18.7</td>
<td>20.9–19.6</td>
<td>256–363</td>
</tr>
</tbody>
</table>

Abbreviations: CL, drug clearance; Vss, volume of distribution at steady state.

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### Table 2. AEs occurring in ≥10% of subjects during the first six cycles of treatment with AT9283

<table>
<thead>
<tr>
<th>AE body system</th>
<th>All AEs, grades 1–5</th>
<th>Related AEs, grades 1–5</th>
<th>Grade 3/4/5 related AEs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood/bone marrow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>27 (12–36.4%)</td>
<td>25 (12–36.4%)</td>
<td>18 (10–30.3%)</td>
</tr>
<tr>
<td>Leucocytes</td>
<td>22 (8–24.2%)</td>
<td>19 (7–21.2%)</td>
<td>7 (4–12.1%)</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>21 (8–24.2%)</td>
<td>17 (5–15.2%)</td>
<td>7 (3–9.1%)</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>15 (9–27.3%)</td>
<td>9 (6–18.2%)</td>
<td>3 (2–6.1%)</td>
</tr>
<tr>
<td>Platelets</td>
<td>13 (9–27.3%)</td>
<td>10 (7–21.2%)</td>
<td>1 (3–1.0%)</td>
</tr>
<tr>
<td>Constitutional symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>13 (12–36.4%)</td>
<td>5 (5–15.2%)</td>
<td>1 (3–0.0%)</td>
</tr>
<tr>
<td>Fever</td>
<td>11 (8–24.2%)</td>
<td>3 (5–9.1%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Dermatology/skin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td>9–7 (21.2%)</td>
<td>5 (3–9.1%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>4 (4–12.1%)</td>
<td>3 (3–9.1%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>26 (15–45.5%)</td>
<td>3 (5–9.1%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Constipation</td>
<td>10 (9–27.3%)</td>
<td>2 (1–3.0%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>7 (7–21.2%)</td>
<td>1 (3–0.0%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>7 (7–21.2%)</td>
<td>3 (5–9.1%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infection—other</td>
<td></td>
<td>6 (4–12.1%)</td>
<td>5 (3–9.1%)</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td></td>
<td>5 (4–12.1%)</td>
<td>5 (4–12.1%)</td>
</tr>
<tr>
<td>Metabolic/laboratory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>8 (5–15.2%)</td>
<td>5 (4–12.1%)</td>
<td>2 (2–6.1%)</td>
</tr>
<tr>
<td>Hypokalemia</td>
<td>8 (4–12.1%)</td>
<td>7 (3–9.1%)</td>
<td>1 (3–0.0%)</td>
</tr>
<tr>
<td>Hypophosphatemia</td>
<td></td>
<td>2 (2–6.3%)</td>
<td>1 (3–0.0%)</td>
</tr>
<tr>
<td>Metabolic—other</td>
<td></td>
<td>0 (0–0.0%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Neurology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Somnolence</td>
<td>9 (7–21.2%)</td>
<td>0 (0–0.0%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Seizure</td>
<td>9 (6–18.2%)</td>
<td>0 (0–0.0%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Ataxia</td>
<td>6 (6–18.2%)</td>
<td>0 (0–0.0%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>6 (6–18.2%)</td>
<td>0 (0–0.0%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Neurology—other</td>
<td></td>
<td>0 (0–0.0%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Pain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain—Head/headache</td>
<td></td>
<td>1 (3–0.0%)</td>
<td>1 (3–0.0%)</td>
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<tr>
<td>Pain—abdomen NOS</td>
<td></td>
<td>1 (3–0.0%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Pain—extremity- limb</td>
<td></td>
<td>0 (0–0.0%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Pain—joint</td>
<td>9 (8–24.2%)</td>
<td>0 (0–0.0%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Pain—back</td>
<td>5 (4–12.1%)</td>
<td>0 (0–0.0%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Pain—other (specify)</td>
<td></td>
<td>0 (0–0.0%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Pulmonary/upper respiratory</td>
<td></td>
<td>0 (0–0.0%)</td>
<td>0 (0–0.0%)</td>
</tr>
<tr>
<td>Pulmonary—other</td>
<td>4 (4–12.1%)</td>
<td>0 (0–0.0%)</td>
<td>0 (0–0.0%)</td>
</tr>
</tbody>
</table>

NOTE: AE terms only given for events occurring in ≥10% of patients. Abbreviations: ALT, Alanine-aminotransferase; NOS, not otherwise specified.

*Patient #25 has received 30 cycles of AT9283. Beyond cycle 6, the following AT9283-related toxicities have occurred: Grade 2 leukopenia, grade 2 lymphopenia, grade 2 neutropenia, grade 1 hypokalemia, all of which resolved. The patient developed ovarian failure believed to be related to cumulative exposure to AT9283 in the context of a patient that received irradiation to the hypothalamic–pituitary axis.
dose levels, consistent with plasma levels and activity observed in 
in vitro models. Neither the Ki67 assay in skin nor the analysis of 
M30:M65 levels in plasma were informative in this study and 
probably reflect the differences between childhood and adult 
cancers. In the adult population, epithelial cancers predominate 
whereas pediatric malignancies are mainly of mesenchymal ori-
gin, and thus do not express CK18, the cleavage of which is the 
basis of the M30:M65 assay. This illustrates that the choice of 
biomarkers is not only related to the mechanism of the study drug, 
but should include consideration of the tumor context and age of 
the patient. At the time of the design of the study there were no 
validated predictive tumor biomarkers for Aurora kinase inhibi-
tors, and therefore analysis of tumor tissue was not included. This 
was a weakness of the study and several putative biomarkers have 
emerged, which would have been of interest to study in terms of 
interrogating responders versus nonresponders. Possible explor-
atory biomarkers include; disturbance of cell-cycle checkpoint 
function, for example, p53 deficiency, or uncontrolled cell-cycle 
entry, that is, loss of pRB or MYC overexpression (3). In preclinical 
studies, AT9283 induced a cell-cycle checkpoint in cells with wild-
type p53 status, returning to the regular cell cycle once AT9283 
administration was withheld, whereas checkpoint-incompetent 
tumor cells (i.e., p53 deficient), underwent endoreduplication 
and apoptosis (20).

A third of evaluable patients had initial stabilization of their 
disease (SD) after two cycles with 3 patients maintaining SD 
beyond four cycles. Another patient with a CNS-PNET experi-
cenced a confirmed PR according to RECIST v1.0 after 16 cycles of 
AT9283 and at the time of the report has had 30 cycles with a 
sustained response. This and a maintained SD in 2 other patients 
with brain tumor indicate that AT9283 does reach sufficient levels 
in the CNS to have an effect. These results are similar to the only 
other reported pediatric Aurora kinase inhibitor study, in which 
there was 1 PR and 6 SD of 23 patients with measurable disease 
treated with the Aurora A selective inhibitor MLN8237 (21).

Aurora kinase inhibitors have so far failed to make a major 
impact in adult solid tumors with more promising activity being 
seen in hematologic malignancies (lymphoma and leukemia; 
refs. 22, 23). Pediatric results indicate that Aurora kinase inhibi-
tion is achieved with tolerable doses of AT9283, but as in the adult 
studies this has not translated into objective responses in the 
majority of cases. Better patient selection with biomarker enrich-
ment based on p53, pRB, and MYC status along with possible 
combination strategies such as other antimitotic inhibitors or 
signal transduction inhibitors would be valuable strategies for 
future studies.

This pediatric phase I study of AT9283 demonstrated significant 
Aurora kinase inhibition at tolerable doses with disease stabiliza-
tion demonstrated in a variety of childhood solid tumors. Future 
studies will focus on hematologic malignancies and possible 
combination studies in solid tumors.

Figure 2.
Summary of pharmacokinetics: AUC versus dose of AT9283.

AUC ng/ml×h

-6,000
-5,000
-4,000
-3,000
-2,000
-1,000
0
Dose (mg/m²/day)

0 5 10 15 20 25

Figure 3.
PD modulation of pHH3, a substrate 
for Aurora kinase B in paired skin 
punch biopsies. Skin punch biopsies 
were optional until a DLT was found 
(11.5 mg/m²/d) when they were 
mandated. A, inhibition of pHH3 
(substrate for Aurora kinase B) can be 
observed in the majority of patients 
from the 11.5 mg/m²/d dose level 
(P < 0.005). B and C, an example of 
skin biopsy from a patient treated 
at the 18 mg/m²/d dose level staining 
for pHH3 pre- (B) and post- 
treatment with AT9283. The arrow, a 
positive cell stained for pHH3.
Disclosure of Potential Conflicts of Interest

L. Moreno is a consultant/advisory board member for AstraZeneca, Novartis, and Roche/Genentech. J.F. Lyons is an employee of Astex Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

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