

# A phase I/II trial of AT9283, a selective inhibitor of aurora kinase in children with relapsed or refractory acute leukemia: challenges to run early phase clinical trials for children with leukemia

B. Vormoor<sup>1,2</sup> | G. J. Veal<sup>1</sup> | M. J. Griffin<sup>1</sup> | A. V. Boddy<sup>1\*</sup> | J. Irving<sup>1</sup> | L. Minto<sup>1</sup> | M. Case<sup>1</sup> | U. Banerji<sup>3,4</sup> | K. E. Swales<sup>3</sup> | J. R. Tall<sup>3</sup> | A. S. Moore<sup>3,4†</sup> | M. Toguchi<sup>5</sup> | G. Acton<sup>6</sup> | K. Dyer<sup>6</sup> | C. Schwab<sup>1</sup> | C. J. Harrison<sup>1</sup> | J. D. Grainger<sup>7,8</sup> | D. Lancaster<sup>4</sup> | P. Kearns<sup>9</sup> | D. Hargrave<sup>10</sup> | J. Vormoor<sup>1,2</sup>

<sup>1</sup>Newcastle Cancer Centre at the Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, UK

<sup>2</sup>Great North Children's Hospital, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK

<sup>3</sup>Cancer Research UK Cancer Therapeutics Unit, The Institute of Cancer Research, London, U.K.

<sup>4</sup>The Royal Marsden Hospital, Sutton, UK

<sup>5</sup>Astex Pharmaceuticals, Cambridge Science Park, Cambridge, UK

<sup>6</sup>Cancer Research UK Centre for Drug Development, London, UK

<sup>7</sup>Royal Manchester Children's Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK

<sup>8</sup>Faculty of Medical & Human Sciences, University of Manchester, Manchester, UK

<sup>9</sup>Institute of Cancer and Genomic Medicine, University of Birmingham, Birmingham, UK

<sup>10</sup>Great Ormond Street Hospital for Children, London, UK

## Correspondence

Britta Vormoor, Newcastle Cancer Centre at the Northern Institute for Cancer Research, Newcastle University, Paul O'Gorman Building, Framlington Place, Newcastle upon Tyne NE2 4HH, UK.

Email: britta.vormoor@ncl.ac.uk

\* Current address: Faculty of Pharmacy, University of Sydney, Australia.

† Current address: The University of Queensland Diamantina Institute, The University of Queensland, Brisbane, Australia.

## Abstract

Aurora kinases regulate mitosis and are commonly overexpressed in leukemia. This phase I/IIa study of AT9283, a multikinase inhibitor, was designed to identify maximal tolerated doses, safety, pharmacokinetics, and pharmacodynamic activity in children with relapsed/refractory acute leukemia. The trial suffered from poor recruitment and terminated early, therefore failing to identify its primary endpoints. AT9283 caused tolerable toxicity, but failed to show clinical responses. Future trials should be based on robust preclinical data that provide an indication of which patients may benefit from the experimental agent, and recruitment should be improved through international collaborations and early combination with established treatment strategies.

## KEYWORDS

aurora kinase, AT9283, leukemia, pediatric, phase I/II trial

## 1 | INTRODUCTION

Leukemia is the most common malignancy in childhood. Despite treatment advances, the outcome of relapsed leukemia remains poor, with

5-year disease-free survival rates for relapsed acute lymphoblastic leukemia (ALL) of 16%–39%,<sup>1</sup> and relapsed acute myeloid leukemia (AML) of 16%–34%.<sup>2</sup> New treatment strategies are needed to improve survival and reduce the toxicity of conventional chemotherapy.

Aurora kinases are a family of serine/threonine kinases involved in mitosis and meiosis. The Aurora kinases are overexpressed in many cancers, including leukemias,<sup>3</sup> and overexpression has been linked to genetic instability.

Abbreviations: ABL, Abelson tyrosine kinase; AE, adverse event; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; AUC<sub>0-t</sub>, area under the plasma concentration time curve; C<sub>max</sub>, maximum concentration; CDD, Centre for Drug Development; CNS, central nervous system; CR-UK, Cancer Research UK; DLT, dose limiting toxicity; FLT3, FMS-like tyrosine kinase 3; JAK, Janus kinase; MTD, maximum tolerated dose; PD, pharmacodynamic; pHH3, phosphohistone H3; PK, pharmacokinetic; SAE, serious adverse event

AT9283 is a multikinase inhibitor affecting not only Aurora A and B, but also FMS-like tyrosine kinase 3 (FLT3), the Janus kinase (JAK) family, and Abelson tyrosine kinase (ABL).<sup>4</sup> All of these are activated in ALL and AML; FLT3 is commonly activated by mutation and ABL by chromosomal translocations.<sup>5-9</sup> A plethora of early phase clinical trials are evaluating small molecule inhibitors against these kinases (reviewed in<sup>10,11</sup>). Preclinical data have shown that inhibition of Aurora A, B and FLT3 kinase by compound 27e led to growth inhibition in an FLT3-ITD-positive AML xenograft.<sup>12</sup> AT9283 has shown *in vitro* activity in cell line models for myeloproliferative disorders with gain of function mutations in JAK2<sup>13</sup> and in adult phase I/II trials for solid tumors and hematological malignancies.<sup>14,15</sup> In adult AML, approximately 33% of patients experienced a reduction in bone marrow blasts.<sup>15</sup> The first-in-child phase I trial with AT9283 in solid tumors leads to a partial response in one patient (diagnosis: central nervous system [CNS]-Primitive neuroectodermal tumour (PNET)) and disease stabilization in 38% of patients, with manageable hematological toxicity.<sup>16</sup> The current study is a first-in-child trial of AT9283 for relapsed/refractory leukemia. The aims of the study were to identify the dose for exploration of AT9283 in a phase II trial (primary objective), to evaluate safety and tolerability of AT9283 by identifying dose-limiting toxicities (DLTs), and to document preliminary activity and investigate its pharmacokinetic (PK) profile (secondary objectives). Tertiary objectives were to assess target inhibition by AT9283 with a validated biomarker assay<sup>17</sup> and identify potential predictive biomarkers.

## 2 | RESULTS

### 2.1 | Study design, eligibility, and dose escalation/drug schedule

Details regarding study design, eligibility criteria, definition of adverse events (AEs)/DLTs, and information on dose escalation, drug schedule, and assessments are summarized in Supplementary Appendix S1. Written and signed informed consent was obtained from all parents/guardians. The trial was sponsored by Cancer Research UK (CR-UK) Centre for Drug Development (CDD) (study number CR0708-12, EudraCT number 2009-016952-36, ClinicalTrials.gov identifier: NCT01431664), and was conducted in accordance with the principles of Good Clinical Practice and CR-UK CDD's Standard Operating Procedures.

## 3 | METHODS

PK analysis was identical to that described previously.<sup>16</sup>

The plasma inhibitory activity assay was used to measure *ex vivo* target kinase inhibition and had been validated for inhibition of Aurora, ABL, and FLT3 kinase.<sup>17</sup>

Immunohistochemistry was used on surrogate tissue (skin punch biopsies) to assess *in vivo* target kinase inhibition of Aurora kinase (change in phosphohistone H3 [pHH3] levels), and changes in p53

(accumulation/stabilization) and Ki67/proliferating cell nuclear antigen levels as biomarkers of antiproliferative responses.<sup>16</sup>

Other pharmacodynamic (PD) measurements included *in vivo* inhibition of JAK-signal transducer and activator of transcription signaling by flow cytometry, genetic screen for mutations in JAK1, 2, 3 and FLT3, and copy number abnormalities of IKZF1 and PAX5 by Multiplex Ligation-dependent Probe Amplification. For details, see Supplementary Appendix S1.

### 3.1 | Patient characteristics

Ten patients underwent screening, seven of whom were eligible for inclusion into the trial. Recruitment started in September 2011 and the first patient was treated in April 2012. Of the seven patients, five (71%) were male and two (29%) were female, with a median age of 3 years (range, 1-18 years). Four patients were diagnosed with relapsed/refractory AML and three patients had relapsed ALL.

Three dose levels of AT9283 were explored: three patients each at 9 and 14.5 mg/m<sup>2</sup>/day, and a further patient at 23 mg/m<sup>2</sup>/day. Each patient received one cycle of treatment. All patients withdrew from the trial due to disease progression during or at the end of cycle 1. The trial closed prematurely in July 2014 due to poor patient recruitment.

### 3.2 | Toxicities

All patients were evaluated for safety. A total of 97 AEs were reported, of which 29 were considered to be related (possible, probable, or highly probable) to the administration of AT9283 (Supplementary Table S1). Five patients experienced a total of 17 serious adverse events (SAEs), of which 13 were considered to be at least possibly related to AT9283. None of the SAEs observed were categorized as a DLT (Supplementary Table S2). There was one patient death during the trial due to disease progression, 22 days after the last administration of AT9283. No maximum tolerated dose (MTD) could be determined in this study.

### 3.3 | Early response signals

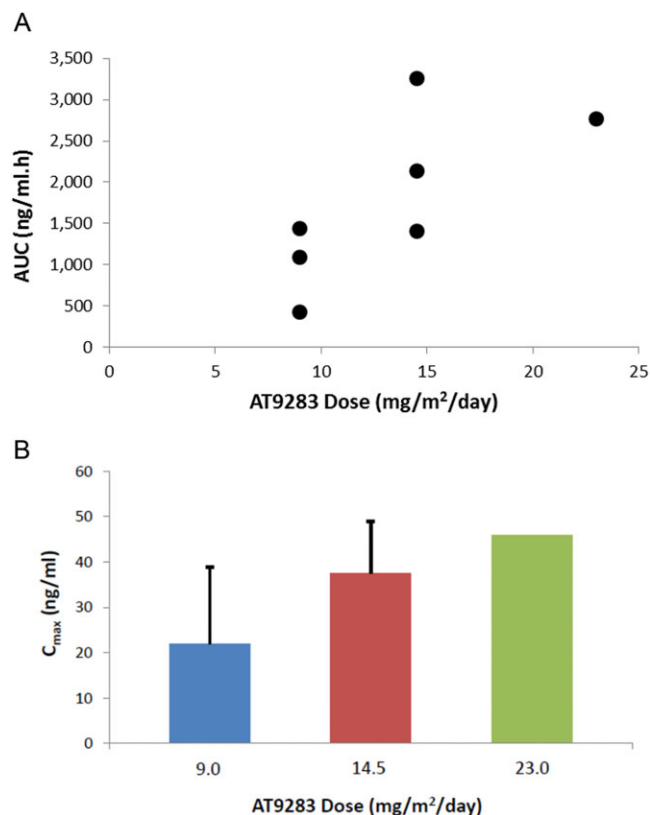
None of the patients achieved a complete remission (<5% blasts in bone marrow), a complete remission with incomplete bone marrow recovery, or a partial remission (<25% blasts in bone marrow).

### 3.4 | Pharmacokinetics and pharmacodynamic assays

The PK parameters  $C_{max}$ , AUC, half-life, and clearance values were broadly comparable to the results reported from the pediatric solid tumor study.<sup>16</sup> Details of results regarding pharmacokinetics and PD assays are presented in Figures 1 and 2 (and Supplementary Appendix S1: Results; Supplementary Table S3 and Figure S1).

## 4 | DISCUSSION

The current trial represents a first-in-child study of AT9283 in hematological malignancies and highlights major challenges encountered

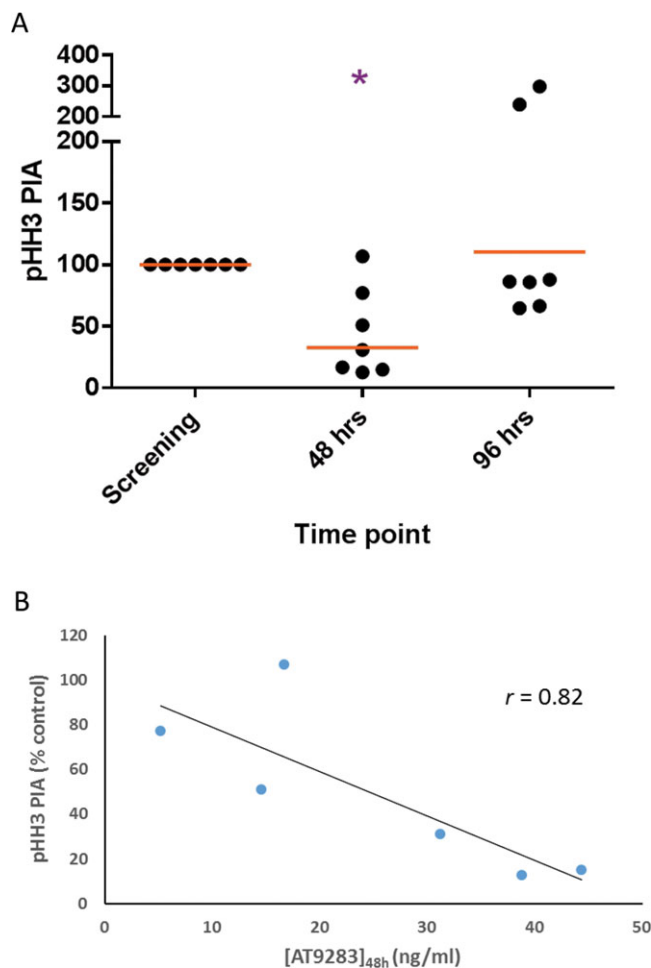


**FIGURE 1** Plot of AT9283 AUC (A) and  $C_{max}$  (B) versus dose level in the leukemic patient population of the study. Despite a high inter-subject variability at both dose levels studied in three patients, there was a general trend towards increased  $C_{max}$  and increased exposure to AT9283 (AUC) with increasing dose levels based on body surface area

when conducting early phase trials in patients with relapsed leukemia. Despite being open for almost 3 years, the trial closed prematurely and the primary endpoint of identifying a dose for phase II exploration was not achieved. AT9283 was well tolerated at the dose levels explored, without any DLTs, but with expected, mainly hematological toxicity. The relatively low starting dose of 9 mg/m<sup>2</sup>/day in comparison with the adult leukemia MTD might have contributed towards the fact that no evidence for clinical efficacy was observed.

Apart from the trial being open at only four sites, the important reasons for the slow accrual were the rarity of eligible patients (e.g., patients with second/higher relapse are rare, CNS disease as exclusion criteria) and competing studies towards the end of the recruitment period (i.e., volasertib, moxetumomab, chimeric antigen receptor (CAR) T cells, dacogen/cytarabine). Furthermore, the slow accrual demonstrated the challenges in conducting early phase studies when eligible patients *per se* are rare and where no preselection regarding pathway activation/underlying mutations is undertaken. In our case, selection for patients with leukemias carrying JAK, ABL, or FLT3 mutations might have increased the chance of observing clinical responses, which could have translated into better recruitment.

In summary, the trial showed that AT9283 has tolerable toxicity and confirmed previous PK data, but it lacked any evidence of efficacy for the doses explored. Our results, taken together with the experience of AT9283 in adult leukemia,<sup>15</sup> provide no evidence for AT9283 as



**FIGURE 2** Plot of plasma inhibitory activity for all patients (A). Phosphorylated histone H3 raw densitometry data normalized to the corresponding Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) signals on the total blot, expressed as a percentage of the screening sample (set at 100%), with subsequent measurements at 48 and 96 h. Six of the seven patients (86%) had a decrease in pHH3 48 hours after the start of AT9283 infusion, with inhibition of phosphorylation ranging from 23% to 87%.  $p < 0.05$  in one-way ANOVA with a multiple comparison test. (B) The relationship between AT9283 concentration and inhibition of pHH3 phosphorylation at the 48 h time point

an active single agent in leukemia, and leave only few indications like megakaryoblastic leukemia,<sup>18</sup> in which future studies might be useful. In general, novel agents like AT9283 should be added to established standard chemotherapy platforms or to upfront single-agent window studies, followed by other treatment elements, as single agents are unlikely to be the way forward in these multiply relapsed patients. Furthermore, successful completion of early phase trials in rare patient populations is difficult in the national setting and such trials are better conducted within international consortia, in order to maximize patient recruitment.

#### ACKNOWLEDGMENTS

This early phase trial was planned and undertaken under the sponsorship and management of Cancer Research Centre for Drug Development. The drug AT9283 for this trial was supplied by Astex

Pharmaceuticals. Recruitment to the trial was facilitated through support from the paediatric Experimental Cancer Medicine Centre (ECMC) network and the respective local comprehensive research networks (LCRN).

### CONFLICT OF INTEREST

MT is an employee of Astex Pharmaceuticals. UB, KES, and JRT are subject to a "Rewards to Inventors Scheme" which may reward contributors to a program that is subsequently licensed. DH was the chief investigator of the AT9283 pediatric solid tumor study. The rest of the authors declared that they have no conflicts of interest.

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### SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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