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

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Key words: leukaemia, pediatric, Aurora kinase, AT9283, phase I/II trial

Abbreviations:

ABL	Abelson tyrosine kinase
AE	Adverse event
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
AUC_{0-t}	Area under the plasma concentration time curve
BSA	Body surface area
C_{max}	maximum concentration
CDD	Centre for Drug Development
Cl	clearance
CNS	Central nervous system
CR-UK	Cancer Research UK
DHPLC	Denaturing high performance liquid chromatography
DLT	Dose limiting toxicity
ECG	Electrocardiogram
Echo	Echocardiogram
FBC	Full blood count
FLT3	FMS-like tyrosine kinase 3
h	hour
JAK	Janus kinase
MLPA	Multiplex Ligation-dependent Probe Amplification
MTD	Maximum tolerated dose
pERK	Phosphorylated ERK
pHH3	Phosphohistone H3
pSTAT	Phosphorylated Signal Transducer and Activator of Transcription
PCNA	Proliferating Cell Nuclear Antigen
PD	pharmacodynamic
PIA	Plasma inhibitory activity
PK	pharmacokinetic
SAE	Serious adverse events
STAT	Signal Transducer and Activator of Transcription
T_{max}	time to reach maximum concentration
$T_{1/2}$	elimination half-life
ULN	Upper limit of normal
Vss	steady state volume of distribution

Abstract

Aurora kinases regulate mitosis and are commonly overexpressed in leukaemia. This phase I/IIa study of AT9283, a multi-kinase inhibitor, was designed to identify maximal tolerated doses, safety, pharmacokinetics and pharmacodynamic activity in children with relapsed/refractory acute leukaemia.

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.

Introduction

Leukaemia is the most common malignancy in childhood. Despite treatment advances, the outcome of relapsed leukaemia remains poor, with 5 year disease-free survival rates for relapsed ALL of 16-39% [1], and relapsed AML of 16-34 % [2]. New treatment strategies are needed to improve survival and reduce 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 leukaemias [3] and overexpression has been linked to genetic instability.

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

Results

Study design, eligibility, dose escalation/drug schedule

Details regarding study design, eligibility criteria, definition of adverse events/dose limiting toxicities, and information on dose escalation, drug schedule and assessments are summarised in the Supplemental 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.

Methods

PK analysis was identical to that described previously [16].

The Plasma Inhibitory Activity (PIA) assay was used to measure *ex vivo* target kinase inhibition and had been validated for inhibition of Aurora, ABL and FLT3 kinase [17].

Immunohistochemistry was used on surrogate tissue (skin punch biopsies) to assess *in vivo* target kinase inhibition of Aurora kinase (change in pHH3 levels), changes in p53 (accumulation/stabilization) and Ki67/proliferating cell nuclear antigen (PCNA) levels as biomarkers of anti-proliferative responses [16].

Other PD measurements included: *in vivo* inhibition of JAK-STAT signalling 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 (MLPA). Details: see Supplemental Appendix S1.

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 7 patients, 5 (71%) were male, 2 (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: 3 patients each at 9 and 14.5 mg/m^2/day, and a further patient at 23 mg/m^2/day. Each patient received 1 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.

Toxicities

All patients were evaluated for safety. A total of 97 Adverse Events (AEs) were reported, of which 29 were considered to be related (possible, probable or highly probable) to the administration of AT9283 (Supplemental Table S1). Five patients experienced a total of 17 Serious Adverse Events (SAE), of which 13 were considered to be at least possibly related to AT9283. None of the SAEs observed were categorised as a DLT (Supplemental Table S2). There was one patient death

during the trial due to disease progression, 22 days after the last administration of AT9283. No MTD could be determined in this study.

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).

Pharmacokinetics and Pharmacodynamic assays

The PK parameters C_{max}, AUC, half-life and Cl values were broadly comparable to the results reported from the paediatric solid tumour study [16]. Details on results regarding pharmacokinetics and pharmacodynamic assays are presented in Figure 1 and 2 (and Supplemental Appendix S1: Results; Supplemental Table S3 and Supplemental Figure S1).

Discussion

The current trial represents a first-in-child study of AT9283 in haematological malignancies and highlights major challenges encountered when conducting early phase trials in patients with relapsed leukaemia. 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 haematological toxicity. The relatively low starting dose of 9 mg/m^2/day in comparison with the adult leukaemia MTD might have contributed towards the fact that no evidence for clinical efficacy was observed.

Apart from the trial being open at only 4 sites, 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, CAR T-cells, Dacogen/cytarabine). Furthermore, it demonstrated the challenges in conducting early phase studies when eligible patients *per se* are rare and where no pre-selection regarding pathway activation/underlying mutations is undertaken. In our case, selection for patients with leukaemias 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 leukaemia [15], provide no evidence for AT9283 as an active single agent in leukaemia, and leave only few

indications like megakaryoblastic leukaemia [18], 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 maximise patient recruitment.

Acknowledgements

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 statement

The author MT is an employee of Astex Pharmaceuticals. The authors UB, KES and JRT are subject to a 'Rewards to Inventors Scheme' which may reward contributors to a programme that is subsequently licensed. DH was the chief investigator of the

AT9283 paediatric solid tumour study. All other authors have declared to have no conflict of interests.

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Legends

Figure 1: Plot of AT9283 AUC (A) and C_{max} (B) versus dose level in the leukaemic patient population of the study. Despite a high inter-subject variability at both dose levels studied in 3 patients, there was a general trend towards increased C_{max} and increased exposure to AT9283 (AUC) with increasing dose levels based on BSA

Figure 2: Plot of Plasma Inhibitory Activity for all patients (A). Phosphorylated histone H3 raw densitometry data normalised to the corresponding 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 out of the seven patients (86%) had a decrease in pHH3 48 hours after the start of AT9283 infusion, with inhibition of phosphorylation ranging from 23-87%. $^{*}p < 0.05$ in one-way ANOVA with multiple comparisons test. Panel 2B shows the relationship between AT9283 concentration and inhibition of pHH3 phosphorylation at the 48 h time point.

Legends to supplemental files:

Supplemental Appendix S1: This section includes details on study design, eligibility criteria, definition of adverse events/dose limiting toxicities, and information on dose escalation, drug schedule and assessments.

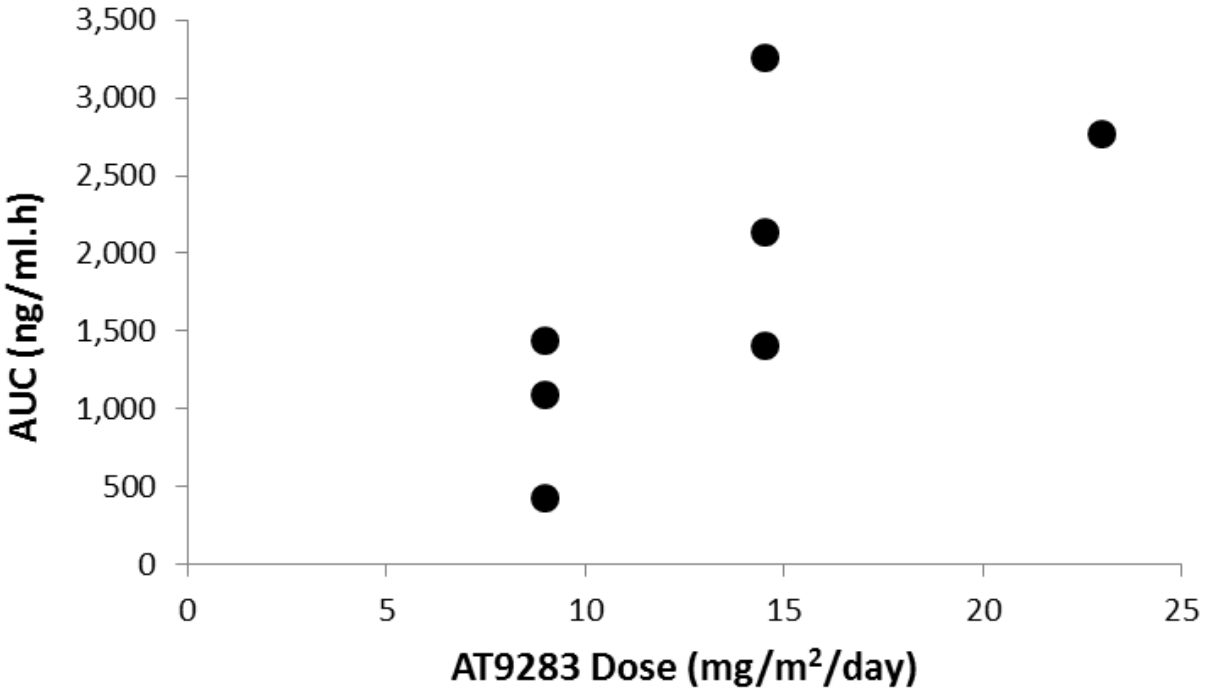
Supplemental Table S1: Frequency of Adverse Events (occurring in ≥20% patients)

Supplemental Table S2: Serious Adverse Events

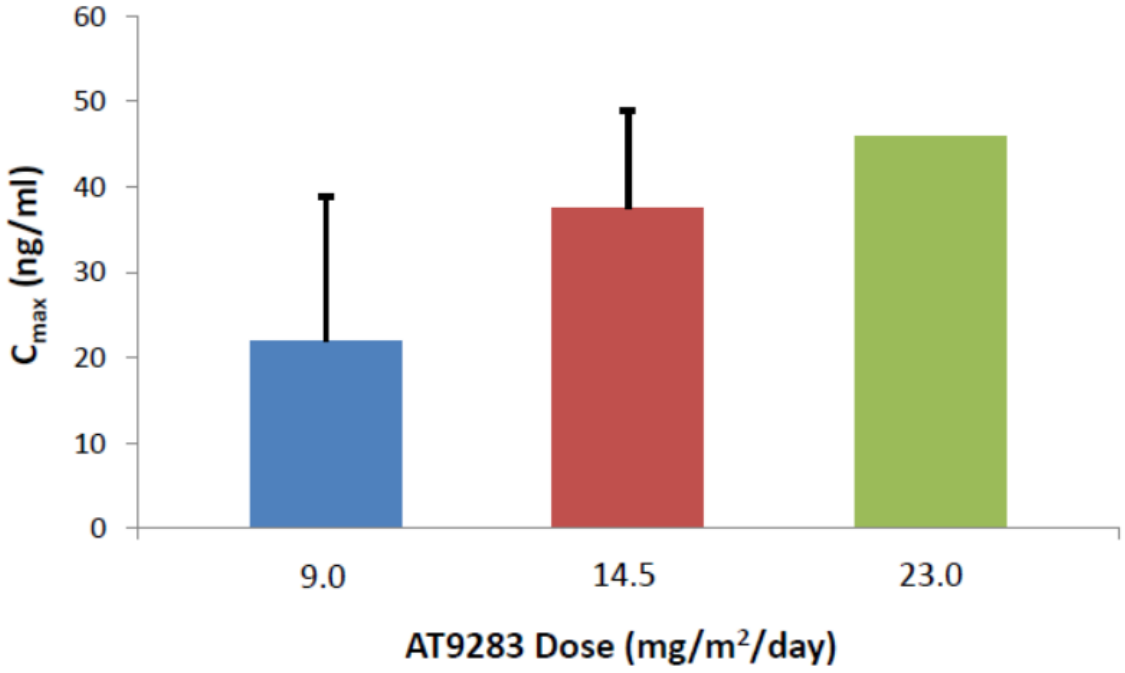
Supplemental Table S3: Summary of pharmacokinetic indices

Supplemental Figure S1: Plot of AT9283 clearance versus body surface area (m^2) for leukaemia patients in the current study (shown in red) and solid tumour patients (from a previous study, shown in black).

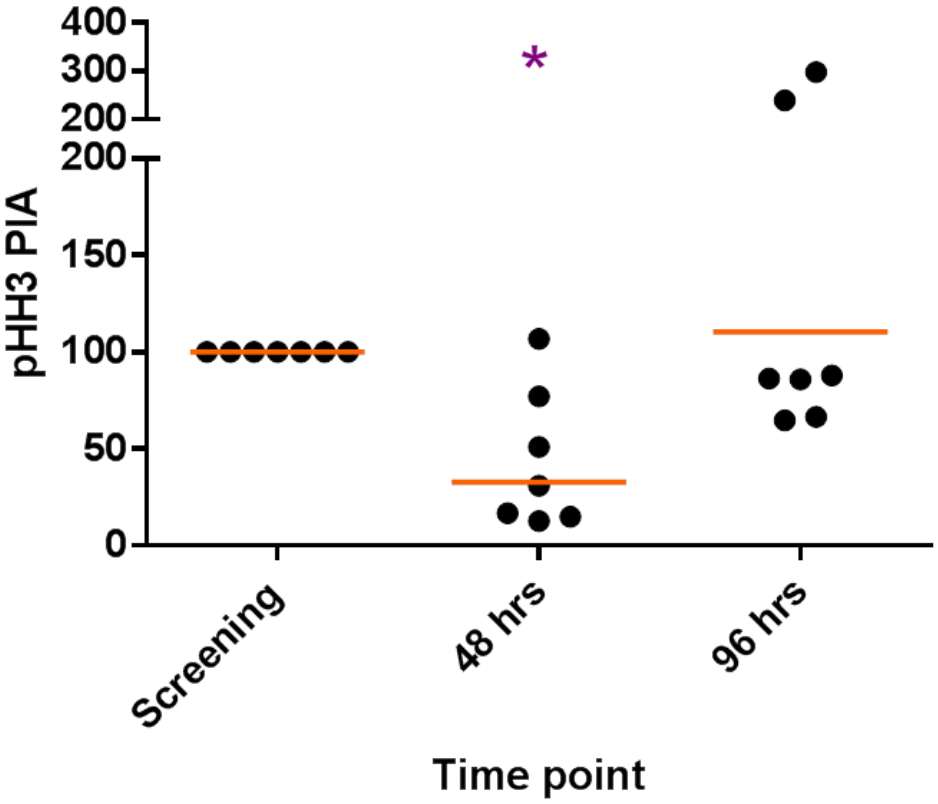
A


3,500
3,000
2,500
2,000
1,500
1,000
500
0
AUC (ng/ml.h)
0
5
10
15
20
25
AT9283 Dose (mg/m²/day)

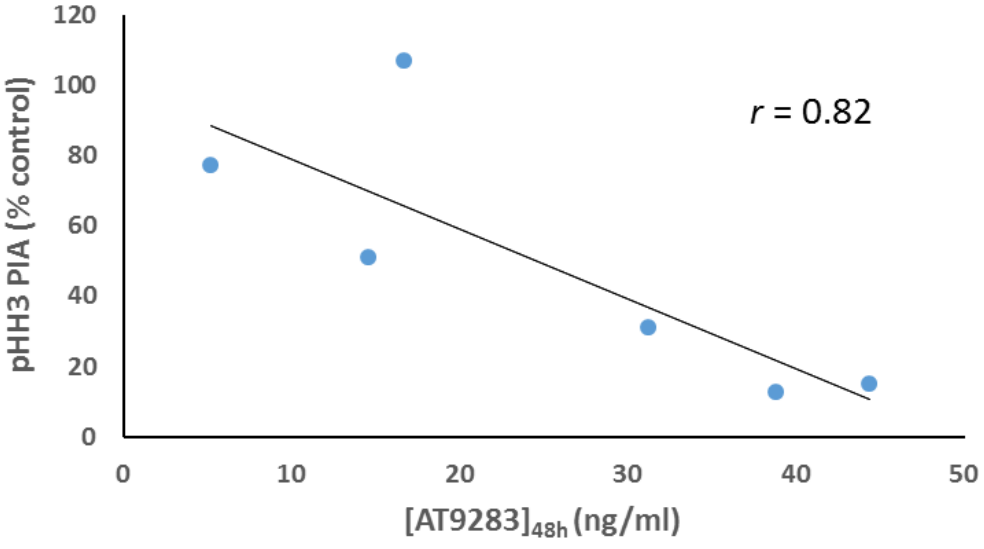
B


60
50
40
30
20
10
0
Cmax (ng/ml)
9.0
14.5
23.0
AT9283 Dose (mg/m²/day)

A


400
300
200
200
150
100
50
0
pHH3 PIA
*
Screening
48 hrs
96 hrs
Time point

B


120
100
80
60
40
20
0
pHH3 PIA (% control)
r = 0.82
0
10
20
30
40
50
[AT9283]48h (ng/ml)

Supplemental Appendix S1

Study design

The trial was an open-label, multi-centre, non-randomized dose-escalation Phase I/IIa paediatric study in patients with relapsed (≥2nd relapse) or refractory leukaemia (ALL and AML). The trial opened to recruitment at 4 sites across England in September, 2011 and closed in July, 2014. The trial was planned to enrol 12-18 patients in a '3+3' design, with the expectation to enrol 3-5 patients per year, thus expecting to remain open for 2.5 years as a minimum. Dose escalation was only allowed in the event of ≤1 DLT per cohort.

Patient eligibility

Patients eligibility criteria included: age >6 months and <19 years, in second (or subsequent) relapse of ALL or AML, or patients who were refractory to induction treatment in first relapse of ALL or AML, the presence of ≥5 % blasts in the bone marrow, a life expectancy of at least 8 weeks, a Karnofsky/Lansky play scale of ≥ 50%, biochemical indices within specific ranges [serum bilirubin < 1.5x upper limit of normal (ULN), Alanine aminotransferase or aspartate aminotransferase <2.5 x ULN, creatinine clearance ≥60 ml/min/1.73m^2 (Schwartz formula)], signed informed consent and being capable of co-operating with treatment and follow-up.

Exclusion criteria were: a diagnosis of chronic myeloid leukaemia, the presence of central nervous system (CNS) disease, cytotoxic chemotherapy within 2 weeks, prior exposure to an Aurora kinase inhibitor, ongoing toxicity from previous treatments (except alopecia and certain grade 1 toxicities), pregnant/lactating women,

participants not agreeing to use highly effective forms of contraception during the trial and for 6 months afterwards, major thoracic or abdominal surgery from which the patient has not yet recovered, positivity for hepatitis B, C or HIV, decreased cardiac function (FS ≤ 29% on Echo), uncontrolled arterial hypertension, congenital heart disease, active graft versus host disease or patients with significant toxicity following haematopoietic stem cell transplant.

Definition of Adverse Events and Dose Limiting Toxicities

Adverse events (AE) were classified according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.02. DLTs were defined as a probably or highly probably drug-related AE to AT9283 with failure to recover an absolute neutrophil count of 0.5 x 10^9/l and a non-transfusion dependent platelet count of 25 x 10^9/l due to documented bone marrow aplasia/hypoplasia by day 42, a >15% reduction in shortening fraction as measured by echocardiogram, any AE with fatal outcome, and any grade 3 or grade 4 non-haematological toxicity (but excluding: alopecia, reversible grade 3 nausea/vomiting, within 48 hours resolving grade 3 mucositis, within 14 days resolving grade 3 transaminitis, and grade 3 fever with neutropenia).

Dose escalation, drug schedule and assessments

The starting dose of AT9283 was 9 mg/m^2/day for 3 days, which is less than 10% of the adult maximum tolerated dose (MTD, 108 mg/m^2/day) in a phase I trial with haematological patients [1]. This conservative approach was chosen as significant fatal cardiac toxicity had been observed at the dose level above the MTD in the adult haematological study and also as the MTD in the adult solid tumour study had been

determined as 9 mg/m^2/day [2]. The MTD in the paediatric solid tumour trial of AT9283 was found to be 18.5 mg/m^2/day, but that was not known at the time point of first patient enrolment, as the two trials were conducted partially overlapping. A further dose escalation to 12 mg/m^2/day was planned, but later revised to 14.5 mg/m^2/day, after review of the safety and PK/PD data of the paediatric solid tumour study (MTD 18.5 mg/m^2/day) [3]. A further dose escalation of up to 100% greater than 14.5 mg/m^2/day was subsequently allowed.

AT9283 was supplied by Astex Therapeutics Ltd; and reconstituted, diluted and given i.v. over 72 hours on days 1-3 of a 21 day cycle as previously described [3]. Treatment was planned to continue for up to 6 cycles, and patients had to have at least a partial remission (PR) after cycle 1 to continue with the next cycle.

At screening, physical examination including vital signs, performance status, height, weight and body surface area (BSA) were obtained, and full blood count (FBC), biochemistry, urinalysis, pregnancy test, chest X-ray, Echo and ECG were performed. Patients had a diagnostic lumbar puncture to exclude CNS disease and a bone marrow aspirate and optional trephine for disease assessment. A skin punch biopsy was taken at baseline and at the end of the first 72 hour infusion for *in vivo* PD assessments. During the study period, patients were reviewed with physical examination, vital signs, assessment of AE and twice weekly haematology and weekly biochemistry laboratory tests. A bone marrow aspirate and trephine was obtained upon recovery of counts to assess disease status. Before a second cycle, Karnofsky/Lansky performance status, BSA, vital signs, height/weight, vital signs, laboratory tests (haematology and biochemistry), Echo and assessment of AE were repeated. At the off-study visit, and in addition to the above assessments, a bone marrow aspirate was repeated if none had been performed during the previous 4

weeks, and could be omitted if rising blast cell counts in peripheral blood signified disease progression.

Methods

Pharmacodynamic studies

Leukaemic patients often have blasts in their peripheral blood which are amenable for biomarker and pharmacodynamics studies. Blood samples were taken at screening and 4, 48 and 96 hours after the start of infusion on cycle 1. A skin punch biopsy was taken at baseline and at 72 hours (+/- 2 hours) following the start of the drug infusion on cycle 1.

Plasma Inhibitory Activity (PIA) assay

Aurora kinase and FLT3 kinase act by phosphorylating histone H3 (HH3) and activating the RAS/RAF/MEK/ERK pathway, respectively, thus levels of pHH3 and pERK1/2 can serve as biomarkers of their kinase activity. The PIA assay involved the incubation of a FLT3-ITD positive AML cell line (MOLM-13) with plasma from patients treated with AT9283 as previously described [4]. Total Histone H3 and ERK1/2 were blotted to confirm modulation of the phosphorylated proteins were not due to changes in total protein levels. Quality controls consisted of human male plasma (SeraLab) spiked with high, medium and low concentrations of AT9283.

Positive and negative controls for pHH3 consisted of specimens of human breast carcinoma with known antigenic reactivity.

Other pharmacodynamics assays

Assessment of the *in vivo* inhibition of the JAK-STAT pathway by AT9283 was performed for patients with white cell counts of $>10x10^9$/l. Phosphorylated STAT5 was measured in leukaemic blasts at baseline and 4 hours post start of AT9283 infusion by flow cytometry using an anti-STAT5 (Tyr 694) antibody (BD Biosciences).

The mean fluorescent intensities of baseline isotype antibody staining compared to pSTAT5 antibody staining, relative to the ratios after 4 hours AT9283 infusion time, were evaluated.

Genetic analyses were performed for mutations in hot spot regions of JAK1, 2, 3 and FLT3 by denaturing high performance liquid chromatography (DHPLC) as previously described [5, 6]. For copy number abnormalities in IKZF1 and PAX5, Multiplex Ligation-dependent Probe Amplification (MLPA) was performed using the SALSA MLPA kit IKZF1 P335 (MRC Holland, Amsterdam, the Netherlands) [7].

Results

Pharmacokinetics

Blood samples for PK measurements were obtained from all 7 patients. In 54% of patients (4/7), plasma concentrations of AT9283 increased to achieve steady-state concentrations within the first 24 hours after the start of infusion. In 3 patients (43%) the plasma concentration of AT9283 continued to rise after 24 hours and peaked at 72 hours. All AT9283 plasma PK parameters are summarized in Table III. C_{max}, AUC, half-life and Cl values were broadly comparable to the results reported from the paediatric solid tumour study.

Despite a high inter-subject variability at both dose levels studied in 3 patients, there was a general trend towards increased C_{max} and increased exposure to AT9283 (AUC) with increasing dose levels based on BSA (see Fig 1, main text). Clearance of AT9283 (uncorrected for SA) plotted against body size suggested a trend towards increased clearance of AT9283 with larger body surface area. However, this correlation was less clear when comparable data from the paediatric solid tumour trial of AT9283 were included in the analysis (Fig 3, supplementary material below).

Pharmacodynamic biomarkers

Plasma Inhibitory Activity assay

All seven patients had blood samples taken pre-treatment and at 48 and 96 hours post start of infusion for *ex vivo* PIA analysis. Six out of the seven patients (86%) had a decrease in pHH3 at serine 10 residue 48 hours after the start of AT9283 infusion, with inhibition of phosphorylation ranging from 23-87%. A dose of 14.5 mg/m^2/day

was required to achieve statistically significant *ex vivo* inhibition of pHH3. The patient who showed no inhibition of pHH3 at 48 hours was treated in the first cohort (9 mg/m^2/day dosing) and showed an inhibition of 33% at the 96 hour time point. Overall, the results of the PIA assay for pHH3 correlate with the dose of AT9283, with the least inhibition in cohort 1 (9 mg/m^2/day dosing) and the greatest inhibition being observed in the cohort 3 patient (23 mg/m^2/day), who achieved 87% inhibition at 48 hours but returned to above pre-screening levels at 96 hours (239%). Combining the data for all patients analysed, the inhibition of pHH3 after 48 hours of AT9283 treatment was statistically significant ($p<0.05$) by one-way ANOVA analysis with multiple comparisons (Fig 2A). Figure 2B shows the relationship between AT9283 concentration at 48 h and inhibition of pHH3 phosphorylation at the same time point. The r value of 0.82 suggests a clear relationship between the two parameters, albeit with a small number of patients (n=6, as a plasma sample from one patient was missing).

Immunohistochemistry

Skin punch biopsies obtained pre-treatment and at 72 hours after the start of drug infusion were used to measure *in vivo* inhibition of Aurora kinase (change in pHH3 levels), possible changes in p53 (accumulation/stabilisation) and anti-proliferative responses (Ki67/ PCNA levels). Six out of seven patients were evaluable for PD analysis, as one patient had not had a 72 hour post-treatment biopsy taken. There was no consistent result for pHH3 staining or quantification of pHH3 staining as expressed by a histoscore (ranging from 0-300), with 3/6 patients (50%) showing an increase in the histoscore post treatment, 2/6 patients (33%) demonstrating

stabilisation (no change), and the remaining patient with minimal scores pre- and post-treatment. Therefore, *in vivo* target inhibition of AT9283 in the form of a reduction in pHH3 could not be confirmed in the present study. Equally, p53 levels were consistently reduced in all patients of cohorts 1 and 2, therefore p53 could not be confirmed as a biomarker of target kinase inhibition. Only 1/6 patients (cohort 3) showed a slight accumulation of p53 after AT9283 treatment. Ki-67 staining and PCNA levels were variable and not consistent in the subjects analysed.

In vivo inhibition of JAK-STAT pathway

Due to low peripheral white cell counts, only three patients with white cell counts >$10x10^9$/l were eligible for assessment of pSTAT5 by phosphoflow and blood samples were obtained from only two of these patients. Both patients showed mean fluorescent intensities at baseline comparable to the negative control cell line and thus neither patient was shown to have constitutive activation of the JAK-STAT pathway. This finding was consistent with the absence of JAK1, JAK2 or JAK3 mutations in the bone marrow of both patients.

Genetic analyses

Genomic DNA from bone marrow samples from all 7 patients was screened for mutations in key exons in FLT3, JAK1, JAK2 and JAK3. In total, 10 PCR amplicons scored aberrant by DHPLC and were sequenced, revealing 9 single nucleotide polymorphisms and 1 activating JAK2 mutation (R683S), which was verified by Sanger sequencing. This mutation has been shown in experimental models to be

oncogenic and confers sensitivity to JAK inhibitors [8]. Unfortunately, this patient who had ALL was not one of the two tested for activated JAK-STAT pathway by flow cytometry due to low peripheral white blood cell counts. MLPA analyses for PAX5 and IKZF1 were performed in 2 of 3 ALL patients. One showed a deletion in IKZF1 (exon 2-7). Copy numbers for PAX5 for both patients were normal.

Legend to supportive Figure 3:

Plot of AT9283 clearance versus body surface area (m^2) for leukaemia patients in the current study (shown in red) and solid tumour patients (from a previous study, shown in black).

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Supplemental TABLE S1 Frequency of Adverse Events (occurring in ≥20% patients)

AE body system	Number of episodes (number of patients, % [n=7])		
AE	**All AEs Grades 1-5**	**Related AEs Grades 1-5**	**Related AEs Grades 3-5**
Blood & lymphatic disorders	**14 (6, 85.7%)**	**6 (4, 57.1%)**	**6 (4, 57.1%)**
Anaemia	7 (4, 57.1%)	2 (1, 14.3%)	2 (1, 14.3%)
Febrile neutropenia	4 (3, 42.9%)	4 (3, 42.9%)	4 (3, 42.9%)
Gastrointestinal disorders	**15 (5, 71.4%)**	**3 (1, 14.3%)**	**1 (1, 14.3%)**
Nausea	2 (2, 28.6%)	1 (1, 14.3%)	1 (1, 14.3%)
vomiting	6 (4, 57.1%)	2 (1, 14.3%)	-
Investigations	**21 (6, 85.7%)**	**10 (5, 71.4%)**	**7 (4, 54.1%)**
Neutrophil count decreased	3 (3, 42.9%)	2 (2, 28.6%)	2 (2, 28.6%)
Platelet count decreased	5 (4, 57.1%)	2 (2, 28.6%)	1 (1, 14.3%)
White blood cell decreased	4 (3, 42.9%)	3 (3, 42.9%)	3 (3, 42.9%)
Metabolism & nutrition disorders	**12 (4, 57.1%)**	**4 (2, 28.6%)**	**1 (1, 14.3%)**
Hypophosphatemia	4 (2, 28.6%)	2 (1, 14.3%)	-
Hypokalemia	2 (2, 28.6%)	1 (1, 14.3%)	1 (1, 14.3%)

AE: Adverse Event

Supplemental TABLE S2 Serious Adverse Events

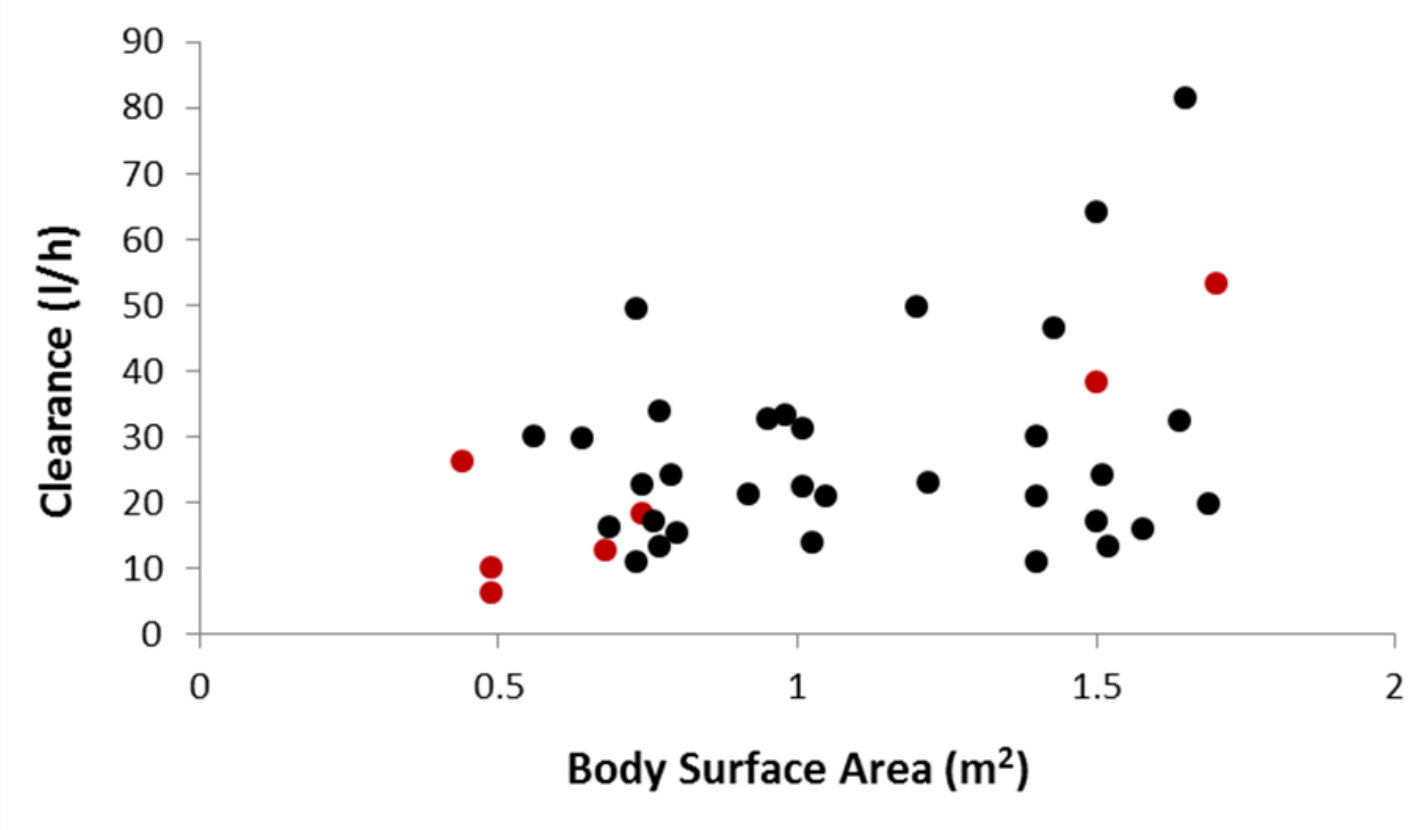
Body System	Total number of SAEs	Total number of related SAEs	Number of patients	% (N=7)
All SAEs	**17**	**13**	**5**	**71.4**
Blood & lymphatic disorders				
Febrile neutropenia	4	4	3	42.9
Other – increase in white cell count	1	1	1	14.3
Cardiac disorders				
Sinus tachycardia	1	1	1	14.3
Gastrointestinal disorders				
Nausea	1	1	1	14.3
Vomiting	2	2	1	14.3
General disorders and administration site conditions				
Death	1	-	1	14.3
Pain	1	-	1	14.3
Infections and Infestations				
Sepsis	2	2	1	14.3
Investigations				
Neutrophil count decreased	1	1	1	14.3
Musculoskeletal and connective tissue disorders				
Bone Pain	1	-	1	14.3
Vascular disorders				
Hypotension	2	2	1	14.3

SAE: Serious Adverse Event

Supplemental TABLE S3 Summary of pharmacokinetic indices

Patient Nr	Dose level ($mg/m^2/day$)	C_{max} (ng/ml)	AUC (ng/ml.h)	Half-life (h)	Cl (l/h)	Vss (l)
1	9	40.9	1443	5.1	12.7	91.9
2	9	8.7	432	4.5	26.1	164.7
3	9	16.0	1096	6.2	38.3	335.2
Mean ± SD	*9*	*21.9 ±16.9*	*990 ± 514*	*5.3 ± 0.9*	*25.7 ± 12.8*	*197 ± 125*
4	14.5	46.8	3256	6.1	6.3	54.6
5	14.5	40.9	2137	4.1	10.0	57.3
6	14.5	24.8	1412	5.7	53.1	427.9
Mean ± SD	*14.5*	*37.5 ± 11.4*	*2268 ± 929*	*5.3 ± 1.1*	*23.1 ± 26.0*	*180 ± 215*
7	23	46.0	2766	5.3	18.4	141.7

C_{max}: maximum concentration
AUC: Area under the plasma concentration time curve
Cl: clearance
Vss: steady state volume of distribution


Clearance (l/h)
90
80
70
60
50
40
30
20
10
0
0
0.5
1
1.5
2
Body Surface Area (m²)