Elsevier Editorial System(tm) for The Lancet Haematology

Manuscript Draft

Manuscript Number: THELANCETHAEMATOLOGY-D-15-00236R2

Title: A phase 1 dose-finding and pharmacokinetic study of the BET bromodomain inhibitor OTX015 in acute leukaemia patients

Article Type: Articles (Original Research)

Keywords: bromodomain inhibitor, OTX015/MK-8628, acute leukaemia, phase I, BRD4

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Manuscript Region of Origin: FRANCE

Abstract: Background Bromodomain (BRD) and extraterminal (BET) proteins are chromatin readers that preferentially impact the transcription of genes with super-enhancers including oncogenes. BET proteins bind acetylated histone tails via their BRD, bringing the elongation complex to the promoter region. OTX015 (MK-8628) specifically binds to BRD2, 3 and 4, preventing BET proteins from binding to the chromatin, thus inhibiting gene transcription. OTX015 inhibits proliferation in a large panel of haematological malignancy cell lines and patient cells, in vitro and in vivo. A phase 1 trial was launched to determine the recommended dose of OTX015 in patients with haematological malignancies. We report here on the leukaemic cohort.
Methods In this multicentre international study, OTX015 was given orally at increasing doses from 10 to 160 mg/day (14/21 days), using a conventional 3+3 design. OTX015 was initially administered once daily, with allowance for exploration of other schedules. Acute leukaemia patients failing or with contraindication to standard therapies were eligible. The primary end point was dose limiting toxicity (DLT) in the first treatment cycle. Secondary objectives were to evaluate safety, pharmacokinetics, pharmacodynamics, and preliminary clinical activity. The study is registered with ClinicalTrials.gov, NCT01713582.
Findings Forty-one patients, 36 with acute myeloid leukaemia, a median age of 70 years and two lines of prior therapy were treated over six dose levels. No DLT was observed until 160 mg when one patient experienced grade 3 diarrhoea and another had grade 3 fatigue. However, concomitant grade 1-2 non-DLT toxicities (gastrointestinal, fatigue, cutaneous) from 120 mg hampered patient compliance and 80 mg was considered the recommended dose with a once daily schedule. Common toxicities included grade 3 fatigue in three patients and grade 3-4 bilirubin increase in two patients. OTX015 plasma concentrations increased proportionally up to 120

mg with trough concentrations in the in vitro active range from 80 mg. Three patients (40, 80, 160 mg) achieved complete remission (CR/CRi) lasting 2 to 5 months, and two additional patients had partial blasts clearance. No predictive biomarkers for response have been identified to date.
Interpretation OTX015 is an orally available, first-in-class BRD inhibitor with linear pharmacokinetics. The single agent recommended dose for a once daily schedule in acute leukaemia patients is 80 mg. Single agent clinical activity in myeloid malignancies was seen at non-toxic doses.
Funding Oncoethix GmbH (formerly Oncoethix SA).

A phase 1 dose-finding and pharmacokinetic study of the BET bromodomain inhibitor OTX015 in acute leukaemia patients

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SUMMARY

Background Bromodomain (BRD) and extraterminal (BET) proteins are chromatin readers that preferentially impact the transcription of genes with super-enhancers including oncogenes. BET proteins bind acetylated histone tails via their BRD, bringing the elongation complex to the promoter region. OTX015 (MK-8628) specifically binds to BRD2, 3 and 4, preventing BET proteins from binding to the chromatin, thus inhibiting gene transcription. OTX015 inhibits proliferation in a large panel of haematological malignancy cell lines and patient cells, *in vitro* and *in vivo*. A phase 1 trial was launched to determine the recommended dose of OTX015 in patients with haematological malignancies. We report here on the leukaemic cohort.

Methods In this multicentre international study, OTX015 was given orally at increasing doses from 10 to 160 mg/day (14/21 days), using a conventional 3+3 design. OTX015 was initially administered once daily, with allowance for exploration of other schedules. Acute leukaemia patients failing or with contraindication to standard therapies were eligible. The primary end point was dose limiting toxicity (DLT) in the first treatment cycle. Secondary objectives were to evaluate safety, pharmacokinetics, pharmacodynamics, and preliminary clinical activity. The study is registered with ClinicalTrials.gov, NCT01713582.

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months, and two additional patients had partial blasts clearance. No predictive biomarkers for response have been identified to date.

Interpretation OTX015 is an orally available, first-in-class BRD inhibitor with linear pharmacokinetics. The single agent recommended dose for a once daily schedule in acute leukaemia patients is 80 mg. Single agent clinical activity in myeloid malignancies was seen at non-toxic doses.

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PANEL: Putting research into context (box located in discussion page of the paper)

Research in context: Intensive cytarabine/anthracycline-based induction therapy, followed by intensification with or without allogeneic stem cell transplantation, allows about 50% of younger acute myeloid leukaemia (AML) patients to be cured. Nonetheless, the outcome for many patients remains poor, either because they are excluded from intensive standard induction and consolidation therapies (older populations), or because their disease is refractory or relapses after such therapies. Among novel therapeutic approaches, epigenetic manipulation has been explored with hypomethylating agents and more recently by inhibition of bromodomains (BRD), histone deacetylase (HDAC), and isocitrate dehydrogenase (IDH). Bromodomain inhibition has shown promising results in preclinical AML studies, including specific AML subsets such as those with NPM1 or IDH2 gene mutation, MLL gene rearrangement, or EVI1 gene overexpression. To date no clinical evaluations of bromodomain inhibition have been published (Pubmed search: leukaemia, bromodomain inhibitor, patient/clinical).

Added value of the study: To our knowledge, this is the first clinical evidence of a novel first-in-class bromodomain inhibitor OTX015/MK-8628 administered to patients with advanced acute leukaemia. OTX015 was safely administered at 80 mg QD with a discontinuous schedule. Evidence of activity was seen in several AML patients treated with OT0X15 doses giving plasma exposures equivalent to those resulting in activity in *in vitro* studies.

Implications of the evidence: In light of demonstrated activity in a fragile elderly AML population along with a manageable safety profile, future development of this bromodomain inhibitor in combination with other compounds active in this population is merited. Extended genetic molecular biology analyses will help guide the selection of populations most likely to benefit from this class of compound.

INTRODUCTION

Acute myeloid leukaemia (AML) is characterized by maturation blockade at an early myeloid progenitor stage, clonal proliferation/accumulation of immature blasts, and bone marrow failure. While almost half of the younger AML patients can be cured,[1] patients who are refractory/relapsing or not fit for intensive therapy (mainly the elderly) have limited therapeutic options.

Epigenetic deregulation is considered to be an important factor in AML genesis, with numerous recurrent gene alterations affecting transcriptional activity in both AML and myelodysplastic syndromes (MDS).[2–4] Hypomethylating agents, including decitabine and azacytidine, were the first epigenetic modulators approved in myeloid malignancies.[5–8] Nonetheless, with limited proven activity, other epigenetic drug families are under investigation, including histone deacetylase (HDAC), isocitrate dehydrogenase (IDH) and bromodomain (BRD) inhibitors.

BRD and extraterminal (BET) proteins are chromatin readers that play a major role in the epigenetic regulation of gene transcription.[9] Their bromodomain-bearing moiety binds to acetylated histone tails, allowing the extraterminal moiety to bring the elongation complex of the transcriptional machinery within proximity of the gene promoter region. Histone acetylation is prevalent at super-enhancer regions that control expression of various oncogenes, rendering them exquisitely sensitive to BRD inhibition.[10] BRD inhibitors are small molecules that specifically bind BRDs,[11] preventing BET proteins from binding to chromatin and thereby inhibiting gene transcription. They have raised considerable interest as a promising novel therapeutic approach in AML[12–17] and other cancers. In AML, several potential biomarkers for BRD inhibition efficacy have been reported. The *NPM1* gene mutation, present in 25-30% of adult AML, favours a BRD4-dependent core transcriptional program that renders cells particularly sensitive to BRD inhibition.[16] Less frequent

abnormalities, such as *IDH2* gene mutation,[15] *EVI1* gene overexpression,[17] or *MLL* gene rearrangement,[14] have also been reported as potential predictors, based on preclinical models.

OTX015 (MK-8628) is a 528 Dalton molecule that binds exclusively to BRD2, 3, and 4 with an EC_{50} of 10-19 nM, inhibiting their binding to acetylated histone H4 with an IC_{50} of 92-112 nM.[18] It exhibits anti-proliferative effects against a large panel of haematological malignancy cell lines with a GI_{50} of 40-500 nM. Ten of 17 leukaemia cell lines tested were considered sensitive with an IC_{50} < 500 nM after 72 hours exposure.[19] Furthermore, primary bone marrow cells from eight of 14 AML patients were found to be sensitive to OTX015 at similar concentrations, and apoptosis was also reported. While growth inhibition has been seen with various *in vivo* tumour models,[20] animal models of acute leukaemia have yet to be explored.

Haematological, gastrointestinal and hepatic toxicities were limiting at the highest OTX015 doses tested in toxicology studies, with no cumulativity seen up to 12 months. No specific target organs were identified in preclinical safety pharmacology studies. To evaluate the clinical tolerance and potential of OTX015, the present phase 1 study was initiated in patients with advanced haematological malignancies, including acute leukaemia patients.

METHODS

Study design and patients

This international phase 1 dose escalation study was performed in seven centres (France, United Kingdom, and Canada; Supplemental Table 1, page 1). The primary objective was to determine the recommended dose of OTX015 single agent in two independent and parallel cohorts, one in acute leukaemia patients and the other in patients with non-leukemic haematological malignancies, with dose limiting toxicity (DLT) as the primary endpoint. Secondary objectives were to determine the safety profile, pharmacokinetics, evaluate

evidence of clinical anti-tumour activity, and identify potential predictive biomarkers for efficacy. We report results here for the acute leukaemia cohort.

To be eligible patients had to be aged ≥ 18 years with relapsed/refractory acute leukaemia (except promyelocytic). Patients aged < 60 years had to have failed at least two standard regimens, while older patients had to have failed at least one regimen and have relapsed < 1 year after previous first-line therapy, or have a contra-indication for standard therapies. Previous allogeneic SCT was allowed, provided relapse had occurred > 90 days later. Graft-*versus*-host disease symptoms or treatment was not permitted at study entry. Patients were required to have an Eastern Cooperative Oncology Group performance status of 0 to 2, normal bilirubin, aminotransferases ≤ three times the upper normal limit, and creatinine clearance ≥ 30 mL/min, with a life expectancy of ≥ 3 months. All patients provided written informed consent. Regulatory approval was obtained from all national authorities, and all local institutional review boards approved the protocol as appropriate. The study was conducted in accordance with the Declaration of Helsinki and registered with ClinicalTrials.gov, number NCT01713582.

Procedures

The study started with a once daily (QD) schedule and allowed for exploration of other schedules according to SMC decision. Eligible patients received OTX015 delivered orally as 10 or 20 mg gelatine capsules, under fasted conditions, daily for 14 consecutive days, followed by a 1-week rest (21-day cycles), at a starting dose of 10 mg QD. A conventional 3+3 dose escalation schedule was used with three patients initially per dose level. Doses were escalated after three patients had received at least one cycle as planned without DLT. Doses were doubled until DLT occurred at which point three additional patients were treated at this dose. If no more than one of the six patients experienced DLT, dose escalation continued using the more conservative, Fibonacci-like model. The highest dose level with no more than one of six patients experiencing DLT was considered the recommended dose. On the basis of

preclinical models, the possibility that continuous exposure at active concentrations was necessary for optimal activity was evaluated after 80 mg QD with a BID schedule (40 mg x 2).

DLT was defined as any one of the following occurring during the first cycle: a National Cancer Institute Common Toxicity Criteria grade 3 or 4 non-haematological related event despite optimal supportive care, grade 3 or 4 asymptomatic non-haematological laboratory abnormality lasting more than 7 days, blast-free aplasia confirmed as lasting ≥ 42 days, or a grade 2 event leading to dose interruption or adaptation. If such toxicities occurred at any time during treatment, OTX015 was interrupted until recovery to grade ≤ 1 (or baseline value) and treatment resumed at the dose level immediately below. A safety monitoring committee (SMC) composed of all principal investigators, the sponsor's medical and safety officers, and an independent expert made all decisions regarding patient safety, DLT qualification, dose escalation, and recommended dose definition. Treatment was continued until progression, intolerable toxicity, treatment interruption for >2 weeks due to toxicity, DLT recurrence despite dose reduction, or withdrawal of consent.

Standard haematology and biochemistry workups were done weekly during the first cycle, then every 3 weeks in the absence of toxicity, and 12-lead ECGs were performed pre-dose and around T_{max} on cycle 1 day 1. Bone marrow aspirations were performed at baseline, days 8, 22 and 43, and thereafter as needed. Response was assessed according to European LeukemiaNet.[21] Partial blast clearance was defined as a >50% reduction in bone marrow blast percentage without persistent circulating blasts.

Mutational analyses were performed in DNA from baseline bone marrow samples to assess a 42-gene panel of genes commonly mutated in AML and MDS (see Supplemental Table 2, page 2) using a MiSeq® platform (Illumina). The library was prepared using the Haloplex™ Target Enrichment System (Agilent Technologies).

Pharmacokinetics

Plasma OTX015 concentrations were measured in blood samples (3 mL) collected into K2-EDTA tubes prior to dosing then at 1, 4, 6, 8, 12, 16, and 24 hours after drug intake, immediately centrifuged and stored at -20 °C. Samples for residual plasma concentration were collected on days 8, 15 and 22 just prior to dosing. Intracellular OTX015 concentrations were measured from bone marrow and blood monocytes collected on day 8, immediately before dosing. Bone marrow and blood were collected into BD Vacutainer CPT^{TM} tubes, stored at room temperature, and mononuclear cells were separated on a Ficoll gradient. OTX015 concentrations were measured using a validated method using Ultra Performance Liquid Chromatography with tandem Mass Spectrometry (UPLC-MS/MS).[22] Analyses were performed using Monolix software (version 4.3).

Analyses

Patients receiving at least 85% of the cumulative intended dose over the first 21 days were evaluable for DLT. All treated patients were evaluated for safety. Patients were evaluable for efficacy according to the European LeukemiaNet.[21] Descriptive analyses were performed with a cut-off of 14 February 2015 using SAS software (version 9.4).

Role of the funding source

This study was sponsored, funded and initiated by Oncoethix SA (now Oncoethix GmbH, a wholly owned subsidiary of Merck Ltd). It was designed by the sponsor in collaboration with the investigators. Data collection, analysis, and interpretation were performed by the investigating research teams, the study CRO and Oncoethix. The manuscript was written by Oncoethix with the corresponding author, then approved by all authors. The corresponding author had access to all study data and confirms the decision to submit.

RESULTS

Between 18 January 2013 and 9 September 2014, 40 acute leukaemia patients and one patient with high-risk MDS were treated over six dose levels. The population was mainly elderly and the majority of patients had AML (36 patients; 88%), more than one-third of whom were secondary to pre-leukaemic conditions or therapy-related (16 patients; 39%) (**Table 1**).

Dose escalation is summarised in **Table 2**, showing that doses were doubled without DLT over the first four dose levels (10 to 80 mg). To test biological activity with continuous exposure at high concentrations, a BID schedule was explored at dose level 4 (40 mg x 2/day). Although no DLT occurred, evaluation of the parallel cohort of non-leukaemic patients showed an excess of thrombocytopenia without an obvious impact on activity for this BID schedule,[23] along with increased frequency of non-haematological toxicities (gastrointestinal, laboratory). In addition, several patients without severe thrombocytopenia at baseline experienced aggravation of thrombocytopenia on treatment, requiring treatment interruption and dose adaptation, with treating physicians deciding against continuing OTX015 treatment with this dosing schedule. Furthermore, given that responses were observed in leukaemia patients with the QD schedule (and not with the BID schedule), dose escalation was resumed with a QD schedule at 120 mg. No DLT was observed until 160 mg. At this dose, two of five evaluable patients experienced DLT, one had grade 3 fatigue and one had grade 3 diarrhoea. The 160 mg QD dose was therefore considered not tolerable and additional patients were enrolled at 120 mg to confirm tolerance and optimize the schedule with both the original intermittent schedule (14 days out of 21) and a continuous schedule. Although DLT was not reported among the 13 patients treated at 120 mg regardless of schedule, the SMC considered that the cumulative prevalence of several gastrointestinal events and fatigue (**Table 3**) was poorly tolerated in this elderly, heavily pre-treated

population with frequent co-morbidities, hampering patient compliance. Furthermore, active plasma trough concentrations achieved at 80 mg (**Table 4**) along with the lack of a clear dose-effect relationship led to the selection of 80 mg, 14 days on/7 days off as the recommended dose for the QD schedule.

Patients received a median of two cycles (range 1-14). Three patients (treated at 40 mg BID and 160 mg QD) had dose reductions due to grade 2-3 non-haematological toxicity. **Table 3** shows the main adverse events considered related to OTX015. Few events (all of which were grade 1 or 2) occurred at the first two dose levels. The most common events were gastrointestinal (diarrhoea: 14 patients, 34%; nausea: 9 patients, 22%), fatigue (11 patients; 27%), and cutaneous (8 patients; 20%), with increasing frequency and severity above 80 mg QD including at 40 mg BID. Asymptomatic laboratory abnormalities included grade 1-2 factor VII decrease (lowest values of 20-30% with a modest impact on INR) in five patients without associated coagulation factor deficiency. Positive de-challenge/re-challenge in one patient confirmed the relationship to study treatment, however treatment discontinuation was not necessary. Three patients experienced isolated (without other liver test dysfunctions) direct bilirubin increases (all grade 3-4 and generally associated with neutropenic sepsis), with a positive de-challenge/re-challenge in one patient suggesting this was related to OTX015. One patient had recurrent transient grade 3 elevation of aminotransferases, although this patient had a similar history when treated with chemotherapy for breast cancer. Grade 3 or 4 toxicities were infrequent, all of which were reversible within ten days of drug interruption. Peripheral blood cytopenia was not considered for the safety assessment, nevertheless in several patients without severe thrombocytopenia at baseline, aggravation of thrombocytopenia occurred on study treatment. No significant changes in QTc interval were seen. No treatment related deaths occurred and no patients discontinued due to related AEs.

Table 4 shows the pharmacokinetics parameters for all patients evaluable for pharmacokinetics. Plasma exposure increased proportionally with dose from 10 to 120 mg

QD. Terminal half-life was 5.79 hours ± 1.12.[24] No accumulation trend was observed until day 15. Intracellular OTX015 concentrations were measured on day 8 pre-dose in 19 patients and showed low concentrations, in the nanomolar range. Although intracellular concentrations increased with dose, they remained in the single-digit nanomolar range, and the magnitude of the concentration increase is much lower in cells (3-4 fold from 10 to 160 mg OTX015) than in plasma (30-40 fold from 10 to 160 mg).

Activity was seen at a range of dose levels. One AML patient achieved complete remission (40 mg QD), another AML patient achieved complete remission with incomplete recovery of platelets (CRi; 80 mg QD), and a third patient with refractory anaemia with excess of blasts (RAEB) also achieved complete remission (160 mg QD)(Supplemental Table 3, page 3). Two other patients with AML secondary to polycythemia vera and MDS, treated at 10 mg and 80 mg QD respectively, experienced partial blast clearance, and the latter patient also had a transient increase of neutrophil counts above 1.0 x 10^9/L. All three patients with responses relapsed on therapy, the two complete remissions lasting 5 and 3 months and the CRi lasting 2 months. The patient with RAEB treated at 160 mg QD had prolonged treatment interruptions that could potentially have contributed to relapse. **Figure 1A** shows maximum variation in bone marrow blast percentages per patient during treatment and **Figure 1B** shows maximum variation in absolute neutrophil counts (ANC). Interestingly, 13 patients had a maximal ANC increase of more than 2 x 10^9/L during treatment relative to baseline, including non-responding patients.

Genetic analyses did not show any obvious correlations between mutations present in the five responders versus those in the 28 non-responders at study entry (**Figure 2**). Of note, neither *NMP1* mutations (one *NPM1* mutated patient was a responder *versus* six non-responders) nor *IDH2* mutations (no responders among two mutated patients) correlated with clinical activity. One responding patient was flt-3 wild-type, while three of the six non-responders had flt-3

internal tandem duplication. No hints of clinical activity were observed in three patients with *EVI1* overexpression or the two patients with *MLL*-rearrangement.

DISCUSSION

Here we report, to our knowledge, the first clinical investigation with a BRD inhibitor. OTX015 was safely administered at a dose of 80 mg QD in pre-treated acute leukaemia patients, with linear pharmacokinetics over the doses evaluated up to 120 mg. At this dose, plasma concentrations were maintained above 400 nM, a concentration known to be active *in vitro*.[19] Nonetheless, low OTX015 intracellular concentrations were found in day 8 bone marrow and peripheral blood monocytes, compared with trough plasma concentrations. This is coherent with preclinical studies reporting that 500 nM extracellular *in vitro* concentrations resulted in intracellular concentrations approximately ten to fifty times lower at which anti-proliferative activity was seen.[25] Taken together, *in vitro* and clinical data show that low intracellular concentrations in the nanomolar range can be active.

Four of the five patients with anti-leukemic activity were treated at 80 mg QD or lower, offering pharmacodynamic evidence, in the absence of known off-target effects, that OTX015 does indeed attain its targets and triggers a biological effect in this dose range. Increasing the dose above this level increased toxicity without a clear impact on efficacy. The responding patient treated at 160 mg QD experienced recurrent toxicities that led to prolonged treatment interruption periods, potentially jeopardising efficacy outcome and/or duration of response.

From preclinical data showing a rapid washout effect,[20] it was anticipated that prolonged high concentrations, equal or superior to active *in vitro* concentrations, would be necessary for optimal clinical activity. A BID schedule was thus tested in order to increase trough concentrations, achieving similar exposure as the equivalent QD dose. While a BID schedule for continuous exposure is desirable, the excess of thrombocytopenia and various non-haematological toxicities seen in the BID cohort resulted in the SMC's decision not to pursue

treating patients with the BID schedule at higher doses. No evidence of clinical activity was seen in the eight patients treated with the BID schedule. In addition, decisions made during the study involving pharmacokinetic data also took into account data from the non-leukemic cohort, for which some trough concentration data were available.[23] While a BID schedule offers the advantage of erasing peak concentrations, there is no evidence that any of the toxicities reported are peak-related. In the absence of evidence of a benefit with a BID schedule over a QD schedule, the latter schedule is selected to optimise patient convenience and compliance.

OTX015-related AEs were manageable, being mainly mild to moderate at the recommended dose, while severe toxicity rapidly resolved after drug interruption. The spectrum of toxicity (*i.e.* gastrointestinal, fatigue, and cutaneous) resembles that of HDAC inhibitors,[26] another family of epigenetic drugs. OTX015-related haematological toxicity, mainly thrombocytopenia, has been found to be dose-limiting in non-leukaemic patients.[23] While this toxicity is not easily evaluable in acute leukaemia patients, the few patients with incomplete bone marrow failure at baseline, experienced severe thrombocytopenia. Similarly, the patient treated at 40 mg who achieved prolonged complete remission had grade 1 thrombocytopenia at day 15 while in remission that recovered completely after 1 week off therapy. Several cases of isolated asymptomatic factor VII decreases were seen in the absence of other vitamin K-dependent factor deficiencies, as was also observed in lymphoma and myeloma patients.[23] In vitro analyses in human HepaRG hepatocyte-like cells exposed to OTX015 suggest that the underlying mechanism involves inhibition of factor VII gene expression (F. Bertoni, personal communication). While two of the three patients with hyperbilirubinemia also experienced factor VII decreases, these events are considered unlikely to be related, with hyperbilirubinemia probably caused by reduced bilirubin transport across the canalicular membrane following MRP/ABCC2 downregulation or by competitive exclusion of bilirubin due to extrusion of OTX015 via the MRP/ABCC2 transporter.[27–29]

During dose escalation, four patients were treated at 80 mg QD without DLT. With six patients treated at 160 mg QD and 13 patients at 120 mg QD (different administration schedules), all without DLT, 80 mg QD was established by the SMC as the recommended dose at which additional patients were to be evaluated in an expansion cohort. Taken together, the treatment-limiting thrombocytopenia and recovery (which was incomplete in some cases) with a 1-week break led to the recommendation of an intermittent schedule to ensure an adequate opportunity to recover from any toxicity. Although it may be argued that a drug holiday can impact efficacy, responses were seen with an intermittent schedule which was deemed necessary not only from a safety perspective but also to allow long-term treatment. In addition, evaluation of BID schedules at doses below the QD recommended dose of 80 mg is anticipated to address presumed on-target effects of thrombocytopenia along with the importance of continuous sustained exposure (steady state C_{trough}), based on the mechanism of action of OTX015.

Preliminary molecular biology analyses did not detect any clinical or genetic predictive factors of clinical activity from the panel of candidates evaluated in this acute leukaemia population, including in patients with putative predictive markers based on preclinical modelling data. Given the small sample size, further efforts are needed to elucidate which patients are more likely to respond to BRD inhibition, so as to enrich populations in future clinical trials. Although many epigenetic changes are driven by genetic alterations, epigenetic rather than genomic investigations may be more successful. Pharmacodynamic analyses evaluating the expression of seven oncogenes (c-MYC, BCL2, CCND1, NF-kB, BRD2/3/4) in bone marrow cells at baseline and after 1 week of treatment were performed (results not shown). mRNA downregulation was not observed for any of the genes evaluated in any patients, including those treated at active doses and those with clinical responses. Given that c-MYC mRNA has been shown to be downregulated in vitro in both leukemic cell lines and in fresh patient bone marrow cells at OTX015 concentrations achieved in the plasma of

patients treated at active doses,[19] these clinical data were considered difficult to interpret. This discrepancy and the failure to show mRNA downregulation in vivo is not fully understood, but may be related to the delay between bone marrow collection and nucleic acid extraction. In addition, low cellularity and a mixture of leukemic and non-leukemic cells in bone marrow samples may account for discrepancies between in vitro and in vivo testing.

The clinical activity of BRD inhibition alone may be insufficient to successfully manage this patient population. All responding patients relapsed, and importantly, several additional patients not formally considered responders showed transient decreases in blasts and/or increases in neutrophils, suggesting the potential value of combination therapy. Supporting this clinical development route, additive or synergistic activity of OTX015 has been observed *in vitro* with several cytotoxic drugs and other epigenetic agents such as HDAC inhibitors and hypomethylating agents against leukaemia cell lines.[30] Combination trials are anticipated, favouring hypomethylating agents in myeloid malignancies due to possible cumulative toxicity with HDAC inhibitors.

With five acute leukaemia patients showing evidence of clinical activity, including three responses in a fragile, elderly, heavily pre-treated population, to our knowledge this is the first demonstration of a single agent BET-BRD inhibitor exhibiting clinical activity in patients with refractory/resistant AML/MDS, rendering further development of this new family of agents of great interest in myeloid malignancies. With limited clues (*i.e.*, few responders in an unselected population), the two most reliable pathways for future research are identification of predictive biomarkers and combination therapy.

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Acknowledgements

We thank the patients and their families, hospital teams, the team at Oncology Therapy Development (OTD), the CRO in charge of the study and the Safety Monitoring Committee. DCT is supported by funding from the NIHR RM/ICR Biomedical Research Centre.

Declaration of potential conflicts of interest

CB, ER, XT, NV, CGR, KY, DCT, CRo, BQ, MM, CRe, FL, KR, FL, CP, and HD received institutional funding from Oncoethix SA for this clinical trial. PH is a former stockholder and chief medical officer of Oncoethix SA. CK is an employee of the CRO managing the study on behalf of Oncoethix SA.

Authors and contributors

Authors: PH and HD were involved in the study design. CB, ER, XT, NV, CGR, KY, DCT, CRo, BQ, MM, CRe, and HD performed data collection. PH, CK, KR, FL and CP performed data analysis and interpretation. PH and HD wrote the manuscript and all authors reviewed and approved the manuscript.

Contributors (non-authors): François Montestruc (eXYSTAT, France) performed statistical analyses and prepared figures, and Sarah MacKenzie (OTD, France) performed manuscript editing.

TABLES

Table 1: Demographic and disease characteristics

	N=41
Age in years, median (range)	70 (19-85)
Males, N (%)	27 (66%)
ECOG performance status, N (%)	
0	13 (32%)
1	24 (58%)
2	4 (10%)
Treated condition, N (%)	
Acute myeloid leukaemia	36 (88%)
De novo	20
Secondary to myelodysplastic syndrome	10
Therapy-related	4
*Secondary to myeloproliferative neoplasms**	2
Prognostic group (fav/inter.I/inter.II/adv)†	4/9/9/12
Median % bone marrow blasts (range)	55 (4-100)
Acute lymphoblastic leukaemia	3 (7%)
Acute undifferentiated leukaemia	1 (2%)
Refractory anaemia with excess of blasts‡	1 (2%)
Previous therapy**	
Median number of lines (range)	2 (1-5)
Standard intensive induction therapy, N (%)	28 (68%)
Allogeneic stem cell transplantation, N (%)	9 (22%)

* Polycythemia vera (1 patient), essential thrombocytemia (1 patient). † LeukemiaNet[21] favourable/intermediate I/intermediate II/adverse (missing data 2 AML patients). ‡ 14% marrow blasts. ** Including treatment for haematological pre-leukaemic conditions.

Table 2: Dose escalation schedule

Dose Level	Dose and schedule	N patients treated (n=41)/evaluable (n=37)	N patients with DLT
1	10 mg QD (14 days/21)	3/3	0
2	20 mg QD (14 days/21)	3/3	0
3	40 mg QD (14 days/21)	4/4	0
4a	80 mg QD (14 days/21)	4/4	0
4b	40 mg BID (14 days/21)	8/6	0
5a	120 mg QD (14 days/21)	7/6	0
5b	120 mg QD (21 days/21)	6/6	0
6	160 mg QD (14 days/21)	6/5	2*

BID (bis in diem), twice a day; DLT, dose limiting toxicity; QD, once a day
*Grade 3 fatigue (1 patient), grade 3 diarrhoea (1 patient)

Table 3: Related non-hematologic adverse events in ≥ 10% of patients (any grade) and all grade 3-5 related events

Daily dose / **Schedule**	**40 mg QD14/21**		**80 mg QD14/21**		**40 mg BID14/21**		**120 mg QD14/21**		**120 mg QD21/21**		**160 mg QD14/21**	
N treated patients	4		4		8		7		6		6	
	G1-2	**G3-4**	G1-2	**G3-4**	G1-2	**G3-4**	G1-2	**G3-4**	G1-2	**G3-4**	G1-2	**G3-4**
Diarrhoea	1				1		3		4		4	**1**
Nausea	1		1	**1**	2		1		2		1	
Anorexia					2		1		2		2	
Vomiting				**1**			1		1			
Skin disorders							4		2		2	
Fatigue	1	**1**			1		4		1		1	**2**
Factor VII decrease	1				2		1		1			
Direct bilirubin increase								**1**		**2***		
Aminotransferase elevation						**1***						

* Grade 4 in 1 patient. No grade 5 events.

Table 4: Pharmacokinetic parameters (mean ± SD values)

	Plasma				Blood and bone marrow monocytes	
Dose level	**N evaluable patients (N=40)**	**C_{max} Day 1 cycle 1 (nM)**	**Trough Pre-dose day 8 cycle 1 concentration (nM)**	**$AUC_{0\rightarrow 24h}$ Day 1 cycle 1 (µg*h /L)**	**N evaluable patients (N=19)**	**Pre-dose day 8 cycle 1 concentration (nM)**
10 mg QD	3	258 (154)	29·0 (18·4)	1,218 (161)	2	1·35
20 mg QD	3	557 (230)	102 (1617)	3,171 (876)	3	1·05
40 mg QD	4	1,522 (854)	NA*	5,792 (1,347)	1	0·20
80 mg QD	5 **	2,230 (931)	274 (142) †	12,890 (4,353)	3	2·27
40 mg BID	*8*	*1,028 (348)*	NA*	*14,410 (4743)*	*NA*	*NA*
120 mg QD	12	3,756 (1,646)	585 (364) ††	18,730 (10,490)	7	2·70
160 mg QD	5	2,627 (1,162)	437 (386) ǂ	14,090 (3761)	3	3·67

NA, not available; * Data for 2 patients only (results not shown); **Includes data from one patient at DL6 (160 mg QD) but who received 80 mg QD on day 1; † Data from 3 patients; †† Data from 11 patients; ǂ Data from 4 patients.

FIGURE LEGENDS

Figure 1: Waterfall plots of maximum absolute variations during OTX015 treatment versus baseline per patient in A) percent bone marrow blasts; B) blood neutrophil counts.

Figure 2: 42-gene panel mutational profile in responders versus non-responders. The five patients with evidence of clinical activity (**A**) (three complete remissions and two partial blasts clearance) did not show a clearly different mutational profile compared to the other patients with no evidence of clinical activity (**B**). Mutated genes are indicated by pink boxes, while the absence of mutation is in blue.

A phase 1 dose-finding and pharmacokinetic study of the BET bromodomain inhibitor OTX015 in acute leukaemia patients

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SUMMARY

Background Bromodomain (BRD) and extraterminal (BET) proteins are chromatin readers that preferentially impact the transcription of genes with super-enhancers including oncogenes. BET proteins bind acetylated histone tails via their BRD, bringing the elongation complex to the promoter region. OTX015 (MK-8628) specifically binds to BRD2, 3 and 4, preventing BET proteins from binding to the chromatin, thus inhibiting gene transcription. OTX015 inhibits proliferation in a large panel of haematological malignancy cell lines and patient cells, *in vitro* and *in vivo*. A phase 1 trial was launched to determine the recommended dose of OTX015 in patients with haematological malignancies. We report here on the leukaemic cohort.

Methods In this multicentre international study, ~~The primary objective of this phase 1 trial was to determine the recommended dose (RD) of OTX015 in acute leukaemia patients failing standard therapies.~~ OTX015 was given orally at increasing doses from 10 to 160 mg/day (14/21 days), using a conventional 3+3 design. OTX015 was initially administered once daily, with allowance for exploration of other schedules. Acute leukaemia patients failing or with contraindication to standard therapies were eligible. The primary end point was dose limiting toxicity (DLT) in the first treatment cycle. Secondary objectives were to evaluate safety, pharmacokinetics, pharmacodynamics, and preliminary clinical activity. The study is registered with ClinicalTrials.gov, NCT01713582.

Findings Forty-one patients, 36 with acute myeloid leukaemia, a median age of 70 years and two lines of prior therapy~~,~~ were treated over six dose levels ~~from 10 to 160 mg/day (14/21 days)~~. No ~~dose limiting toxicity (~~DLT~~)~~ was observed until 160 mg when one patient experienced grade 3 diarrhoea and another had grade 3 fatigue. However, concomitant grade 1-2 non-DLT toxicities (gastrointestinal, fatigue, cutaneous) from 120 mg hampered patient compliance and 80 mg wa~~i~~s considered the recommended dose ~~RD~~ with a once daily schedule. Common toxicities included grade 3 fatigue in three patients and grade 3-4 bilirubin increase in two patients. OTX015 plasma concentrations increased proportionally up to 120

mg with trough concentrations in the *in vitro* active range from 80 mg. Three patients (40, 80, 160 mg) achieved complete remission (CR/CRi) lasting 2 to 5 months, and two additional patients had partial blasts clearance. No predictive biomarkers for response have been identified to date.

Interpretation OTX015 is an orally available, first-in-class BRD inhibitor with linear pharmacokinetics. The single agent recommended dose ~~RD~~ for a once daily schedule in acute leukaemia patients is 80 mg. Single agent clinical activity in myeloid malignancies was seen at non-toxic doses.

Funding Oncoethix GmbH (formerly Oncoethix SA).

PANEL: Putting research into context (box located in discussion page of the paper)

Research in context: Intensive cytarabine/anthracycline-based induction therapy, followed by intensification with or without allogeneic stem cell transplantation, allows about 50% of younger acute myeloid leukaemia (AML) patients to be cured. Nonetheless, the outcome for many patients remains poor, either because they are excluded from intensive standard induction and consolidation therapies (older populations), or because their disease is refractory or relapses after such therapies. Among novel therapeutic approaches, epigenetic manipulation has been explored with hypomethylating agents and more recently by inhibition of bromodomains (BRD), histone deacetylase (HDAC), and isocitrate dehydrogenase (IDH). Bromodomain inhibition has shown promising results in preclinical AML studies, including specific AML subsets such as those with NPM1 or IDH2 gene mutation, MLL gene rearrangement, or EVI1 gene overexpression. To date no clinical evaluations of bromodomain inhibition have been published (Pubmed search: leukaemia, bromodomain inhibitor, patient/clinical).

Added value of the study: To our knowledge, this is the first clinical evidence of a novel first-in-class bromodomain inhibitor OTX015/MK-8628 administered to patients with advanced acute leukaemia. OTX015 was safely administered at 80 mg QD with a discontinuous schedule. Evidence of activity was seen in several AML patients treated with OT0X15 doses giving plasma exposures equivalent to those resulting in activity in *in vitro* studies.

Implications of the evidence: In light of demonstrated activity in a fragile elderly AML population along with a manageable safety profile, future development of this bromodomain inhibitor in combination with other compounds active in this population is merited. Extended genetic molecular biology analyses will help guide the selection of populations most likely to benefit from this class of compound.

INTRODUCTION

Acute myeloid leukaemia (AML) is characterized by maturation blockade at an early myeloid progenitor stage, clonal proliferation/accumulation of immature blasts, and bone marrow failure. While almost half of the younger AML patients can be cured,[1] patients who are refractory/relapsing or not fit for intensive therapy (mainly the elderly) have limited therapeutic options.

Epigenetic deregulation is considered to be an important factor in AML genesis, with numerous recurrent gene alterations affecting transcriptional activity in both AML and myelodysplastic syndromes (MDS).[2–4] Hypomethylating agents, including decitabine and azacytidine, were the first epigenetic modulators approved in myeloid malignancies.[5–8] Nonetheless, with limited proven activity, other epigenetic drug families are under investigation, including histone deacetylase (HDAC), isocitrate dehydrogenase (IDH) and bromodomain (BRD) inhibitors.

BRD and extraterminal (BET) proteins are chromatin readers that play a major role in the epigenetic regulation of gene transcription.[9] Their bromodomain-bearing moiety binds to acetylated histone tails, allowing the extraterminal moiety to bring the elongation complex of the transcriptional machinery within proximity of the gene promoter region. Histone acetylation is prevalent at super-enhancer regions that control expression of various oncogenes, rendering them exquisitely sensitive to BRD inhibition.[10] BRD inhibitors are small molecules that specifically bind BRDs,[11] preventing BET proteins from binding to chromatin and thereby inhibiting gene transcription. They have raised considerable interest as a promising novel therapeutic approach in AML[12–17] and other cancers. In AML, several potential biomarkers for BRD inhibition efficacy have been reported. The *NPM1* gene mutation, present in 25-30% of adult AML, favours a BRD4-dependent core transcriptional program that renders cells particularly sensitive to BRD inhibition.[16] Less frequent

abnormalities, such as *IDH2* gene mutation,[15] *EVI1* gene overexpression,[17] or *MLL* gene rearrangement,[14] have also been reported as potential predictors, based on preclinical models.

OTX015 (MK-8628) is a 528 Dalton molecule ~~(Figure 1)~~ that binds exclusively to BRD2, 3, and 4 with an EC_{50} of 10-19 nM, inhibiting their binding to acetylated histone H4 with an IC_{50} of 92-112 nM.[18] It exhibits anti-proliferative effects against a large panel of haematological malignancy cell lines with a GI_{50} of 40-500 nM. Ten of 17 leukaemia cell lines tested were considered sensitive with an IC_{50} < 500 nM after 72 hours exposure.[19] Furthermore, primary bone marrow cells from eight of 14 AML patients were found to be sensitive to OTX015 at similar concentrations, and apoptosis was also reported. While growth inhibition has been seen with various *in vivo* tumour models,[20] animal models of acute leukaemia ~~(AL)~~ have yet to be explored.

Haematological, gastrointestinal and hepatic toxicities were limiting at the highest OTX015 doses tested in toxicology studies, with no cumulativity seen up to 12 months. No specific target organs were identified in preclinical safety pharmacology studies. To evaluate the clinical tolerance and potential of OTX015, the present phase 1 study was initiated in patients with advanced haematological malignancies, including acute leukaemia ~~AL~~ patients.

METHODS

Study design and patients

This international phase 1 dose escalation study was performed in seven centres (France, United Kingdom, and Canada; Supplemental Table 1, page 1). The primary objective was to determine the recommended dose ~~(RD)~~ of OTX015 single agent in two independent and parallel cohorts, one in acute leukaemia ~~AL~~ patients and the other in patients with non-leukemic haematological malignancies, with dose limiting toxicity (DLT) as the primary endpoint. Secondary objectives were to determine the safety profile, pharmacokinetics ~~(PK)~~,

evaluate evidence of clinical anti-tumour activity, and identify potential predictive biomarkers for efficacy. We report results here for the acute leukaemia ~~AL~~ cohort.

To be eligible patients had to be aged ≥ 18 years with relapsed/refractory acute leukaemia ~~AL~~ (except promyelocytic). Patients aged < 60 years had to have failed at least two standard regimens, while older patients had to have failed at least one regimen and have relapsed < 1 year after previous first-line therapy, or have a contra-indication for standard therapies. Previous allogeneic SCT was allowed, provided relapse had occurred > 90 days later. Graft-*versus*-host disease symptoms or treatment was not permitted at study entry. Patients were required to have an Eastern Cooperative Oncology Group performance status of 0 to 2, normal bilirubin, aminotransferases ≤ three times the upper normal limit, and creatinine clearance ≥ 30 mL/min, with a life expectancy of ≥ 3 months. All patients provided written informed consent. Regulatory approval was obtained from all national authorities, and all local institutional review boards approved the protocol as appropriate. The study was conducted in accordance with the Declaration of Helsinki and registered with ClinicalTrials.gov, number NCT01713582.

Procedures

The study started with a once daily (QD) schedule and allowed for exploration of other schedules according to SMC decision. Eligible patients received OTX015 delivered orally as 10 or 20 mg gelatine capsules, under fasted conditions, daily for 14 consecutive days, followed by a 1-week rest (21-day cycles), at a starting dose of 10 mg ~~once daily~~QD. A conventional 3+3 dose escalation schedule was used with three patients initially per dose level. Doses were escalated after three patients had received at least one cycle as planned without ~~dose limiting toxicity (~~DLT~~)~~. Doses were doubled until DLT occurred at which point three additional patients were treated at this dose. If no more than one of the six patients experienced DLT, dose escalation continued using the more conservative, Fibonacci-like model. The highest dose level with no more than one of six patients experiencing DLT was

considered the ~~RD~~recommended dose. On the basis of preclinical models, the possibility that continuous exposure at active concentrations was necessary for optimal activity was evaluated after 80 mg QD with a BID schedule (40 mg x 2).

DLT was defined as any one of the following occurring during the first cycle: a National Cancer Institute Common Toxicity Criteria grade 3 or 4 non-haematological related event despite optimal supportive care, grade 3 or 4 asymptomatic non-haematological laboratory abnormality lasting more than 7 days, blast-free aplasia confirmed as lasting ≥ 42 days, or a grade 2 event leading to dose interruption or adaptation. If such toxicities occurred at any time during treatment, OTX015 was interrupted until recovery to grade ≤ 1 (or baseline value) and treatment resumed at the dose level immediately below. A safety monitoring committee (SMC) composed of all principal investigators, the sponsor's medical and safety officers, and an independent expert made all decisions regarding patient safety, DLT qualification, dose escalation, and recommended dose ~~RD~~ definition. Treatment was continued until progression, intolerable toxicity, treatment interruption for >2 weeks due to toxicity, DLT recurrence despite dose reduction, or withdrawal of consent.

Standard haematology and biochemistry workups were done weekly during the first cycle, then every 3 weeks in the absence of toxicity, and 12-lead ECGs were performed pre-dose and around T_{max} on cycle 1 day 1. Bone marrow aspirations were performed at baseline, days 8, 22 and 43, and thereafter as needed. Response was assessed according to European LeukemiaNet.[21] Partial blast clearance was defined as a >50% reduction in bone marrow blast percentage without persistent circulating blasts.

Mutational analyses were performed in DNA from baseline bone marrow samples to assess a 42-gene panel of genes commonly mutated in AML and MDS (see Supplemental Table 2, page 2) using a MiSeq® platform (Illumina). The library was prepared using the Haloplex™ Target Enrichment System (Agilent Technologies).

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Pharmacokinetics

Plasma OTX015 concentrations were measured in blood samples (3 mL) collected into K2-EDTA tubes prior to dosing then at 1, 4, 6, 8, 12, 16, and 24 hours after drug intake, immediately centrifuged and stored at -20 °C. Samples for residual plasma concentration were collected on days 8, 15 and 22 just prior to dosing. Intracellular OTX015 concentrations were measured from bone marrow and blood monocytes collected on day 8, immediately before dosing. Bone marrow and blood were collected into BD Vacutainer CPT™ tubes, stored at room temperature, and mononuclear cells were separated on a Ficoll gradient. OTX015 concentrations were measured using a validated method using Ultra Performance Liquid Chromatography with tandem Mass Spectrometry (UPLC-MS/MS).[22] Analyses were performed using Monolix software (version 4.3).

Analyses

Patients receiving at least 85% of the cumulative intended dose over the first 21 days were evaluable for DLT. All treated patients were evaluated for safety. Patients were evaluable for efficacy according to the European LeukemiaNet.[21] Descriptive analyses were performed with a cut-off of 14 February 2015 using SAS software (version 9.4).

Role of the funding source

This study was sponsored, funded and initiated by Oncoethix SA (now Oncoethix GmbH, a wholly owned subsidiary of Merck Ltd). It was designed by the sponsor in collaboration with the investigators. Data collection, analysis, and interpretation were performed by the investigating research teams, the study CRO and Oncoethix. The manuscript was written by Oncoethix with the corresponding author, then approved by all authors. The corresponding author had access to all study data and confirms the decision to submit.

RESULTS

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Between 18 January 2013 and 9 September 2014, 40 acute leukaemia ~~AL~~ patients and one patient with high-risk MDS were treated over six dose levels. The population was mainly elderly and the majority of patients had AML (36 patients; 88%), more than one-third of whom were secondary to pre-leukaemic conditions or therapy-related (16 patients; 39%) (**Table 1**).

Dose escalation is summarised in **Table 2**, showing that doses were doubled without DLT over the first four dose levels (10 to 80 mg). To test biological activity with continuous exposure at high concentrations, a BID schedule was explored at dose level 4 (40 mg x 2/day). Although no DLT occurred, evaluation of the parallel cohort of non-leukaemic patients showed an excess of thrombocytopenia without an obvious impact on activity for this BID schedule,[23] along with increased frequency of non-haematological toxicities (gastrointestinal, laboratory). In addition, several patients without severe thrombocytopenia at baseline experienced aggravation of thrombocytopenia on treatment, requiring treatment interruption and dose adaptation, with treating physicians deciding against continuing OTX015 treatment with this dosing schedule. Furthermore, given that responses were observed in leukaemia patients with the QD schedule (and not with the BID schedule), dose escalation was resumed with a QD schedule at 120 mg. No DLT was observed until 160 mg. At this dose, two of five evaluable patients experienced DLT, one had grade 3 fatigue and one had grade 3 diarrhoea. The 160 mg QD dose was therefore considered not tolerable and additional patients were enrolled at 120 mg to confirm tolerance and optimize the schedule with both the original intermittent schedule (14 days out of 21) and a continuous schedule. Although DLT was not reported among the 13 patients treated at 120 mg regardless of schedule, the SMC considered that the cumulative prevalence of several gastrointestinal

events and fatigue (**Table 3**) was poorly tolerated in this elderly, heavily pre-treated population with frequent co-morbidities, hampering patient compliance. Furthermore, active plasma trough concentrations achieved at 80 mg (**Table 4**) along with the lack of a clear dose-effect relationship led to the selection of 80 mg, 14 days on/7 days off as the recommended dose ~~RD~~ for the QD schedule.

Patients received a median of two cycles (range 1-14). Three patients (treated at 40 mg BID and 160 mg QD) had dose reductions due to grade 2-3 non-haematological toxicity. **Table 3** shows the main adverse events considered related to OTX015. Few events (all of which were grade 1 or 2) occurred at the first two dose levels. The most common events were gastrointestinal (diarrhoea: 14 patients, 34%; nausea: 9 patients, 22%), fatigue (11 patients; 27%), and cutaneous (8 patients; 20%), with increasing frequency and severity above 80 mg QD including at 40 mg BID. Asymptomatic laboratory abnormalities included grade 1-2 factor VII decrease (lowest values of 20-30% with a modest impact on INR) in five patients without associated coagulation factor deficiency. Positive de-challenge/re-challenge in one patient confirmed the relationship to study treatment, however treatment discontinuation was not necessary. Three patients experienced isolated (without other liver test dysfunctions) direct bilirubin increases (all grade 3-4 and generally associated with neutropenic sepsis), with a positive de-challenge/re-challenge in one patient suggesting this was related to OTX015. One patient had recurrent transient grade 3 elevation of aminotransferases, although this patient had a similar history when treated with chemotherapy for breast cancer. Grade 3 or 4 toxicities were infrequent, all of which were reversible within ten days of drug interruption. Peripheral blood cytopenia was not considered for the safety assessment, nevertheless in several patients without severe thrombocytopenia at baseline, aggravation of thrombocytopenia occurred on study treatment. No significant changes in QTc interval were seen. No treatment related deaths occurred and no patients discontinued due to related AEs.

Table 4 shows the ~~PK~~ pharmacokinetics parameters for all patients evaluable for pharmacokinetics~~PK~~. Plasma exposure increased proportionally with dose from 10 to 120 mg QD. Terminal half-life was 5.79 hours ± 1.12.[24] No accumulation trend was observed until day 15. Intracellular OTX015 concentrations were measured on day 8 pre-dose in 19 patients and showed low concentrations, in the nanomolar range. Although intracellular concentrations increased with dose, they remained in the single-digit nanomolar range, and the magnitude of the concentration increase is much lower in cells (3-4 fold from 10 to 160 mg OTX015) than in plasma (30-40 fold from 10 to 160 mg).

Activity was seen at a range of dose levels. One AML patient achieved complete remission (~~CR;~~ 40 mg QD), another AML patient achieved complete remission ~~CR~~ with incomplete recovery of platelets (CRi; 80 mg QD), and a third patient with refractory anaemia with excess of blasts (RAEB) also achieved complete remission~~CR~~ (160 mg QD)(~~Table 5~~Supplemental Table 3, page 3). Two other patients with AML secondary to polycythemia vera and MDS, treated at 10 mg and 80 mg QD respectively, experienced partial blast clearance, and the latter patient also had a transient increase of neutrophil counts above 1.0 x 10^9/L. ~~**Figure 2** illustrates the kinetics of response in the patient with CR treated at 40 mg QD. Some signs of activity were apparent during cycles 1 and 2, however the maximum effect was not seen until cycles 3 and 4.~~ All three patients with responses relapsed on therapy, the two complete remissions ~~CRs~~ lasting 5 and 3 months and the CRi lasting 2 months. The patient with RAEB treated at 160 mg QD had prolonged treatment interruptions that could potentially have contributed to relapse. **Figure 1~~3~~A** shows maximum variation in bone marrow blast percentages per patient during treatment and **Figure ~~3~~1B** shows maximum variation in absolute neutrophil counts (ANC). Interestingly, 13 patients had a maximal ANC increase of more than 2 x 10^9/L during treatment relative to baseline, including non-responding patients.

Genetic analyses did not show any obvious correlations between mutations present in the five responders versus those in the 28 non-responders at study entry (**Figure 24**). Of note, neither *NMP1* mutations (one *NPM1* mutated patient was a responder *versus* six non-responders) nor *IDH2* mutations (no responders among two mutated patients) correlated with clinical activity. One responding patient was flt-3 wild-type, while three of the six non-responders had flt-3 internal tandem duplication. No hints of clinical activity were observed in three patients with *EVI1* overexpression or the two patients with *MLL*-rearrangement.

DISCUSSION

Here we report, to our knowledge, the first clinical investigation with a BRD inhibitor. OTX015 was safely administered at a dose of 80 mg QD in pre-treated acute leukaemia ~~AL~~ patients, with linear pharmacokinetics ~~PK~~ over the doses evaluated up to 120 mg. At this dose, plasma concentrations were maintained above 400 nM, a concentration known to be active *in vitro*.[19] Nonetheless, low OTX015 intracellular concentrations were found in day 8 bone marrow and peripheral blood monocytes, compared with trough plasma concentrations. This is coherent with preclinical studies reporting that 500 nM extracellular *in vitro* concentrations resulted in intracellular concentrations approximately ten to fifty times lower at which anti-proliferative activity was seen.[25] Taken together, *in vitro* and clinical data show that low intracellular concentrations in the nanomolar range can be active.

Four of the five patients with anti-leukemic activity were treated at 80 mg QD or lower, offering pharmacodynamic evidence, in the absence of known off-target effects, that OTX015 does indeed attain its targets and triggers a biological effect in this dose range. Increasing the dose above this level increased toxicity without a clear impact on efficacy. The responding patient treated at 160 mg QD experienced recurrent toxicities that led to prolonged treatment interruption periods, potentially jeopardising efficacy outcome and/or duration of response.

From preclinical data showing a rapid washout effect,[20] it was anticipated that prolonged high concentrations, equal or superior to active *in vitro* concentrations, would be necessary for optimal clinical activity. A BID schedule was thus tested in order to increase trough concentrations, achieving similar exposure as the equivalent QD dose. While a BID schedule for continuous exposure is desirable, the excess of thrombocytopenia and various non-haematological toxicities seen in the BID cohort resulted in the SMC's decision not to pursue treating patients with the BID schedule at higher doses. No evidence of clinical activity was seen in the eight patients treated with the BID schedule. In addition, decisions made during the study involving pharmacokinetic data also took into account data from the non-leukemic cohort, for which some trough concentration data were available.[23] While a BID schedule offers the advantage of erasing peak concentrations, there is no evidence that any of the toxicities reported are peak-related. In the absence of evidence of a benefit with a BID schedule over a QD schedule, the latter schedule is selected to optimise patient convenience and compliance.

OTX015-related AEs were manageable, being mainly mild to moderate at the recommended dose~~RD~~, while severe toxicity rapidly resolved after drug interruption. The spectrum of toxicity (*i.e*. gastrointestinal, fatigue, and cutaneous) resembles that of HDAC inhibitors,[26] another family of epigenetic drugs. OTX015-related haematological toxicity, mainly thrombocytopenia, has been found to be dose-limiting in non-leukaemic patients.[23] While this toxicity is not easily evaluable in acute leukaemia ~~AL~~ patients, the few patients with incomplete bone marrow failure at baseline, experienced severe thrombocytopenia. Similarly, the patient treated at 40 mg who achieved prolonged complete remission ~~CR~~ had grade 1 thrombocytopenia at day 15 while in remission that recovered completely after 1 week off therapy ~~**(Figure 2)**~~. Several cases of isolated asymptomatic factor VII decreases were seen in the absence of other vitamin K-dependent factor deficiencies, as was also observed in lymphoma and myeloma patients.[23] In vitro analyses in human HepaRG hepatocyte-like cells

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exposed to OTX015 suggest that the underlying mechanism ~~may relate to~~involves inhibition of factor VII gene expression (F. Bertoni, personal communication). While two of the three patients with hyperbilirubinemia also experienced factor VII decreases, these events are considered unlikely to be related, with hyperbilirubinemia probably caused by reduced bilirubin transport across the canalicular membrane following MRP/ABCC2 downregulation or by competitive exclusion of bilirubin due to extrusion of OTX015 via the MRP/ABCC2 transporter.[27–29]

~~Taken together, these safety outcomes~~ During dose escalation, four patients were treated at 80 mg QD without DLT. With six patients treated at 160 mg QD and 13 patients at 120 mg QD (different administration schedules), all without DLT, 80 mg QD was established by the SMC as the recommended dose at which additional patients were to be evaluated in an expansion cohort. Taken together, the treatment-limiting thrombocytopenia and recovery (which was incomplete in some cases) with a 1-week break led to the recommendation of an intermittent schedule to ensure an adequate opportunity to recover from any toxicity. Although it may be argued that a drug holiday can impact efficacy, responses were seen with an intermittent schedule which was deemed necessary not only from a safety perspective but also to allow long-term treatment. In addition, evaluation of ~~alternative~~ BID ~~dose~~ schedules at doses below the ~~once daily~~QD recommended dose ~~RD~~ of 80 mg is anticipated to address presumed on-target effects of thrombocytopenia along with the importance of continuous sustained exposure (steady state C_{trough}), based on the mechanism of action of OTX015.

Preliminary molecular biology analyses did not detect any clinical or genetic predictive factors of clinical activity from the panel of candidates evaluated in this acute leukaemia ~~AL~~ population, including in patients with putative predictive markers based on preclinical modelling data. Given the small sample size, further efforts are needed to elucidate which patients are more likely to respond to BRD inhibition, so as to enrich populations in future

clinical trials. Although many epigenetic changes are driven by genetic alterations, epigenetic rather than genomic investigations may be more successful. Pharmacodynamic analyses evaluating the expression of seven oncogenes (c-MYC, BCL2, CCND1, NF-kB, BRD2/3/4) in bone marrow cells at baseline and after 1 week of treatment were performed (results not shown). mRNA downregulation was not observed for any of the genes evaluated in any patients, including those treated at active doses and those with clinical responses. Given that c-MYC mRNA has been shown to be downregulated in vitro in both leukemic cell lines and in fresh patient bone marrow cells at OTX015 concentrations achieved in the plasma of patients treated at active doses,[19] these clinical data were considered difficult to interpret. This discrepancy and the failure to show mRNA downregulation in vivo is not fully understood, but may be related to the delay between bone marrow collection and nucleic acid extraction. In addition, low cellularity and a mixture of leukemic and non-leukemic cells in bone marrow samples may account for discrepancies between in vitro and in vivo testing.

The clinical activity of BRD inhibition alone may be insufficient to successfully manage this patient population. All responding patients relapsed, and importantly, several additional patients not formally considered responders showed transient decreases in blasts and/or increases in neutrophils, suggesting the potential value of combination therapy. Supporting this clinical development route, additive or synergistic activity of OTX015 has been observed *in vitro* with several cytotoxic drugs and other epigenetic agents such as HDAC inhibitors and hypomethylating agents against leukaemia cell lines.[30] Combination trials are anticipated, favouring hypomethylating agents in myeloid malignancies due to possible cumulative toxicity with HDAC inhibitors.

With five acute leukaemia ~~AL~~ patients showing evidence of clinical activity, including three responses in a fragile, elderly, heavily pre-treated population, to our knowledge this is the first demonstration of a single agent BET-BRD inhibitor exhibiting clinical activity in patients with refractory/resistant AML/MDS, rendering further development of this new family of

agents of great interest in myeloid malignancies. With limited clues (*i.e.*, few responders in an unselected population), the two most reliable pathways for future research are identification of predictive biomarkers and combination therapy.

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Acknowledgements

We thank the patients and their families, hospital teams, ~~and~~ the team at Oncology Therapy Development (OTD), the CRO in charge of the study and ~~, in particular Fabrice Bourdel (project manager), Eugenia Riveiro (senior scientist), Sarah MacKenzie (medical writer), and the Clinical Research Associates. We also thank Prof. Michel Marty (Hôpital Saint-Louis, Paris) as an independent consultant on~~ the ~~-~~Safety Monitoring Committee ~~and François Montestruc (eXYSTAT, France) for statistical analyses~~. DCT is supported by funding from the NIHR RM/ICR Biomedical Research Centre.

Declaration of potential conflicts of interest

CB, ER, XT, NV, CGR, KY, DCT, CRo, BQ, MM, CRe, FL, KR, FL, CP, and HD received institutional funding from Oncoethix SA for this clinical trial. PH is a former stockholder and chief medical officer of Oncoethix SA. CK is an employee of the CRO managing the study on behalf of Oncoethix SA.

Authors and contributors

Authors: PH and HD were involved in the study design. CB, ER, XT, NV, CGR, KY, DCT, CRo, BQ, MM, CRe, and HD performed data collection. PH, CK, KR, FL and CP performed data analysis and interpretation. PH and HD wrote the manuscript and all authors reviewed and approved the manuscript.

Contributors (non-authors): François Montestruc (eXYSTAT, France) performed statistical analyses and prepared figures, ~~Eugenia Riveiro prepared figures,~~ and Sarah MacKenzie (OTD, France) performed manuscript editing.

TABLES

Table 1: Demographic and disease characteristics

	N=41
Age in years, median (range)	70 (19-85)
Males, N (%)	27 (66%)
ECOG performance status, N (%)	
0	13 (32%)
1	24 (58%)
2	4 (10%)
Treated condition, N (%)	
Acute myeloid leukaemia	36 (88%)
De novo	20
Secondary to myelodysplastic syndrome	10
Therapy-related	4
*Secondary to myeloproliferative neoplasms**	2
Prognostic group (fav/inter.I/inter.II/adv)[†]	4/9/9/12
Median % bone marrow blasts (range)	55 (4-100)
Acute lymphoblastic leukaemia	3 (7%)
Acute undifferentiated leukaemia	1 (2%)
Refractory anaemia with excess of blasts[‡]	1 (2%)
Previous therapy[**]	
Median number of lines (range)	2 (1-5)
Standard intensive induction therapy, N (%)	28 (68%)
Allogeneic stem cell transplantation, N (%)	9 (22%)

* Polycythemia vera (1 patient), essential thrombocytemia (1 patient). [†] LeukemiaNet[21] favourable/intermediate I/intermediate II/adverse (missing data 2 AML patients). [‡] 14% marrow blasts. ** Including treatment for haematological pre-leukaemic conditions.

Table 2: Dose escalation schedule

Dose Level	Dose and schedule	N patients treated (n=41)/evaluable (n=37)	N patients with DLT
1	10 mg QD (14 days/21)	3/3	0
2	20 mg QD (14 days/21)	3/3	0
3	40 mg QD (14 days/21)	4/4	0
4a	80 mg QD (14 days/21)	4/4	0
4b	40 mg BID (14 days/21)	8/6	0
5a	120 mg QD (14 days/21)	7/6	0
5b	120 mg QD (21 days/21)	6/6	0
6	160 mg QD (14 days/21)	6/5	2*

BID (bis in diem), twice a day; DLT, dose limiting toxicity; QD, once a day
*Grade 3 fatigue (1 patient), grade 3 diarrhoea (1 patient)

Table 3: Related non-hematologic adverse events in ≥ 10% of patients (any grade) and all grade 3-45 related events

Daily dose Schedule	40 mg QD14/21		80 mg QD14/21		40 mg BID14/21		120 mg QD14/21		120 mg QD21/21		160 mg QD14/21	
N treated patients	4		4		8		7		6		6	
	AnyG1-2	G3-4	G1-2Any	G3-4	G1-2Any	G3-4	G1-2Any	G3-4	G1-2Any	G3-4	G1-2Any	G3-4
Diarrhoea	1				1		3		4		54	**1**
Nausea	1		21	**1**	2		1		2		1	
Anorexia					2		1		2		2	
Vomiting			1	**1**			1		1			
RashSkin disorders							4		2		2	
Fatigue	21	**1**			1		4		1		31	**2**
Factor VII decrease	1				2		1		1			
Direct bilirubin increase							1	**1**	2	**2***		
Aminotransferase elevation					1	**1***						

* Grade 4 in 1 patient. No grade 5 events.

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Table 4: Pharmacokinetic parameters (mean ± SD values)

	Plasma				Blood and bone marrow monocytes	
Dose level	**N evaluable patients (N=40)**	**C_{max} Day 1 cycle 1 (nM)**	**Trough Pre-dose day 8 cycle 1 concentration (nM)**	**$AUC_{0\rightarrow24h}$ Day 1 cycle 1 (µg*h /L)**	**N evaluable patients (N=19)**	**Pre-dose day 8 cycle 1 concentration (nM)**
10 mg QD	3	258 (154)	29·0 (18·4)	1,218 (161)	2	1·35
20 mg QD	3	557 (230)	102 (1617)	3,171 (876)	3	1·05
40 mg QD	4	1,522 (854)	NA*	5,792 (1,347)	1	0·20
80 mg QD	5**	2,230 (931)	274 (142) †	12,890 (4,353)	3	2·27
40 mg BID	*8*	*1,028 (348)*	NA*	*14,410 (4743)*	*NA*	*NA*
120 mg QD	12	3,756 (1,646)	585 (364) ††	18,730 (10,490)	7	2·70
160 mg QD	5	2,627 (1,162)	437 (386) ǂ	14,090 (3761)	3	3·67

NA, not available; * Data for 2 patients only (results not shown); **Includes data from one patient at DL6 (160 mg QD) but who received 80 mg QD on day 1; † Data from 3 patients; †† Data from 11 patients; ǂ Data from 4 patients.

Table 5: Characteristics of patients with standard responses

	Patient 1 (CR)	**Patient 2 (CRi)**	**Patient 3 (CR)**
OTX015 dose level	40 mg QD	80 mg QD	160 mg QD
Age/gender	74/F	72/M	62/M
Significant prior history / known pre-leukaemic condition	Monoclonal IgM-induced neuropathy controlled with 9 cycles of RFC	MDS treated with azacitidine	Inv(16) AML controlled with standard induction therapy and high-dose busulfan/ASCT
Treated condition	Post-treatment AML	Post-MDS AML	Post-treatment RAEB
Prior systemic therapy for the treated condition	LDAC	Idarubicin/cytarabine investigational agent	Azacytidine
Bone marrow blasts at baseline	94%	85%	14%
Presence of peripheral blood blasts at baseline	YES	YES	NO
Myelodysplastic features	YES	YES	YES
Karyotype	Normal	Normal	Complex; without Inv(16)
Molecular biology	No abnormality	NPM1 *mut** *flt3 wt*	P53 *mut*

ASCT: autologous stem cell transplantation; LDAC: low-dose cytarabine; MDS: myelodysplastic syndrome; *mut*: mutated; NPM1: nucleophosmin 1 gene; *wt*: wild type; RAEB: refractory anaemia with excess of blasts; RFC: rituximab, fludarabine, cyclophosphamide.
* NPM1/ABL1 mRNA ratio 1937% at baseline; dropped to 0.039% at the end of cycle 1.

FIGURE LEGENDS

Figure 1: OTX015 chemical formula. 2-[(6S)-4-(4-Chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo-[4,3-a]-[1,4]diazepin-6-yl]-N-(4-hydroxyphenyl)acetamide dehydrate.

Figure 2: Response kinetics in a patient with complete remission (40 mg QD). This 74-year-old female with post-therapy AML had no evidence of activity before the end of cycle 1, with decreased marrow blasts at day 22. Clearance of peripheral blood blasts was seen during cycle 2. Transfusions were no longer required from cycle 3, and neutrophils and platelets gradually increased to normal values. The maximum therapeutic effect was seen at the end of cycle 4.

Figure ~~3~~1: Waterfall plots of maximum absolute ~~variations~~ variations during OTX015 treatment versus baseline per patient in A) percent bone marrow blasts; B) blood neutrophil counts.

Figure 2~~4~~: 42-gene panel mutational profile in responders versus non-responders. The five patients with evidence of clinical activity (**A**) (three complete remissions and two partial blasts clearance) did not show a clearly different mutational profile compared to the other patients with no evidence of clinical activity (**B**). Mutated genes are indicated by pink boxes, while the absence of mutation is in blue.

Lan-Lan Smith, PhD
Editor-in-Chief
The Lancet Haematology

02 November 2015

Ref: THELANCETHAEMATOLOGY-D-15-00236R1

Dear Dr Smith,

Following your email of 16 October 2015, we are pleased to re-submit our revised manuscript entitled "A phase 1 dose-finding and pharmacokinetic study of the BET bromodomain inhibitor OTX015 in acute leukaemia patients".

Below are our responses to the editorial comments made by Lancet Haematology. A tracked changes version of the article is provided integrating these responses, along with a clean version.

Sincerely,

Prof Hervé Dombret, MD, PhD
Institut Universitaire d'Hématologie
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75010 Paris, France

Editorial comments

1. Please spell out two word abbreviations (eg acute leukaemia). Done (AL, RD, PK, CR)
2. Please delete figure 1 from the manuscript. Done
3. We do not include representative patient data as figures in articles, please add some additional figures for other patients as well (both responders and non-responders). Figure 2 has been deleted. Putting in data for several patients will make this very difficult to read and including data for non-responders would not make a lot of sense for the reader.
4. Please move table 5 to the appendix. Done
5. As multiple reviewers requested that the pharmacodynamics data (and because it was a pre-specified endpoint of the trial), please include in your discussion the reasons for not having this data and discuss the limitations to the interpretation of your results from not having it. Done (discussion) – note that the conclusion already highlights that further biomarker research is the way forward.
6. Reviewer1, point 3: Please mention that three patients had both bilirubin elevation and decrease in factor VII, but that you do not think this was connected. Done (discussion section; this was in fact two of the three pts).
7. Reviewer 2, point 1: Please add this information about myelotoxicity at 40 BID. Done (results section).
8. Reviewer 5, point 2, 3, and 4: Please add additional discussion on the dosing schedule. Done – point 3 (drug holiday) was already added to the discussion.

9. Please check with your co-authors, and confirm, that all names are spelt correctly, and affiliations listed correctly. We cannot guarantee that we will be able to correct names and affiliations after publication of your article. Modifications made in track changes to the Word version.

10. The study title should have a descriptor—ie, randomised trial, case-control study, prospective analysis, population-based study etc… the study title already includes "phase I dose-finding and pharmacokinetic" and has not been further modified.

11. Please indicate in the authorship if any authors are full professors. "Prof." added where appropriate. Note that several authors pointed out they have a PhD also- however I understand only the highest degree is included. I have added them in case.

12. Summary: Your abstract should conform to the CONSORT guidelines for abstracts (CONSORT for Abstracts: Lancet 2008; 371: 281-83), and must include:

a) Background: A sentence indicating the aim of this study.

b) Methods: A brief summary of the main patient characteristics (ie, main entry criteria)

c) Methods: Details of the regimens used.

d) Methods: An explicit description of the actual primary endpoint.

e) Methods: The nature by which analyses were done (eg, intention to treat, per protocol).

f) Methods: The trial registration number.

g) Methods: The status of the trial - is it ongoing/still enrolling/is this an interim analysis, etc?

h) Findings: Efficacy data for the primary endpoint only.

i) Findings: The most common (grade 3-4) adverse events (incl actual numbers of patients affected); any serious adverse events.

j) Interpretation: please do not just restate your findings. What do they mean, clinically? What are their implications?

See recent issues of the journal for examples. At this stage, please do not worry about the word length of the abstract - accuracy and completeness here are essential.

Done (with adaptation for a phase 1)

13. Please confirm that your study conforms to the CONSORT guidelines by completing and returning the checklist (please disregard the section on randomisation).

CONSORT - for RCTs - http://download.thelancet.com/flatcontentassets/authors/tlo-consort-checklist.pdf

Done (with adaptation for a phase 1)

14. If you have included such data for a drug(s), please confirm that the dose, route, and frequency of administration (and the form: eg, a particular salt) are correct.

Done

15. It is Lancet style is to give actual numbers (numerator and denominator) together with percentages.

Done where feasible – as a phase I study with small numbers, % are not always relevant.

16. Lancet style is to provide p values to two significant figures, unless $p<0.0001$ (if this is the case, then please revise to the latter). Not applicable

17. Methods, patient selection. Please ensure that the following items are included:

a) Disease setting, including permitted histologies and/or molecular abberations.

b) Age limit for eligibility.

c) Indicate the means of disease evaluation (eg, RECIST, etc)
d) Permitted performance status(es) defined.
e) An indication of estimated survival of eligible patients.
f) Laboratory tests required to assess eligibility.
g) Previous treatments permitted/not permitted (and washout period(s)).
h) If the trial is second-line (or above), mention criteria for determining progression at inclusion.
i) Comorbidities permitted/not permitted.
j) Nature of consent sought and IRB approval.

Done where applicable

18. Methods: procedures. Please ensure that the following items are included:

a) Planned doses of treatment(s).
b) Duration of treatment/number of cycles planned.
c) Criteria for a patient to be removed from the study.
d) Details of permitted dose reductions/interruptions.
e) Type and frequency of radiographic assessments.
f) If applicable, whether or not the primary endpoint was centrally reviewed.
g) Frequency and type of laboratory monitoring.

Done

19. Methods: Outcomes: Please ensure the following items are included:

a) Definition of the primary endpoint.
b) Definition of all secondary endpoints.

Done

20. Methods: Statistical analysis. Please ensure the following items are included:

a) Hypothesis, power calculation, and derivation of sample size.
b) Definitions of population assessed for primary and secondary outcomes, and for safety (eg, ITT, per protocol, etc).
c) Rules for defining patients as not assessable.
d) Statistical methods for analysis of the primary and secondary outcomes.
e) Any sensitivity analyses, etc.
f) Software package (and version number) used for statistical analyses.
g) The trial registration number - "This study is registered [as an International Standard Randomised Controlled Trial/with ClinicalTrials.gov/similar], number [ISRCTNxxxxxxxx/NCTxxxxxxx/similar]."

Done where applicable

21. As I am sure you are aware, the Lancet group are very supportive of protocol-based research and so have recently decided to encourage authors to post the protocol document on a publicly accessible website; a margin link to the website will then be put in the paper. Would you like to do this for your protocol? If so, please send us the protocol link with your final corrections. Please note that if you do wish to do this then the weblink should not be temporary.

The Sponsor's policy is to allow a journal to post on its website at the time of publication and once the article has been accepted, the critical sections of the protocol that are relevant to evaluating the study, specifically the study objectives and hypotheses, patient inclusion and exclusion criteria, study design and procedures, efficacy and safety measures, statistical analysis plan, and any amendments

relating to those sections. Merck reserves the right to redact proprietary information. Merck will also retain copyright of the protocol but will allow the journal to publish the critical sections described above on its website with a link to the published paper. Any further use of the protocol, including these critical sections, will require Merck's permission. Lancet Haematology will receive a message from Merck on acceptance of the article with details of who to contact to obtain the relevant protocol sections for posting. Is this suitable for you?

22. Results, please give the exact dates between which patients were enrolled—ie, day, month, year. Done

23. Results: please explicitly state the number of patients included in analyses, and the number of patients deemed ineligible (and reasons why). Done

24. Results: Please give median follow-up (and IQR) for the analyses presented Not applicable

25. Results: Safety and tolerability data. Please ensure that the following items are included:

a) Data regarding number of patients who required dose reductions.

b) Data regarding number of patients who discontinued for drug-related toxicity.

c) Data regarding the number of treatment-related deaths.

Done

26. The adverse events table should be stratified by grades 1-2, 3, 4 and 5. For adverse events of grade 1 or 2, any occurring in ≥10% of patients should be reported. All grade 3, 4, and 5 events should be reported. Done

27. Please add 95% CI to all time-to-event data and other data derived from Kaplan-Meier analyses. Not applicable

28. The Discussion should start with a summary of the main findings of this study. Done

29. If you have claimed a first, please reword to: "To our knowledge… this is the first time...", since you can never be 100% sure. Done

30. We require completed author contribution forms from all authors listed (that they agree with the submission and content and to being listed), declaring their contribution to the article, and stating the role of the funding source. The form can be downloaded at http://download.thelancet.com/flatcontentassets/authors/tlhae-author-signatures.pdf
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31. We require completed ICMJE declaration forms from all authors listed declaring any potential conflicts of interest. The form can be found at http://download.thelancet.com/flatcontentassets/authors/icmje-coi-form.pdf

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32. We require written and signed consent from any individuals who are cited in the acknowledgments section. The following format can be used and a signed statement submitted with the revised manuscript:

* "I permit <corresponding author> et al to list my name in the acknowledgments section of their manuscript and I have seen a copy of the paper <full article title>." Text modified for some and document attached for DCT.

33. Please ensure that reference numbering throughout the manuscript is not inserted with electronic referencing software, such as Endnote. Done

34. Please supply figures as high-resolution EPS format, exported directly from your statistical

package if possible, rather than embedded in a Word file. For more information, see download.thelancet.com/flatcontentassets/authors/artwork-guidelines.pdf Done for figure 1a/b; Fig 2 is jpeg (as recommended in guidelines)

35. As your study reports on a multi-centred trial, please provide a list for the appendix including each site from which patients were recruited, the name of the principle investigator responsible for this site, and the number of patients which were recruited from that particular site. This list should be ordered from the centres which contributed the greatest number of patients to the trial being listed first, to that which contributed the least listed last. Done

36. We are not able to refer to specific tables and figures in the appendix. Please ensure all your supplementary materials are compiled into a single document and paginated, and then please refer to the page on which the item appears in the manuscript to reference that item.

37. It is no longer Lancet policy to edit or style supplementary material for the web; however, this material will still be hosted on our website as a pdf of the author supplied file. Please style your supplementary material as per the guidelines below. Please note that we will be unable to correct any errors in the web appendix following publication; as such, please check carefully when submitting. Please supply the webappendix as a single PDF file, with the pages paginated.
Done

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* Text should be in 10 point Times New Roman font, single spaced
* Headings should be in 10 point BOLD

Tables

* Main table heading should be in 10 point Times New Roman font BOLD
* Legends should be in 10 point, single spaced
* Tables should be in 8 point Times New Roman font, single spaced
* Headings within tables should be in 8 point BOLD

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* SI units are required
* Numbers in text and tables should always be provided if % is shown.
* Means should be accompanied by SDs, and medians by interquartile range.
* Exact p values should be provided, unless p<0·0001

Drug names

* Recommended international nomenclature (rINN) is required

References

* Vancouver style (eg, Smith A, Jones, B, Clements S. Clinical transplantation of tissue-engineered airway. Lancet 2008; 372: 1201-09. Hourigan P. Ankle injuries. In: Sports medicine. Chan D, ed. London: Elsevier, 2008: 230-47.)
* Numbered in order of mention in Web Appendix and numbered separately from references in the full paper

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* Written consent from all parties must be supplied at submission

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* Audio material should be submitted as an mp3 file, duration up to 10 minutes

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* Video material should preferably be submitted in .mpg (or .mov, .avi, or .gif) format with Aspect ratio of 16:9, no larger than 40 Mb

Figure1A

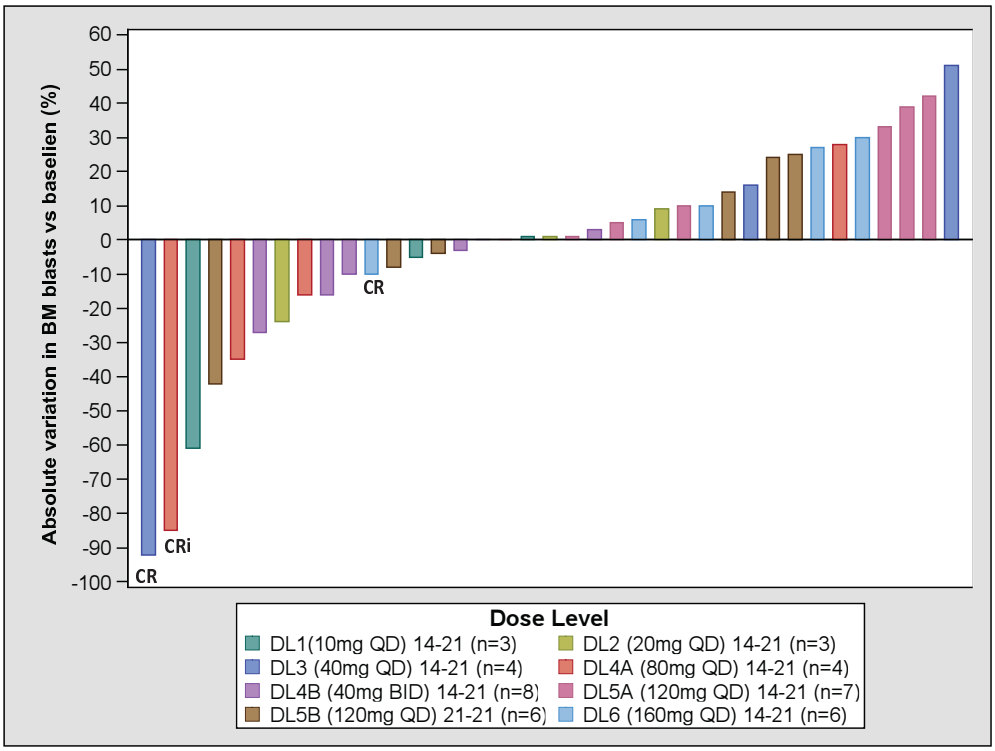
Absolute variation in BM blasts vs baselien (%)
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
CR
CRi
CR
Dose Level
DL1(10mg QD) 14-21 (n=3)
DL2 (20mg QD) 14-21 (n=3)
DL3 (40mg QD) 14-21 (n=4)
DL4A (80mg QD) 14-21 (n=4)
DL4B (40mg BID) 14-21 (n=8)
DL5A (120mg QD) 14-21 (n=7)
DL5B (120mg QD) 21-21 (n=6)
DL6 (160mg QD) 14-21 (n=6)

Figure1B

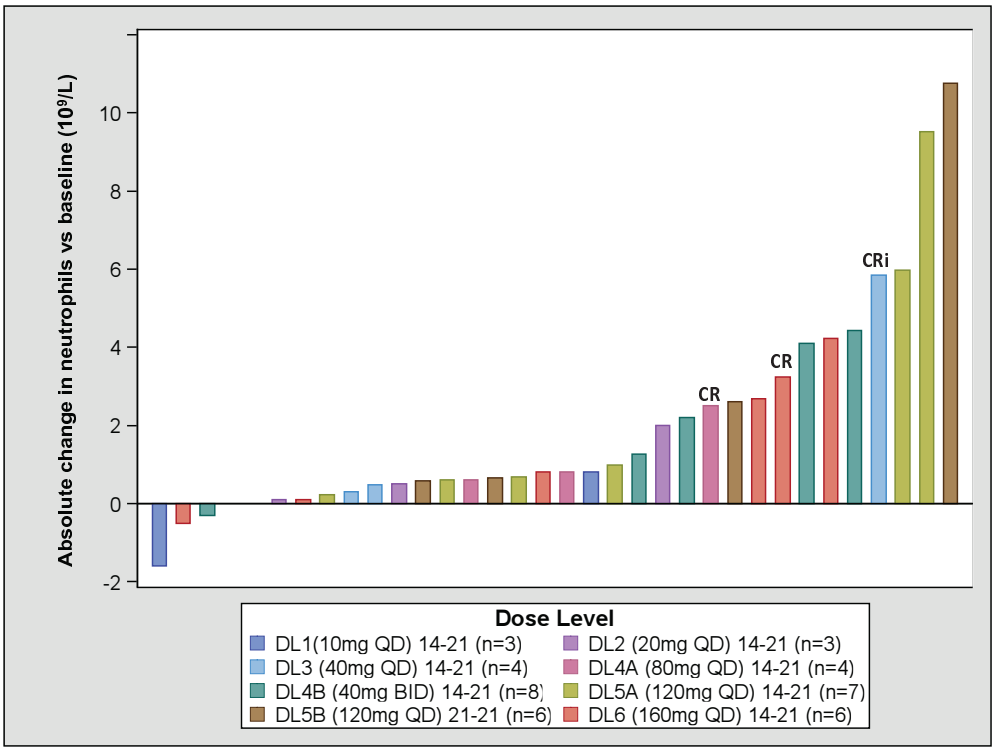
Absolute change in neutrophils vs baseline (10⁹/L)
10
8
6
4
2
0
-2
CRi
CR
CR
Dose Level
DL1(10mg QD) 14-21 (n=3)
DL2 (20mg QD) 14-21 (n=3)
DL3 (40mg QD) 14-21 (n=4)
DL4A (80mg QD) 14-21 (n=4)
DL4B (40mg BID) 14-21 (n=8)
DL5A (120mg QD) 14-21 (n=7)
DL5B (120mg QD) 21-21 (n=6)
DL6 (160mg QD) 14-21 (n=6)

Figure2

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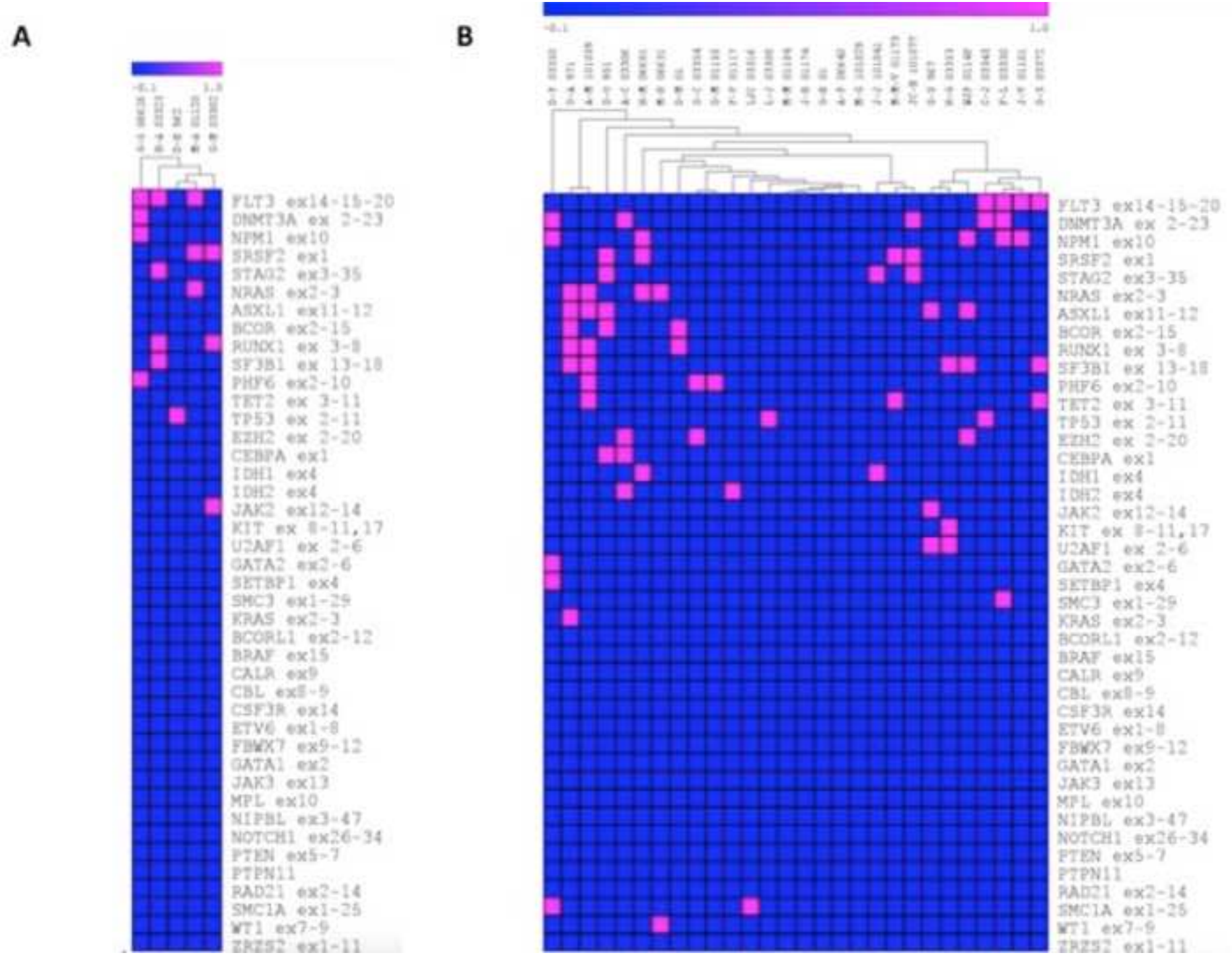

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