

## A randomised evaluation of low-dose cytosine arabinoside(ara-C) plus tosedostat versus low-dose ara-C in older patients with acute myeloid leukaemia: results of the LI-1 trial

### Summary

Older patients with acute myeloid leukaemia (AML) account for nearly half of those with the disease. Because they are perceived to be unfit for, unwilling to receive, or unlikely to benefit from conventional chemotherapy they're present an important unmet need. Tosedostat is a selective oral aminopeptidase inhibitor, which in phase I/II trials showed acceptable toxicity and encouraging efficacy. We report the only randomised study of lowdose cytosine arabinoside (LDAC) combined with tosedostat (LDAC-T) versus LDAC in untreated older patients not suitable for intensive treatment. A total of 243 patients were randomised 1:1 as part of the 'Pick-a-Winner' LI-1 trial. There was a statistically non-significant increase in the complete remission (CR) rate with the addition of tosedostat, LDAC-T 19% versus LDAC 12% [odds ratio (OR) 0.61, 95% confidence interval (CI) 0.30–1.23;  $P=0.17$ ]. For overall response (CR+CR with incomplete recovery of counts), there was little evidence of a benefit to the addition of tosedostat (25% vs. 18%; OR 0.68, 95% CI 0.37–1.27;  $P=0.22$ ). However, overall survival (OS) showed no difference (2-year OS 16% vs. 12%, hazard ratio 0.97, 95% CI 0.73–1.28;  $P=0.8$ ). Exploratory analyses failed to identify any subgroup benefitting from tosedostat. Despite promising pre-clinical, early non-randomised clinical data with acceptable toxicity and an improvement in response, we did not find evidence that the addition of tosedostat to LDAC produced a survival benefit in this group of patients with AML. International Standard Randomised Controlled Trial Number: ISRCTN40571019 Keywords: AML, acute leukaemia, chemotherapy, elderly.

### Introduction

A major current challenge in the treatment of acute myeloid leukaemia (AML) is to find effective, convenient and safe treatment for older patients.<sup>1,2</sup> Almost half of patients with AML are aged >70 years. To date, intensive therapy, even for those considered fit enough to receive it, delivers poor survival particularly for patients with comorbidities, poor performance score or adverse disease biology. Ever since, in the overdue clinical trials in this population, it has been assumed that unless remission was achieved, little benefit was anticipated. Standards of care include low-dose cytosine arabinoside (ara-C) (LDAC)<sup>3</sup> and the hypomethylating agents azacitidine<sup>4</sup> or decitabine,<sup>5</sup> each of which has low remission rates, although the hypomethylating agents may prolong survival without achieving remission. Several new treatments tested in this context have substantially improved remission rates, but not overall survival (OS), although the recently published results of combining venetoclax with azacytidine have for the first time prolonged survival in this patient group with a non-intensive approach.<sup>6</sup> Tosedostat is an example of a new class of orally administered metalloenzyme inhibitors with anti-proliferative and antiangiogenic activity in vivo and in vitro against a wide range of haematological and solid human cancer cells.<sup>7</sup> The exposure of cells to tosedostat results in the intracellular accumulation of an acid metabolite, CHR-79888, which exerts a powerful inhibitory effect on intracellular metalloenzymes resulting in anti-proliferative, pro-apoptotic, and anti-angiogenic activity.<sup>8</sup> The intracellular metalloenzyme targets for tosedostat are likely to be members of the M1 family of aminopeptidases, so tosedostat is an aminopeptidase inhibitor. Aminopeptidases play a critical role in the final steps of protein recycling downstream of proteasomal degradation and inhibition of aminopeptidases by tosedostat may, like proteasome inhibition, disrupt the turnover of cellular proteins in such a way that it impacts cancer cell growth.<sup>9</sup> Natural product inhibitors of aminopeptidases, particularly bestatin, exhibit similar, albeit weaker, pharmacological actions to tosedostat, including its pro-apoptotic, anti-proliferative and anti-angiogenic effects and its ability to induce amino acid deprivation response (AADR)-related gene expression changes.<sup>10</sup> Tosedostat synergises in vitro with a very wide range of chemotherapeutic and targeted agents in inducing anti-proliferative effects in many haematological and non-haematological cancer cell lines. We previously showed evidence

of synergy with ara-C in pre-clinical studies with human AML cells.<sup>11</sup> A number of early stage clinical trials established a daily dose level of 120 mg, with little toxicity and some encouraging clinical activity. The initial phase I study defined 180 mgs the maximum tolerated dose with the limitation being protracted thrombocytopenia, and demonstrated good tolerance at a daily dose of 130 mg. In a total of 51 patients with relapse/refractory disease in the study, the overall marrow response was 24%.<sup>12</sup> A second study, (OPAL; Clinical Trials. gov Identifier: NCT00780598),<sup>13</sup> also in relapsed/refractory older patients, assessed more prolonged administration at two dose levels (240 mg for 2 months, then 120 mg for 4 months, or 120 mg for 6 months). Initially 35 patients were allocated to each schedule, which resulted in an overall response rate (ORR) of 22%. From this study, the dose for prolonged treatment emerged as 120 mg once a day. Based on the pre-clinical evidence of synergy, Mawad et al.<sup>14</sup> in a phase II study, which included 26 untreated older patients, combined tosedostat (120 mg) daily with conventional dose ara-C (1 g/m<sup>2</sup>/days 1–5) or decitabine (20 mg/m<sup>2</sup>/days 1–5). A subsequent eight patients received a higher tosedostat dose. Complete remission/complete remission with incomplete recovery of counts (CR/CRi) was achieved in 53% of the patients and it was concluded that the 120 mg dose was preferable. Finally, Visani et al.<sup>15</sup> conducted a non-randomised phase II study on 33 older untreated patients with the LDAC and tosedostat combination and showed a CR/CRi rate of 54%, the majority of which were CRs. Of additional interest was that they suggested that those patients who achieved CR could be predicted with a 212 gene panel. A microarray analysis performed in 29 of the 33 patients identified 188 genes associated with clinical response (CR vs. noCR). Three of them [cluster of differentiation 93 (CD93), Golgi reassembly stacking protein 1 (GORASP1), C-X-C motif chemokine ligand 16 (CXCL16)] were validated by quantitative polymerase chain reaction.<sup>16</sup> This potential improvement in efficacy and tolerability suggested that it may be especially relevant in the management of older patients who frequently have resistant disease and tolerate traditional therapies poorly. We therefore investigated whether tosedostat combined with LDAC was superior to LDAC alone as first-line therapy for older patients with AML who were not considered fit for intensive therapy.

## Method

This evaluation of tosedostat was a component of our 'Pick-a-Winner' trial strategy in the LI-1 trial (ISRCTN40571019) where patients are randomised between a control arm (LDAC) and one of a number of experimental options.<sup>17</sup> The comparison is only between each experimental option and LDAC, and not between the experimental options. Patients allocated to LDAC only act as controls to patients who have been contemporaneously randomised to an experimental arm. Patients were eligible if they had *de novo* or secondary AML or high-risk myelodysplastic syndrome (MDS), defined as >10% marrow blasts, and were aged >60 years and considered unfit for intensive chemotherapy. 'Unfitness' was determined by the investigator/attending clinician and not specifically protocol defined, and documented by collection of comorbidity using components of the Sorrow Index.<sup>18</sup> Patients with a prior diagnosis of MDS [>10% blasts, i.e. refractory anaemia with excess of blasts (RAEB) 2] who had received azacitidine were not eligible, but patients with a prior diagnosis of MDS with <10% blasts who had failed a demethylation agent and then developed AML were. Patients were categorised for response and survival using the validated multiparameter Wheatley risk score,<sup>19</sup> which predicted survival based on age, performance status, cytogenetics and *de novo* or secondary disease. This score has been prospectively validated in older patients treated both non-intensively with LDAC and with intensive chemotherapy. Diagnosis and response definitions described below were designated by the local investigator. Cytogenetics (a minimum of 20 meta-phases) and immunophenotypic characterisation were carried out in regional reference laboratories that participate in national quality assurance schemes. In this study, patients were randomised 1:1 to LDAC or LDAC combined with tosedostat (LDAC-T). LDAC treatment comprised ara-C 20 mg twice a day for 10 days by sub-cutaneous injection for four courses given at 4–6 weeks intervals (there was no placebo). Tosedostat was given orally at 120 mg once a day continuously for

up to 6 months. Patients who were considered to be benefiting, by demonstrating stable disease or continuing response, were permitted to continue on their allocated treatment. Patients were required to provide written consent, and the trial was sponsored by Cardiff University and approved by the Wales Research Ethics Committee in compliance with the Declaration of Helsinki. Endpoints and assessments The primary endpoint was OS, following international guide-lines OS is defined as the time from randomisation to death. The protocol defined CR as a normocellular bone marrow aspirate containing <5% leukaemic blasts and showing evidence of normal maturation of other marrow elements. Persistence of myelodysplastic features did not preclude the diagnosis of CR. To achieve CR, patients required neutrophil recovery to  $\geq 1.09 \times 10^9/l$  and also platelets to  $\geq 100 \times 10^9/l$ , without evidence of extramedullary disease. Patients who achieved CR according to the protocol, but without evidence of adequate count recovery are denoted here as CRi, patients were required to be platelet-transfusion independent indicating sufficient time for marrow regeneration. Overall response was defined as CR/CRi, as we do not have complete data on partial response and morphological leukaemia-free state. For remitters, relapse-free survival (RFS) was the time from remission (CR or CRi) until relapse or death. Survival from CR is defined as the time from CR/CRi (first report) until death.

**Toxicity** Adverse events and toxicity were recorded as defined by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 3.

**Statistical Methods** All analyses were by intention-to-treat. Categorical endpoints (e.g. CR rates) were compared using Mantel–Haenszel tests, giving Peto odds ratios (ORs) and confidence intervals (CIs). Continuous/scale variables were analysed by non-parametric (Wilcoxon rank sum) tests. Time-to-event outcomes were reanalysed using the log-rank test, with Kaplan–Meier survival curves. ORs/hazard ratios (HRs) <1 indicate benefit for the investigational therapy. In the ‘Pick-a-Winner’ design, analyses are performed for each investigational arm separately versus the control arm of LDAC. In addition to overall analyses, exploratory analyses were performed stratified by the randomisation stratification parameters and other important variables, with suitable tests for interaction. Because of the well-known dangers of subgroup analysis, these were interpreted cautiously. The power calculation for the trial as a whole specified that final analysis was to be performed after 340 events (deaths) had been reported. Under the rules of the ‘Pick-a-Winner’ design, the Data Monitoring Committee (DMC) initially examined outcomes after response data were available for the first 100 patients in each randomisation (50 patients in each arm). At this point, in order to show sufficient promise to be carried forward, there had to be at least a 25% improvement in remission rates (CR+CRi) for the experimental arm over the control arm. At this time, the DMC also assessed survival and toxicity as additional criteria to be satisfied, although there was no formal stopping rule for either of these endpoints. If the DMC believed there was sufficient promise in the arm, the trial would continue to accrue until ~100 patients were in each arm. Once 170 deaths had been recorded a further interim analysis was performed and the HR for survival was required to be <0.85 in order for the trial to consider continuing to 400 patients and 340 events. At this point, the decision to stop or continue is made on the basis of the HR for OS. The aspiration of the study is a doubling of survival from 11% to 22% at 2 years, which is inequivalent to an average HR of 0.69. At the time of this final analysis the median (range) follow-up for OS is 48 (0.2–405) months. Surviving patients are censored at the date last known to be alive.

## Results

**Patient characteristics** Between June 2014 and February 2017, 243 patients with a median (range) age of 76 (60–88) years entered the randomisation, of whom 60% were male and 40% female. Overall, 66% had de novo AML, 28% secondary AML and 6% high-risk MDS. Cytogenetic analysis identified 1% had favourable, 65% intermediate and 22% adverse cytogenetics (Table I). By the validated Wheatley index, 19% were good risk, 36% standard risk and 58% poor risk. This validated score would predict an expected 12 months survival of 36%, 42% and 14% for LDAC monotherapy in the three risk groups based on historical data, and would be equivalent to a predicted overall 12-month survival of ~25%. The disposition of

the patients is shown in the Fig 1 Consolidated Standards of Reporting Trials (CONSORT) diagram. A median (range) of 2 (1–8) courses was delivered in either arm. For LDAC-T the mean number of courses delivered was 29 and comprised: none, 6%; one, 38%; two, 24%; three, 5%; four, 6%; five, 5%; six, 4%; seven, 3%; and eight, 13%. For LDAC alone, the mean number of courses delivered was 23 and comprised: none, 5%; one, 34%; two, 18%; three, 4%; four, 10%; five, 3%; six, 9%; seven, 2%; and eight, 15%;  $P=0.03$ ). The reasons provided by investigators for not receiving intensive therapy were age in 90% of cases, fitness in 45% of cases (both together in 38% of cases), and other reasons in 5% of cases, of which over half were patient choice. The haematopoietic cell transplantation-comorbidity index (HCT-CI) was 0 in 42%, 1–2 in 30% and  $\geq 3$  in 28%. Of the comorbidities listed on entry, the most frequent were those described as prior tumour (14%), diabetes (13%); cardiac (9%); infection (9%); mild-to-moderate pulmonary (8%); rheumatological (8%); obesity (8%) and arrhythmia (5%) (Table I). No other comorbidity was present in  $>5\%$  of patients.

Response Initial assessment by the DMC after the first 100 patients in September 2015 agreed that the randomisation should continue. In February 2017, the DMC performed an out-comes assessment on the LDAC-T versus LDAC arms of the LI-1 trial ( $n=243$ ), at which point additional randomisations were suspended pending the review. At the second interim analysis in November 2017 after 183 events, while there was a benefit in remission rates LDAC-T failed to show a sufficiently promising HR for survival and therefore on the recommendation of the DMC the arm was closed. Patients who were benefitting from tosedostat were permitted to stay on treatment. The data presented here represent an analysis undertaken after the DMC recommendation with cleaner data and more mature follow-up. Overall, CR was achieved in 16% of patients with a further 6% achieving a CRi (total ORR 21%). There was a non-significant increase in CR rate with tosedostat (LDAC-T 19% vs. LDAC 12%; OR 0.61, 95% CI 0.30–1.23;  $P=0.17$ ). For the overall response (CR+CRi), there was little evidence that a benefit of the addition of tosedostat could be seen (25% vs. 18%; OR 0.68, 95% CI 0.37–1.27;  $P=0.22$ ). A non-significant reduction in resistant disease was observed by the addition of tosedostat (60% vs. 68%; OR 0.68, 95% CI 0.40–0.16;  $P=0.16$ ). The 30-day mortality was not significantly increased (16% vs. 14%; HR 1.26, 95% CI 0.65–2.46;  $P=0.05$ ; Table II).

Treatment compliance Following remission, treatment was given to 19 of 22 LDAC patients (five patients received one course, four patients received two courses, one patient received three courses, two patients received four courses and seven patients received six or more courses) and 26 of 30 tosedostat patients treated (three patients received one course, four patients received two courses, six patients received three courses, four patients received four courses, one patient received five courses and 12 patients received six or more courses). No patient allocated to LDAC alone received tosedostat; however, two patients randomised to receive LDAC-T received one and three courses of LDAC alone.

Survival of responders For the total 52 patients who achieved a CR/CRi, the median OS from remission was 218 months. Although there was an apparent modest benefit in 2-year survival from response (47% vs. 36%), this failed to reach statistical significance (HR 0.88, 95% CI 0.43–1.80;  $P=0.07$ ; Fig 2B). For patients who relapsed, there was no significant difference in the survival following relapse between treatment arms (1-year survival post-relapse 30% vs. 17%; HR 0.93, 95% CI 0.45–1.92;  $P=0.08$ ; (Fig 2C). In the patients who did not achieve CR/CRi, the survival was not different between the arms.

Relapse-free survival Although remission rates were higher in the tosedostat arm, there was no significant difference in duration of remission RFS (HR 0.82, 95% CI 0.46–1.47;  $P=0.05$ ; Fig 2D). Toxicity Although rates of Grade  $\geq 3$  toxicity were low overall, tosedostat was associated with significantly increased diarrhoea, and cardiac toxicity (two Grade 4 events that led to tosedostat discontinuation—atrial fibrillation and raised troponin) in course one, and with greater cardiac and liver alanine transaminase toxicity in course two. Resource usage (blood product support, antibiotics and hospital utilisation) tended to be consistently higher in the tosedostat arm, although the only significant difference between arms was an increased use of platelets in course one (mean 50 vs. 35 pools,  $P=0.006$ ); (Fig 3A,B).

**Exploratory subgroup analysis** Exploratory analyses were carried out on survival to determine if there was an identifiable subgroup with a differential effect of treatment. Baseline covariates including age, sex, diagnosis, cytogenetics, white blood count, performance status, and Wheatley risk group were explored (Figure S1). Additional analysis by nucleophosmin 1 (NPM1) and FMS-like tyrosine kinase 3 internal tandem duplication/tyrosine kinase domain (FLT3-ITD/TKD) status was additionally explored. More detailed molecular analyses were not available. Although the power of such analyses is limited by small numbers in some subgroups, there were no significant inter-actions between baseline variables and treatment for survival. In particular, no subgroup could be identified where there was a benefit for LDAC-T.

## Discussion

For older patients compared to younger patients with AML, the decision in treatment strategy is not always obvious. At one end of the spectrum there are patients who have several comorbidities where even if the prognostic assessment of their disease biology is not adverse, are at high risk of not surviving a version of standard chemotherapy. At the other are patients who are chronologically old, but have few comorbidities combined with good performance status. In these cases intensive chemotherapy may be of benefit, but the decision to offer conventional chemotherapy may be negatively influenced by adverse disease biology, where chemotherapy may have a low chance of success. Some patients who are 'fit' may decline treatment in preference for more time out of hospital, particularly if facilitated by outpatient or oral medication. At the centre of this is the physician, indeed in our previous AML14 trial where an intensive and non-intensive treatment approach was available, the physician emerged as an independent factor in treatment choice. Many prognostic scoring systems have been developed for younger patients to guide treatment decisions and such scores can be developed for older patients, but few have been prospectively validated in recipients of non-intensive therapy. We developed the WheatleyScore,<sup>19</sup> which is useful in predicting expected outcomes for non-intensive treatment approaches. In the present study based on the Wheatley score, 4% of patients were favourable, 31% intermediate and 65% were at adverse risk with respective expected 12-month OS of 36%, 42% and 14% respectively. The predicted 12-month OS was 25%, which is what was achieved. We developed LDAC as a standard of care at a time when no other randomised trials in this patient population had suggested an alternative. We found that clinical toxicities were no greater than best supportive care.<sup>3</sup> However, durable benefit was only seen in the 18% of patients who entered CR, where the median OS was 575 days compared to only 66 days for those that did not respond. This experience led to the development of a 'Pick-a-Winner' design, which depended on an initial improvement in remission rate as a surrogate for future survival benefit. A number of novel treatments that produced encouraging results in non-randomised trials have been included, but failed the scrutiny of randomisation.<sup>20–23</sup> Others were able to double the remission rates, but did not improve OS.<sup>24,25</sup> Another observation has been that in different cohorts of LDAC patients the remission rate varied from 14% to 21% and the 12-month OS from 25% to 32%, without obvious differences in patients' characteristics.<sup>26</sup> To date, 2480 randomisations have been undertaken in 1753 patients to evaluate 13 agents or combinations.<sup>21–26</sup> The evaluation is complete on 11 options, and two are ongoing. The use of remission as a surrogate end-point helps identify and exclude unpromising treatments, but should not replace survival as an endpoint in trials in this population. Mechanistically, tosedostat has several properties that could be particularly helpful in older patients.<sup>8</sup> The developmental phase I/II experience in relapse and in combination was both feasible from the toxicity point of view and appeared to offer an improved clinical response. The oral formulation is also helpful in the elderly population. We therefore initiated the randomised comparison reported here. Disappointingly, the combination failed to meet the DMC criteria to continue the trial. In reaching their recommendation the DMC looked not only at the strict continuation criteria set down, based upon remission, but also relied upon safety data, and in particular early mortality when deciding whether to continue. The DMC closed the tosedostat arm based on

a failure to improve survival as assessed by the CI at the time of their analysis, which depended on observing aHR of 0.69, representing the requirement to improve 2-year survival from 11% to 22%. It was therefore concluded that even with more patients included the drug was unlikely to demonstrate the sort of benefit required by the design of the trial. As is observed in many such studies, the primary reason for discontinuation was refractory disease. For responding patients, the median OS was an impressive 21.8 months, although we were unable to identify any clinical or laboratory findings that could reliably identify such patients, a recent publication by Visani *et al.*<sup>15</sup> has proposed a gene expression profile that could predict such a response and could warrant further evaluation. The introduction of hypomethylating agents has improved survival without substantially improving the rate of remission<sup>4</sup> and globally considered the standard of care for the frail unfit patient with AML. New combinations (including venetoclax, enasidenib, ivosidenib and glasdegib) show considerable promise, and indeed have received regulatory approval for this patient group, mostly based on non-randomised data.<sup>27–31</sup> As described above there are several examples of early promise that fail in the rigour of randomisation. Although recently published data from the VIALE-A study (NCT02993523), in perhaps a more selected frail elderly AML population, combining venetoclax with azacitidine has demonstrated a significant improvement in OS, this combination may ultimately become considered the new standard of care in this setting.<sup>6</sup> In conclusion, tosedostat demonstrated promising early data and acceptable tolerability, its addition to LDAC did achieve a modest improvement in response rates, but we did not find evidence that it produced a survival benefit in this group of patients. Strategies other than aminopeptidase inhibition appear to demonstrate more rational approaches for future non-intensive combined therapy in AML.

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#### Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article. Fig S1. Tests for subgroup interactions