A RANDOMISED EVALUATION OF LOW-DOSE ARA-C PLUS TOSEDOSTAT VERSUS LOW DOSE ARA-C IN OLDER PATIENTS WITH ACUTE MYELOID LEUKAEMIA: RESULTS OF THE LI-1 TRIAL

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Abstract

Older patients with acute myeloid leukaemia 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 represent 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 low dose ara-C (LDAC) combined with tosedostat (LDAC-T) versus LDAC in untreated older patients not suitable for intensive treatment. 243 patients were randomised 1:1 as part of the “Pick a Winner” LI-1 trial. There was a non-significant increase in CR rate with the addition of tosedostat (LDAC-T vs LDAC; 19% vs 12%, OR 0.61 (0.30-1.23) p=0.17). For overall response (CR+CRi), there was little evidence of a benefit to the addition of tosedostat (25% vs 18%; OR 0.68 (0.37-1.27) p=0.22). However overall survival (OS) showed no difference (2-year OS 16% vs 12%, HR 0.97 (0.73-1.28) p=0.8). Exploratory analyses failed to identify any subgroup benefitting from tosedostat. Despite promising pre-clinical, early unrandomised 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 AML patients.

Trial Reference ISRCTN40571019

Introduction

A major current challenge in the treatment of acute myeloid leukaemia (AML) is to find effective, convenient and safe treatment for older patients[1,2]. Almost half of patients with AML are over 70 years of age. To date, intensive therapy, even for those considered fit enough to receive it, delivers poor survival particularly for patients with co-morbidities, 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 ara-C (LDAC)[3] and the hypomethylating agents azacitidine[4] or decitibine[5], each of which have 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, although the recently published results of combining venetoclax with azacitidine have for the first time prolonged survival in this patient group with a non-intensive approach[6]

Tosedostat is an example of a new class of orally administered metalloenzyme inhibitors with anti-proliferative and anti-angiogenic activity in vivo and in vitro against a wide range of haematological and solid human cancer cells[7]. 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[8]. 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[9]. 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[10].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[11].

A number of early stage clinical trials established a daily dose level of 120mg, with little toxicity and some encouraging clinical activity. The initial phase 1 study defined 180mg as the maximum tolerated dose (MTD) with the limitation being protracted thrombocytopenia, and demonstrated good tolerance at a daily dose of 130mg. In a total of 51 patients with relapse/refractory disease in the study, the overall marrow response was 24%[12]. A second study, (OPAL)[13], also in relapsed/refractory older patients, assessed more prolonged administration at two dose levels (240mg for 2 months then 120mg for 4 months or 120mg 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 120mg once a day. Based on the pre-clinical evidence of synergy Mawad and colleagues[14], in a phase 2

study which included 26 untreated older patients combined tosedostat (120mg) daily with conventional dose ara-C (1g/m^2 days 1-5) or decitibine (20mg/m^2/days 1-5). A subsequent 8 patients received a higher tosedostat dose. Complete remission (CR)/complete remission with incomplete recovery of counts (CRi) CR/CRi was achieved in 53% and it was concluded that the 120mg dose was preferable. Finally Visani and colleagues[15] conducted an unrandomised 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 33 patients identified 188 genes associated with clinical response (CR vs no CR). Three of them (CD93, GORASP1, CXCL16) were validated by quantitative polymerase chain reaction[16].

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.

Methods.

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[17]. 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 older than 60 years and considered unfit for intensive chemotherapy. “Unfitness” was determined by the investigator/attending clinician- not specifically protocol defined and documented by collection of co-morbidity using components of the Sorror index[18]. Patients with a prior diagnosis of MDS (>10% blasts, RAEB 2) who had received azacitidine were not eligible, but patients with a prior diagnosis of MDS with <10% blasts who have failed a demethylation agent and then developed AML were. Patients were categorised for response and survival using the validated multi-parameter Wheatley risk score[19] 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 metaphases) and immunophenotypic characterization were carried out in regional reference laboratories which 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 20mg twice a day for 10 days by subcutaneous injection for 4 courses given at 4 to 6 weeks intervals (there was no placebo). Tosedostat was given orally at 120mg 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 overall survival (OS), following international guidelines OS is defined as the time from randomisation to death. The protocol defined complete remission (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 $\geq 1.0 \times 10^9$/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 date on partial response and morphologic 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 are by intention-to-treat. Categorical endpoints (e.g. CR rates) were compared using Mantel-Haenszel tests, giving Peto odds ratios and confidence intervals. Continuous/scale variables were analysed by non-parametric (Wilcoxon rank sum) tests. Time-to-event outcomes were analysed using the log-rank

test, with Kaplan-Meier survival curves. Odds/hazard ratios (OR/HR) less than 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 2.5% 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 approximately 100 patients were in each arm. Once 170 deaths had been recorded a further interim analysis was performed and the hazard ratio for survival was required to be less than 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 hazard ratio for OS. The aspiration of the study is a doubling of survival from 11% to 22% at two years which is equivalent to an average hazard ratio of 0.69.

At the time of this final analysis the median follow-up for OS is 48 months (range 0.2-40.5). 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 age of 76 years (range 60-88) entered the randomisation, of whom 60% were male and 40% female. Sixty-six percent had de novo AML, 28% secondary AML, and 6% high risk MDS. Cytogenetic analysis identified 1% had favourable, 65% intermediate and 22% adverse cytogenetics (table1). By the validated Wheatley index[19], 5% were good risk, 36% standard risk and 58% poor risk. This validated score would predict an expected 12 month 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 approximately 25%.

The disposition of the patients is shown in figure 1 (CONSORT diagram). A median of 2 courses (range 1-8) was delivered in either arm. For LDAC-T the mean was 2.9; and number of courses was 0=6%; 1=38%; 2=24%; 3=5%; 4=6%; 5=5%; 6=4%; 7=3%; 8=13%. For LDAC alone, the mean was 2.3, and the number of courses was 0=5%; 1=34%; 2=18%; 3=4%; 4=10%; 5=3%; 6=9%; 7=2%; 8=15%; p= 0.3).

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 HCT-CI was (0 = 42%, 1-2 = 30%, 3+ =28%). Of the co-morbidities 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 1). No other co-morbidity was present in more than 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 outcomes 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, however LDAC-T failed to show a sufficiently promising hazard ratio 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 represents 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 vs LDAC) (19% vs 12%, OR 0.61 (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% vs18%; OR 0.68 (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 (0.40-0.16) p=0.16. The thirty-day mortality was not significantly increased (16% vs 14%, HR 1.26 (0.65-2.46) p=0.5. (table 2).;

Treatment Compliance: Following remission, treatment was given to 19/22 LDAC patients (5 patients received 1 course, 4 patients 2 courses, 1 patient 3 courses, 2 patients 4 courses, and 7 patients 6 or more courses) and 26/30 Tosedostat patients treated (3 patients received 1 course, 4 patients 2 courses, 6 patient 3 courses, 4 patients 4 courses, 1 patient 5 courses, and 12 patients 6 or more courses). No patient

allocated to LDAC alone received tosedostat; however 2 patients randomised to receive LDAC-T received 1 and 3 courses of LDAC alone.

Overall Survival The OS did not differ by treatment arm (LDAC-T vs LDAC) (2-year OS 16% vs 12%, HR 0.97 (0.73-1.28) p=0.8; figure 2a).

Survival of Responders: For the total 52 patients who achieved a CR/CRi, the median OS from remission was 21.8 months. Although there was an apparent modest benefit in 2 years survival from response (447% vs 36%), this failed to reach statistical significance (HR 0.88 (0.43-1.80) p=0.7) (figure 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 (0.45-1.92) p=0.8; (figure 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 (0.46-1.47) p=0.5; figure 2d).

Toxicity: Although rates of grade 3+ toxicity were low overall, tosedostat was associated with significantly increased diarrhoea, and cardiac toxicity (2 grade 4 events that led to tosedostat discontinuation- AF and raised troponin) in course 1, and with greater cardiac and liver alanine transaminase (ALT) toxicity in course 2 . Resource usage (blood product support, antibiotics and hospital utilisation) tended to be consistently higher in the tosedostat arm, though the only significant difference between arms was an increased use of platelets in course 1 (mean 5.0 vs 3.5 pools p=0.006); (figure 3a and 3b).

Exploratory Subgroup Analysis:

Exploratory analyses were carried out on survival, to find out 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 (Supplemental Figure 2). Additional analysis by NPM1 and 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 interactions between baseline variables and treatment for survival. In particular, no subgroup could be identified where there was a benefit for LDAC-T.

Discussion:

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 co-morbidities 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 co-morbidities 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 were 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 Wheatley Score[19], which is useful in predicting expected outcomes for non-intensive treatment approaches. In this study based on the Wheatley score 4% of patients were favourable, 31% intermediate and 65% were adverse risk with respective expected 12-month OS 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[3]. However durable benefit was only seen in the 18% of patients who entered CR, where 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 unrandomised trials have been included, but failed the scrutiny of randomisation[20-23]. Others were able to double the remission rates but did not improve overall survival[24,25]. Another observation has been that in different cohorts of LDAC patients the remission rate varied from 14% to 21% and the 12 month survival from 25% to 32%, without obvious differences in patients’ characteristics[26]. To date 2480 randomisations have been undertaken in 1753 patients to evaluate 13 agents or combinations[21-26]. The evaluation is complete on 11 options, and 2 are ongoing. The use of remission as a surrogate endpoint helps identify and exclude unpromising treatments, but should not replace survival as an endpoint in trials in this population.

Mechanistically tosedostat has several properties which could be particularly helpful in older patients[8]. 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 IDMC criteria to continue the trial. In reaching their recommendation the IDMC 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 or not to continue. The IDMC closed the tosedostat arm based on a failure to improve survival as assessed by the confidence intervals at the time of their analysis which depended on observing a hazard ratio 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 which could reliably identify such patients a recent publication by Visani[15] 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[4] and globally considered the standard of care for the frail unfit AML patient. New combinations (including venetoclax, enasidenib, ivosidenib and glasdegib) show considerable promise, and indeed have

received regulatory approval for this patient group, mostly based on unrandomised data[27-31]. As described above there are several examples of early promise which fail in the rigour of randomization. Although recently published data from the VIALE-A study, in perhaps a more selected frail elderly AML population, combining venetoclax with azacitidine has demonstrated a significant improvement in overall survival, this combination may ultimately become considered the new standard of care in this setting[6].

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 combination therapy in AML.

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Disclosure of Conflicts of Interest

AKB was an employee of CTI Biopharma 2015-2017. REC has received research funding and honoraria from Novartis and Bristol Myers Squibb, and honoraria from Pfizer, Jazz Pharmaceuticals and Abbvie. MC has received research funding from Novartis, Bristol-Myers Squibb, Cyclacel and Takeda/Incyte, is/has been an advisory board member for Bristol-Myers Squibb, Novartis, Incyte, Daiichi Sankyo, Jazz and Pfizer and has received honoraria from Astellas, Bristol-Myers Squibb, Novartis, Incyte, Pfizer and Gilead. The other authors have nothing to disclose.

Author Contributions: MD: chief investigator; reviewed the data and wrote the manuscript AKB: designed the trial; wrote protocol; chief investigator until Q3 2014; RKH: designed the trial, wrote the protocol, analysed the data. CA analysed the data with extended follow up, IT supervised the data collection, reviewed the data. MTS, CH, and PG were major recruiters. NHR: designed trial; reviewed the data. MC Co-CI, and REC reviewed the data. All authors reviewed the manuscript.

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