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SHORT REPORT

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A randomised evaluation of low-dose Ara-C plus pegylated recombinant arginase BCT-100 versus low dose Ara-C in older unfit patients with acute myeloid leukaemia: Results from the LI-1 trial

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Summary

The survival of acute myeloid leukaemia (AML) patients aged over 60 has been suboptimal historically, whether they are treated using hypomethylating agents, low-dose cytarabine (LDAC) or venetoclax-based regimens. Progress is being made, however, for subgroups with favourable molecular or cytogenetic findings. Arginine metabolism plays a key role in AML pathophysiology. We report the only randomised study of LDAC with recombinant arginase BCT-100 versus LDAC alone in older AML patients unsuitable for intensive therapy. Eighty-three patients were randomised to the study. An overall response rate was seen in 19.5% (all complete remission [CR]) and 15% (7.5% each in CR and CR without evidence of adequate count recovery [CRi]) of patients in the LDAC+BCT-100 and LDAC arms respectively (odds ratio 0.73, confidence interval 0.23–2.33; $p = 0.592$). No significant difference in overall or median survival between treatment arms was seen. The addition of BCT-100 to LDAC was well tolerated.

KEYWORDS
arginase, AML, BCT-100, cytarabine

INTRODUCTION

Acute myeloid leukaemia (AML) is one of the most common blood cancers in children and adults. Over the last 50 years, there has been a steady improvement in prognosis for younger patients and those with favourable genetic features. However, for patients with AML over the age of 60, a considerable number are unsuitable for intensive remission-induction chemotherapy due to frailty and comorbidities. For this patient group, treatment may be based on combining drugs with hypomethylating agents or low-dose cytarabine (LDAC).[1–3] Latterly, venetoclax-based regimens have become the standard approach.[3] However, the prognosis is poor, with most patients relapsing and only very few patients experiencing long-term survival.[4] Thus, there is an urgent need for multidrug combination therapy with non-toxic, novel agents to improve patient outcomes.

Arginine is a semi-essential amino acid consumed in the diet and essential to a number of cell pathways including protein synthesis and cell signalling. Arginine metabolism

plays a key role in disease pathogenesis by enabling AML blast division and contributing to immune escape.[5] Arginine is taken up through the expression of cell surface transporters and is catabolised principally by the enzymes arginase I, arginase II or nitric oxide synthase. In the majority of non-malignant cell types, low arginine conditions are well tolerated due to the expression of argininosuccinante synthase (ASS1), ornithine transcarbamylase (OTC), and argininosuccinate lyase (ASL) enzymes, which allow the recycling of arginine from precursors. However, in AML blasts, and a number of other cancers, the expression of ASS1 or OTC may be low or absent, making the cells reliant on extracellular arginine, termed arginine auxotrophy.[6]

BCT-100 is a pegylated recombinant human arginase that leads to a rapid depletion of arginine in murine models and sustained arginine deprivation in the blood of solid cancer patients.[7–9] BCT-100 demonstrated activity as a single-agent against AML cell lines, AML xenografts and primary AML blasts from newly diagnosed or relapsed patients, and was synergistic in combination with cytarabine.[5]

Here we investigated the efficacy of LDAC+BCT100 versus LDAC alone in patients aged over 60, considered by the investigator to be unsuitable for intensive therapy.

METHODS

Design and eligibility

The LI-1 trial (ISRCTN40571019) evaluates novel therapies as part of a 'Pick-a-Winner' strategy.[2,10] This design allows several treatments to be assessed simultaneously in a randomised fashion, with the aim of doubling 2-year survival from 11% to 22% (hazard ratio 0.69). Interim assessments are performed after 50 and 100 patients per arm are recruited to assess safety data.

Patients aged older than 60 years, with de novo or secondary AML or high-risk myelodysplastic syndrome (MDS; >10% marrow blasts), and considered unfit for intensive therapy by the treating clinician, were eligible for the study. Patients with a prior diagnosis of MDS with less than 10% blasts, who had failed a demethylation agent but subsequently developed AML, were also eligible. Impaired renal or hepatic function (defined as total bilirubin ≥1.5 times the upper limit of normal [ULN]), aspartate aminotransferase or alanine aminotransferase 2.5 times or more than the ULN, and serum creatinine more than $174\,\mu mol/L$ were exclusion criteria. Patients with a history of myocardial infarction, unstable angina, or cerebrovascular accident/transient ischaemic attack within the previous 6 months were also excluded.

Patients were randomised 1:1 between the LDAC control arm and the LDAC+BCT100 recombinant human arginase arm. Cytarabine (LDAC) was administered as 20 mg twice daily for 10 days by subcutaneous injection, with cycles repeated every 28–42 days. BCT-100 was administered as a single infusion of 1600 U/kg intravenously on days 1, 8, 15 and 22 of every course. Patients continued to receive their

allocated treatment as long as the disease was stable or there was a continued response. A minimum of four courses was recommended for both treatment arms.

All patients provided written informed consent. The LI1 trial was sponsored by Cardiff University and approved by the Wales Research Ethics Committee in compliance with the Declaration of Helsinki (ISRCTN No: ISRCTN40571019).

Endpoints and toxicity

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 less than 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 more than $1.0 \times 10^9/l$ and also platelets equal to or greater than $100 \times 10^9/l$, 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 a 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.

Adverse events and toxicity were defined by the National Cancer Institute Common Terminal Criteria for Adverse Events (NCI CTCAE) version 3.

Statistical analysis

All analyses are by intention-to-treat with planned interim assessments after 50 and 100 patients per arm are recruited. Categorical endpoints (e.g. CR rates) were compared using logistic regression, giving odds ratios and confidence intervals. Continuous/scale variables were analysed by nonparametric (Wilcoxon rank sum) tests. Time-to-event outcomes were analysed using Kaplan–Meier survival curves and the log-rank test, which were used to report hazard ratios, confidence intervals and p-values.[11]

Arginine enzyme-linked immunosorbent assay

Blood samples were taken immediately prior to the administration of the first four doses of BCT-100. The concentration of arginine was quantified using a competitive enzyme linked immunoassay (K7733, Immunodiagnostik) according to the manufacturers' instructions. In brief, the assay uses a competitive enzyme immunoassay in which L-arginine is derivatised
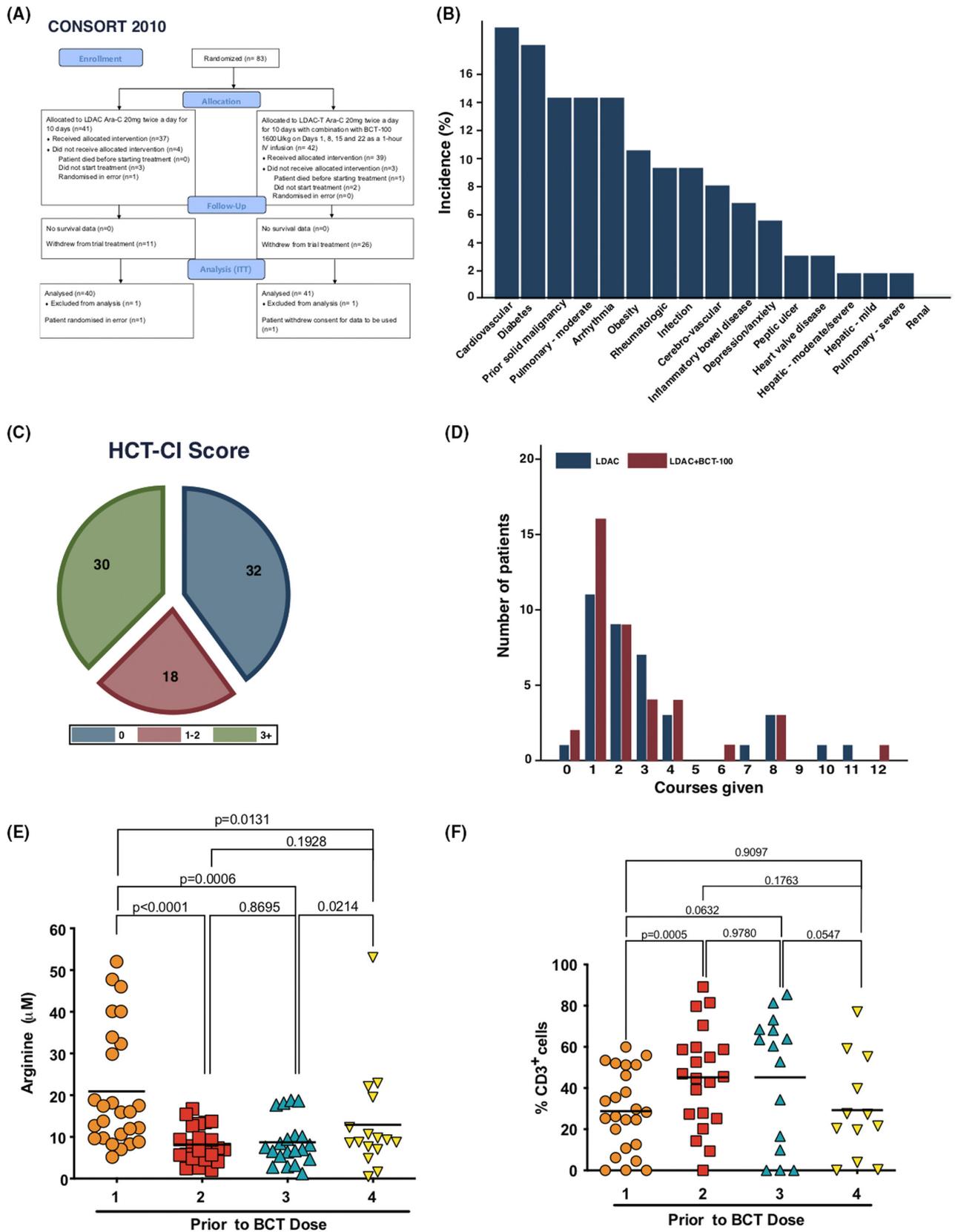
FIGURE 1 (A) Consolidated standards of reporting trials (CONSORT) flow diagram. Ara-C, cytosine arabinoside; LDAC, low-dose cytosine arabinoside; BCT-100 recombinant PEG-arginase. (B) Incidence of baseline comorbidities in trial patients. (C) Haematopoietic cell transplantation-specific Comorbidity Index score (HCT-CI) in trial patients at baseline. (D) Number of LDAC or LDAC+BCT-100 courses received by patients on trial. (E) Plasma arginine concentrations prior to the first four doses of BCT-100. (F) frequency of CD3+ T cells in the blood of patients prior to the first four doses of BCT-100.
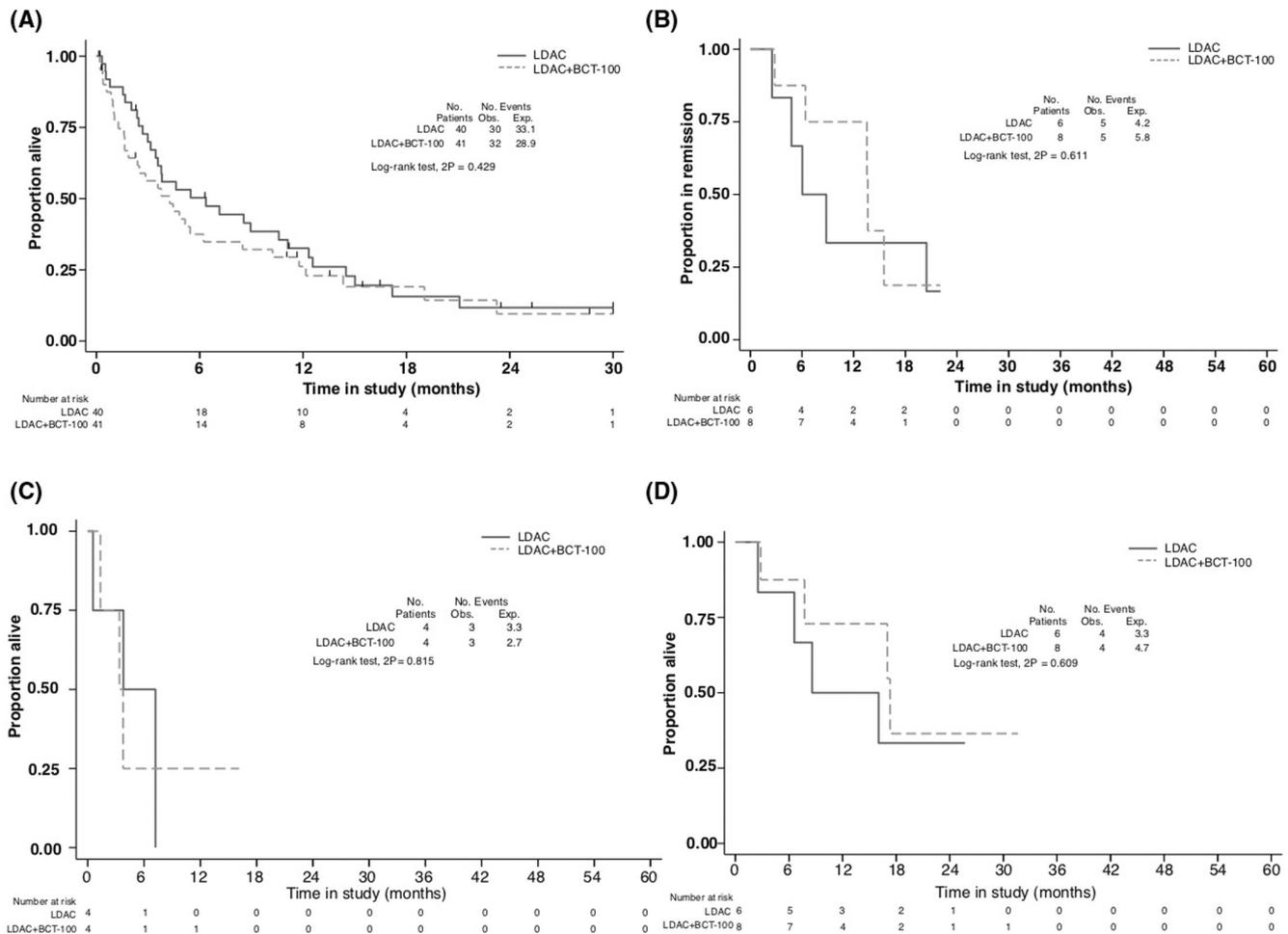
FIGURE 2 (A) Overall survival (OS), (B) relapse-free survival (RFS). (C) Survival after relapse, (D) survival after remission. BCT-100, recombinant PEG-arginase; HCT-CI, haematopoietic cell transplantation-specific Comorbidity Index score; LDAC, low-dose cytosine arabinoside.

from samples and competes with an L-arginine-tracer for binding of polyclonal antibodies in the microtiter wells.[5,12]

Flow cytometric analysis

To determine any effect of arginine depletion on circulating T cell frequency, following red cell lysis (Qiagen) whole blood was stained with anti-human CD3 antibody (Biolegend) on ice for 30 min. Cells were resuspended in fluorescence-activated cell sorting buffer. Propidium (Biolegend) was used to assess viability. Cells were analysed using a Beckman Coulter Cytoflex flow cytometer and analysed using FlowJo and CytExpert software (Tree Star Inc).

RESULTS

Eighty-three patients were randomised between September 2018 and December 2020 across 30 UK hospitals, prior to the study being closed early due to the COVID-19 pandemic. (Figure 1A) Fifty-seven patients had de novo AML, 19 had secondary AML, and five were high-risk MDS patients. With

regard to cytogenentics, 2.5% of patients had favourable cytogenentics, 59.3% had normal/intermediate, 23.5% had adverse and 14.8% had unknown cytogenetics (Table S1). The most frequent comorbidities were diabetes, cardiovascular disease, prior solid malignancy, or arrythmia; other less frequent comorbidities are shown in Figure 1B. The haematopoietic cell transplantation-comorbidity index (HCT-CI) was 0 in 41%, 1–2 in 23%, and 3 or more in 36% of patients. (Figure 1B,C).

Seventy-six patients received their allocated treatment (LDAC $n = 37$, LDAC+BCT-100 $n = 39$) with a median time in study of 4.6 months and median follow-up of 23.4 months, as calculated by the reverse Kaplan–Meier method. A median of two courses (range 0–21) was delivered in each arm (Figure 1D). The maximum number of courses of LDAC was 21 and of LDAC+BCT-100 was 12 ($n = 1$ patient each).

Pharmacodynamics

BCT-100 led to a depletion of plasma arginine in all patients (Figure 1E). No reduction in the frequency of circulating T cells was seen (Figure 1F).

Response

An overall response rate (CR + CRi) was seen in eight of 41 patients (19.5%; all CR) and six of 40 patients (15%; 7.5% each of CR + CRi) in the LDAC+BCT-100 and LDAC arms, respectively (odds ratio [OR] 0.73, CI 0.23–2.33; p = 0.592) (Table S2). OS did not differ between treatment arms (Figure 2A) and no significant difference in median survival was seen (4.3 months LDAC+BCT-100 vs. 6.4 months LDAC). Although there was an improvement in 1 year RFS by the addition of BCT-100 (6/8 patients), compared to LDAC alone (2/6 patients), this did not achieve statistical significance (75% LDAC+BCT-100 vs. 33.3% LDAC, OR 0.48, CI 0.09–2.63; p = 0.398) (Figure 2B–D). Analysis by patient or AML characteristics did not identify any subgroup in which the addition of BCT-100 led to significant benefit in OS.

Toxicity

Rates of Grade 3 or higher toxicity were very low in both arms. The addition of BCT-100 did not lead to any significant increase in toxicities. Although there was an increase in overnight stays for patients receiving BCT-100 in course 1 (median four nights for LDAC vs. 12 nights LDAC+BCT-100, p = 0.01), no other significant differences in supportive-care measures were seen in the two arms (Table S3). As patients receiving BCT-100 all attended hospital for the drug infusion, it is likely that low-grade toxicities were more commonly detected in this group, leading to the increase in overnight stays. Patients in the LDAC+BCT-100 arm did not experience significantly increased 30 and 60 day mortality (30 day mortality 17.% vs. 10.8%; 60 day mortality 35.7% vs. 16.2%) (Table S2). The addition of BCT-100 did not significantly change the median time to recovery of neutrophil or platelet counts.

CONCLUSION

In summary, we demonstrated that BCT-100 recombinant arginase can be administered alongside LDAC to older AML patients, with an acceptable toxicity profile. The low toxicity in this patient population, despite using a drug combination, is encouraging, as cytopenia can limit the delivery of venetoclax-based therapies.[13] However, no improvement in response rates or survival were seen, despite confirmed arginine depletion. Some analyses may be underpowered as the trial closed early with 83 patients recruited rather than the 100 patients as planned in the study design.

Although we have previously shown that the majority of AML blasts have low to absent ASS or OTC expression, enrichment for ASS negative AML patients could be one strategy to enhance response. Similar biomarker-enriched approaches have been taken for BCT-100 and other arginine depleting enzymes still under clinical investigation.[14,15] BCT-100 continues to undergo early phase clinical evaluation in other adult and paediatric solid and haematological malignancies.[16]

CONFLICT OF INTEREST

Mhairi Copland has received research funding from Cyclacel and Incyte, is an advisory board member for Novartis, Incyte, Jazz Pharmaceuticals, Pfizer and Servier, and has received honoraria from Astellas, Novartis, Incyte, Pfizer and Jazz Pharmaceuticals. All other authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Data from this trial will be made available upon request to the study sponsor.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.