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SUPPORTING INFORMATION

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A Phase I dose-escalation study of DCLL9718S, an antibody-drug conjugate targeting C-type lectin-like molecule-1 (CLL-1) in patients with acute myeloid leukemia

To The Editor:

Acute myeloid leukemia (AML) is an aggressive malignancy with especially dismal outcomes in relapsed or refractory patients. C-type lectin-like molecule-1 (CLL1) is a transmembrane glycoprotein expressed on the surface of AML blast cells.[1] CLL1 is expressed on committed myeloid cells in bone marrow, but is absent on normal HSCs. Its expression is detectable on neutrophils and monocytes/macrophages but there is no known expression on non-hematopoietic stem cells (HSC) or tissues.[2] The absence of CLL1 expression on HSC may translate into less myelosuppression than other AML targets. Reduced myelosuppression may increase the therapeutic window while also enabling better combinability with standard AML therapies.

DCLL9718S is a THIOMAB™ antibody-drug conjugate (TDC) consisting of a humanized monoclonal IgG1 anti-CLL1 antibody (MCLL0517A) linked to two pyrrolobenzodiazepine (PBD) dimer drugs via a cleavable disulfide linker. The development of DCLL9718S was under a Research Collaboration and License Agreement with Spirogen Ltd., a member of the AstraZeneca group. The PBDs bind covalently in the minor groove of DNA and form inter-strand and intra-strand cross-linked adducts as well as mono-adducts.[3,4] Following binding of the TDC to target cells expressing CLL1, the TDC is internalized, degraded in the lysosome, and the active PBD dimer drug is released, resulting in DNA damage and cytotoxicity.[4]

Patients ≥18 years with relapsed or refractory AML, Eastern Cooperative Oncology Group (ECOG) Performance Status score 0–2, adequate end-organ function, and who were were willing and able to undergo pre-treatment and on-treatment bone marrow aspirates were eligible. The primary objectives were to assess the safety and tolerability of DCLL9718S and to determine the maximum tolerated dose (MTD). Secondary objectives included analysis of pharmacokinetics, pharmacodynamic biomarkers, target expression profile, and preliminary assessment of anti-tumor efficacy (see detailed inclusion criteria, DLT definitions, and endpoints in supplemental methods). Efficacy was measured by the rate of complete remission (CR), complete remission with incomplete blood count recovery (CRi), and complete remission with incomplete platelet count recovery (CRp) per the International Working Group Response Criteria for AML,[5] assessed at the end of Cycles 1 and 3, then every three cycles.

Eighteen safety evaluable patients were assigned to five escalating dose level cohorts (dose levels 10–160 μg/kg). Baseline characteristics and molecular markers at screening are presented in Table 1. Patients received DCLL9718S (10–160 μg/kg) given in 21-day cycles with DCLL9718S dosed on Day 1 of each cycle. The median number of DCLL9718S treatment cycles administered was two (range 1–4).

Most patients experienced at least one AE, irrespective of attribution (Table S1). Half of the safety evaluable patients experienced at least one AE that was considered related to the study drug. Twelve patients (67%) experienced at least one AE of Grade ≥3 intensity; the most common were febrile neutropenia (six patients [33%]) and pneumonia (five patients [28%]). No DLTs were reported during the 21-day DLT assessment window. Two patients had Grade 4 AEs (thrombocytopenia) assessed as related to the study drug. The SAEs experienced by ≥25% of patients were febrile neutropenia (six patients [33%]) and pneumonia (four patients [22%]). No deaths were reported as related to DCLL9718S.

TABLE 1 Patient demographic and disease characteristics at baseline

	All patients (*N* = 18)
Age, median (range)	72 (19–89)
Age group (years)	
<65	7 (39%)
≥65	11 (61%)
Gender	
Male	12 (67%)
Female	6 (33%)
ECOG performance status at screening	
0	1 (6%)
1	17 (94%)
No. prior cancer regimens	
Average	3
Range	(1–10)
R/R AML with prior history of MDS	
Yes	5 (28%)
No	13 (72%)
ELN Classification	
Favorable	2 (11%)
Intermediate	5 (28%)
Adverse	10 (55%)
Unknown	1 (6%)
De novo versus therapy-related/secondary AML	
Primary AML	12 (67%)
Secondary AML	6 (33%)
Prior ASCT	0 (0%)
Molecular markers at screening	**All patients (*N* = 19)**
NPM1	
WT	11 (58%)
Mutated	1 (5%)
Unknown	7 (37%)
CEBPA	
WT	10 (53%)
Mutated	1 (5%)
Unknown	8 (42%)
FLT3-ITD	
WT	13 (68%)
Mutated	1 (5%)
Unknown	5 (26%)
FLT3-TKD	
WT	11 (58%)
Mutated	1 (5%)
Unknown	7 (37%)
TP53	
WT	9 (47%)
Mutated	1 (5%)
Unknown	9 (47%)
IDH1	
WT	8 (42%)
Mutated	2 (11%)
Unknown	9 (47%)

Note: Data not available for IDH2 mutation.
Abbreviations: AML, acute myeloid leukemia; ASCT, autologous stem cell transplant; ECOG, Eastern Cooperative Oncology Group; ELN, European LeukemiaNet; MDS, myelodysplastic syndrome.

Two patients in the 160 μg/kg dose developed elevated liver function tests. One patient experienced a Grade 3 increased ALT, Grade 2 increased AST, and Grade 1 increased alkaline phosphatase, without concomitant bilirubin elevation (all related to study drug) on Cycle 1 Day eight, but had a Grade 1 bilirubin elevation on Day 17 (unrelated) that resolved on its own on Cycle 1 Day 19. The patient then developed progression of disease and was taken off protocol on Cycle 1 Day 19. The ALT/AST elevations had both improved to Grade 1 at that time. The second patient experienced Grade 3 AST/ALT, bilirubin, and alkaline phosphatase increases on Cycle 1 Day eight, initially thought to be due to the concomitant administration of posaconazole (Figure S1). Posaconazole was discontinued. The AST/ALT elevations resolved on study Day 23, though bilirubin and alkaline phosphatase remained elevated for several weeks. The delayed recovery raised the concern that a causal relationship to DCLL9718S could not be ruled out. A maximum tolerated dose was not identified; based on the hepatic events observed at the highest dose and lack of anti-leukemic activity dose escalation was stopped.

The mean acPBD maximum concentrations (Cmax) occurred immediately after the infusion and increased with dose; acPBD PK showed a multi-exponential decline. The acPBD and the total antibody analytes demonstrated dose-dependent rapid clearance of ADC from circulation and large IIV (up to 182% CV) across the tested doses. Increases in Cycle 1 doses generally resulted in an increase in systemic exposure for all the three analytes; the Cycle 1 $AUC_{0\text{-}21d}$ increased disproportionately with dose for all the three analytes suggesting non-linear PK. The unconjugated PBD was consistently low. Minimal accumulation was observed for the acPBD, total antibody and unconjugated PBD analytes upon repeated dosing on the q3w schedule and steady-state appeared to be reached within the first dose in Cycle 1. The summary of PK parameters after the first dose in Cycle 1 are shown in Figure S2 and Table S2. No post-baseline ADA evaluable patients tested positive for ADA.

Among the 18 patients who received DCLL9718S, no patients achieved an objective IWG2003 CR or PR response. Response data was missing in three of these 18 patients (two patients in 160 μg/kg and one patient in 40 μg/kg) due to death or discontinuation of treatment before assessments (Figure 1).

Expression of CLL1 was detected in all patients except one (95%, 17/18), with varying expression intensity. While the majority of patients showed an expected unimodal CLL1 expression, a bimodal expression

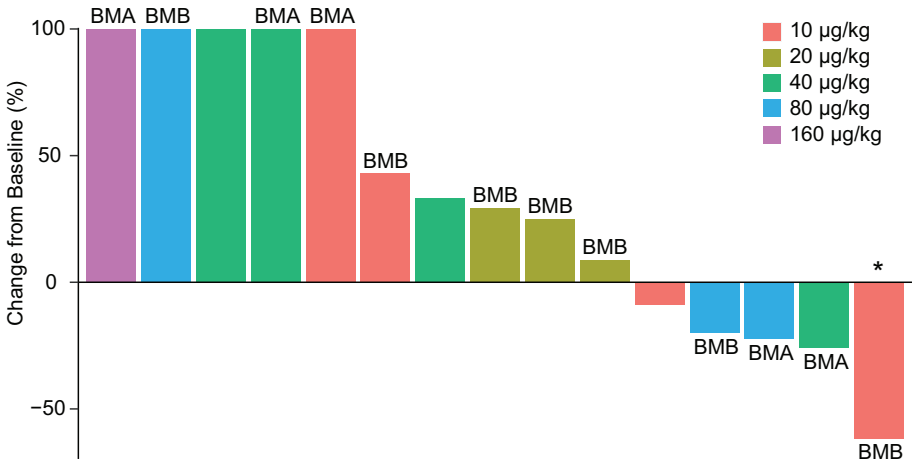

FIGURE 1 Waterfall plot showing patient-level changes in marrow blasts by dose cohort. For columns not marked by BMA or BMB, changes in blast levels were calculated from peripheral blood. *Patient did not meet criteria for response. Bone marrow aspirate demonstrated 25% increased blasts. BMA, bone marrow aspirate; BMB, bone marrow biopsy

of CLL1 with positive and negative population on CD34+ blasts was observed in some AML samples (3/18 patients). We were not able to assess whether the CLL1 bimodal expression and intensity had an impact on the clinical activity since no responses were observed.

DCLL9718S is the first anti-CLL1 ADC to enter the clinic. Preclinical evidence suggested that as compared to CD33 which is expressed on normal HSC, the degree and duration of marrow suppression with an anti-CLL1 ADC would be reduced. Also, DCLL9718S was designed with a highly potent PBD payload with the potential to have anti-leukemic activity even in chemotherapy resistant AML cells.[6,7]

The study was stopped during the dose escalation phase based on an assessment of safety, efficacy and pharmacokinetic data. With the limited data, the cause of the hepatic injury cannot be definitely determined, but may plausibly be attributed to the PBD payload. Emerging data from other ADCs in development in multiple indications suggest that hepatic injury may be a class effect of the PBD or modified PBD-like payloads.[8] Although anecdotal, both patients on this study with hepatic AEs had relatively higher DCLL9718S exposures (C_{max}/AUC) for all the three measured analytes. Hepatic injury by direct targeting of the liver by DCLL9718S is considered unlikely as CLL1 is not known to have any hepatic expression. Other mechanisms of hepatic injury could include non-specific uptake of antibody in the liver, metabolism of the unbound PBD in the liver, covalent binding of the PBD dimer to the hepatic tissues, or possible bystander killing if AML blasts are present in the liver. Ultimately though the mechanism of hepatic toxicity remains speculative.

Evaluation of CLL1 as a potential target in AML was incomplete as dose escalation was stopped due to toxicity unlikely to be attributable to the fact that DCLL9718S targets CLL1. We cannot determine whether dose levels were sufficiently high to drive antileukemic activity. Rapid clearance was observed in the majority of patients suggestive of targeted mediated clearance indicating engagement of CLL1 and internalization of DCLL9718S. Expression of CLL1 was observed in 95% of patients on study. These data support that CLL1 remains a viable target to pursue in AML with future ADCS, bispecific antibodies and CART approaches. As recently reported, a subset of AML patients have bimodal CLL1 expression.[9] In these patients with bimodal CLL1 AML blast expression, a subpopulation of blasts were CLL1 negative. No evidence of selective clearance of CLL1 positive blasts was observed in this study.

Given the limited tolerability and anti-tumor activity of DCLL9718S in this study, the program will not move forward in Phase II trials. In general, drugs (e.g., ADCs) with a highly potent payload, a narrow therapeutic index, and high PK variability pose significant challenges to development. CLL1 as a target in AML continues to remain viable and the data presented here helps to elucidate its role, guide future development and potential pitfalls that should be considered with future CLL1 directed therapies in myeloid malignancies.

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CONFLICT OF INTEREST

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Writing of the paper: all authors. All authors reviewed the manuscript critically and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

Qualified researchers may request access to individual patient level data through the clinical study data request platform (https://vivli.org/). Further details on Roche's criteria for eligible studies are available here (https://vivli.org/members/ourmembers/). For further details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here (https://www.roche.com/research_and_development/who_we_are_how_we_work/clinical_trials/our_commitment_to_data_sharing.htm).

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Increase of plasma erythroferrone levels during high-altitude exposure: A sub-analysis of the TOP OF HOMe study

To the Editor:

In recent years, erythroferrone (ERFE) has been described as the key endocrine regulator that connects erythropoiesis with iron metabolism.[1] In animal models, erythropoietin (EPO), which is the main driver of erythropoiesis, will induce ERFE production, which subsequently suppresses hepcidin synthesis, probably by inhibiting the hepatic BMP/SMAD pathway.[1] Low hepcidin itself will allow increased iron release from enterocytes and iron-recycling macrophages and thus augment iron availability for erythropoiesis. The same pathways are of clinical importance for the pathophysiology of iron disorders in humans, particularly in patients suffering from ineffective erythropoiesis.[1]

However, the specific roles of EPO and ERFE in physiological responses in healthy individuals who are exposed to high-altitude are less well understood.[2,3]

In a field experiment, eight healthy volunteers living in Homburg, Germany (233 meters above sea level [MASL]) spent 4 days at the "Schneefernerhaus" (Garmisch-Partenkirchen, Germany), an environmental research station at 2656 MASL. Our study was initially designed to study phosphorus regulation at high-altitude. We therefore randomized the eight volunteers into two groups, who were subsequently put either on a normal phosphorus diet (1300–1400 mg phosphorus/day) or on a low phosphorus diet (700–800 mg phosphorus/day). Detailed information about the project and the baseline characteristics are summarized in an earlier publication of our group, which focused upon phosphorus regulation at high-altitude.[4] The mean atmospheric air pressure at 233 and 2656 MASL is 1012 hPa (212.01 hPa oxygen partial pressure) and 740 hPa (155.03 hPa oxygen partial pressure).

Before, during and after high-altitude exposure, blood and urine samples were collected twice (Time Point [TP] 1–2), seven times (TP 3–9) and three-times (TP 10–12), respectively, for measurements of plasma ERFE, hepcidin and EPO; at day 2, maximal aerobic exercise was performed (∼10 km high-altitude hiking with a total ascent of ∼700 m), directly before TP 6.

We discarded the hepcidin, ERFE and EPO measurements after TP 9 from further analysis, as earlier work from our group suggested that a short-term increase in plasma EPO will increase plasma ERFE for approximately 7 days[5]; thus, we did not expect plasma ERFE to return to baseline values within 48 h (i.e., at TP 10–12) after high-altitude exposure.

The study was approved by the local ethics committee and conducted in concordance with the Declaration of Helsinki. All participants provided written informed consent.

Note, ERFE was measured from plasma samples by an enzyme-linked sandwich immunosorbent assay described previously.[5] Hepcidin was measured by an enzyme-linked immunosorbent assay (DRG hepcidin 25; DRG International Inc., USA). All other metabolites were measured according to the standardized methods of the Saarland University central laboratory.

Statistical analyses were performed by SPSS 20. Continuous data are presented as median (interquartile range [IQR]). Comparison as time progressed was performed by linear mixed model analysis, with prespecified time points as the dependent variable and high-altitude exposure (TP 3–9) versus no high-altitude exposure (TP 1–2) as the independent variable. Two-sided p values <.05 were considered significant.

As shown before, EPO increased compared to baseline (TP 1: 7.12 [6.06; 11.36] mIU/mL) soon after arriving at 2656 MASL, reaching its highest levels at TP 5 (20.80 [17.48; 21.59] mIU/mL). After descending to 233 MASL, EPO returned to baseline. Plasma hepcidin initially increased from 8.36 [8.03; 22.03] ng/mL to 22.30 [11.03; 44.06] ng/mL at TP 4 and subsequently decreased. Compared to levels measured at 233 MASL (TP 1: 2.56 [1.00; 5.88] ng/mL), plasma ERFE successively increased at high altitude, reaching its peak at TP 8 (10.30 [5.87; 17.47] ng/mL) (Figure 1, Table S2), and remaining elevated after a return to low altitude until TP 12. Strenuous physical exercise had no immediate effect upon either EPO, hepcidin or ERFE. In subgroup analyses, the changes in ERFE were particularly pronounced in individuals randomized to low phosphorus intake (Tables S1A and S1B).

In the linear mixed model analysis, plasma EPO was significantly associated with high-altitude exposure (Estimate 7.78 CI [5.59; 9.97]; $p < .001$). Plasma hepcidin, however, showed no association with high-altitude exposure (Estimate 0.52 CI [−5.55; 6.58]; $p = .858$). While