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Exposure–response analysis of blinatumomab in patients with relapsed/refractory acute lymphoblastic leukaemia and comparison with standard of care chemotherapy

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Aims: The relationship between blinatumomab exposure and efficacy endpoints (occurrence of complete remission [CR] and duration of overall survival [OS]) or adverse events (occurrence of cytokine release syndrome [CRS] and neurological events) were investigated in adult patients with relapsed/refractory acute lymphoblastic leukaemia (r/r ALL) receiving blinatumomab or standard of care (SOC) chemotherapy to evaluate appropriateness of the blinatumomab dosing regimen.

Methods: Exposure, efficacy and safety data from adult patients (n = 646) with r/r ALL receiving stepwise (9 then 28 μg/day, 4-week cycle) continuous intravenous infusion (n = 537) of blinatumomab or SOC (n = 109) chemotherapy were pooled from phase 2 and 3 studies. The occurrence of CR, neurological and CRS events, and duration of OS were analysed using Cox proportional hazards models or logistic regression, as appropriate. Confounding factors were tested multivariately as needed.

Results: Blinatumomab steady-state concentration following 28 μg/day dosing was associated with the probability of achieving CR (odds ratio and 95% confidence interval: 1.073 [1.033–1.114]), and a longer duration of OS compared to SOC (hazard ratio and 95% confidence interval: 0.954 [0.936–0.973], P < .05) in multivariate analyses. The exposure–safety analyses indicated that blinatumomab steady-state concentration following the 9 or 28 μg/day dose was not associated with increased probability of CRS or neurological events, after accounting for blinatumomab treatment effect (P > .05).

Conclusions: Blinatumomab step-dosing regimen of 9/28 μg/day provided treatment benefit in achieving CR and increasing the duration of OS over SOC and was appropriate in management of CRS and neurological events in patients with r/r ALL.

KEYWORDS

acute lymphoblastic leukaemia, blinatumomab, exposure–response analysis

1 | INTRODUCTION

Treatment in adult acute lymphoblastic leukaemia (ALL) has improved considerably in the past decades, with first-line treatment complete remission (CR) rates increased to 85–90%,[1] and 5-year overall survival (OS) rates in newly diagnosed ALL increased to approximately 40%.[2] Unfortunately, disease relapse is still a major therapeutic challenge, with at least 1/3 of standard-risk patients and up to 2/3 of high-risk patients eventually experiencing relapse.[3] Patients who relapse have a 5-year OS rate of approximately 7%.[2] In addition, standard chemotherapy can be associated with significant toxicity, such as myelosuppression, and infections[4]; hence, new therapies with improved efficacy or safety profiles are needed for the treatment of relapsed or refractory ALL (r/r ALL).

Blinatumomab is a novel single-chain antibody construct in the class of the bispecific T-cell engager (BiTE). Blinatumomab is designed to transiently connect CD19-positive cells[5] with T cells; causing the formation of a cytolytic synapse between the T cell and the tumour cell,[6] and thereby releasing the pore-forming protein perforin and the apoptosis-inducing proteolytic enzymes granzymes A and B. The subsequent serial lysis of multiple malignant cells by a single T cell closely resembles a natural cytotoxic T-cell reaction. Blinatumomab-mediated T-cell activation involves the transient release of inflammatory cytokines and the proliferation of T cells.[7]

Blinatumomab was granted breakthrough therapy designation by the US Food and Drug Administration in June 2014 for the treatment of adult patients with Philadelphia chromosome-negative (Ph−) r/r ALL. In the USA, blinatumomab has received accelerated approval (2014) and full approval (2017) for the treatment of Ph− or Ph-positive (Ph+) r/r B-cell precursor ALL in adult and paediatric patients. Recently, blinatumomab received accelerated approval for the treatment of minimal residual disease–positive B-cell precursor ALL.[8] Blinatumomab continues to be investigated for the treatment of non-Hodgkin lymphoma (NHL) in adults.

Blinatumomab exhibited linear pharmacokinetics (PK) under continuous intravenous infusion (cIV) for 4–8 weeks per cycle over a dose range of 5–90 µg/m²/day. Estimated mean (standard deviation) clearance, volume of distribution and elimination half-life were reported to be 3.11 (2.98) L/h, 4.35 (2.45) L and 2.10 (1.41) h, respectively.[8] PK were similar in patients with ALL and NHL, and no dose adjustment was required based on patient demographics or renal function in the evaluated patient populations. A previously published population PK analysis[9] concluded that disease related factors such as effects of baseline B-cell counts, T-cell counts, B-cell/T-cell ratio, and percentage of blasts in the bone marrow did not show any significant effect on CL. Hence lower exposure with higher disease burden is not expected. In clinical studies, <2% of patients treated with blinatumomab tested positive for binding anti-blinatumomab antibodies. Of the 9 patients who developed anti-blinatumomab antibodies, 7 (78%) had in vitro neutralizing activity.[8]

The objectives of the present analysis were to investigate the relationships between blinatumomab exposure and select efficacy (CR and OS) and safety (cytokine release syndrome [CRS] and

What is already known about this subject

- Blinatumomab is approved to treat relapsed/refractory acute lymphoblastic leukaemia in adults and children

- Blinatumomab may cause cytokine release syndrome and neurological toxicities

- Blinatumomab increases median overall survival to 7.7 months *vs* 4.0 months with chemotherapy

What this study adds

- Increasing blinatumomab steady-state concentrations were associated with higher probabilities of achieving complete remission

- Increasing blinatumomab steady-state concentration was associated with longer durations of overall survival

- After accounting for blinatumomab treatment effect, magnitude of blinatumomab steady-state concentration was not associated with an increased probability of cytokine release syndrome or neurological events

neurological events [NEs]) endpoints from patients diagnosed with Ph + or Ph− r/r ALL receiving blinatumomab or standard of care (SOC) chemotherapy in studies MT103–211 (blinatumomab alone),[10] 20120216 (blinatumomab alone),[11] and 00103311 (blinatumomab or SOC).[12] An exposure–response (ER) analysis of blinatumomab reported for a phase 2 study (MT103–211 [NCT01466179]; n = 189) in r/r adult ALL[13] found an association of higher blinatumomab exposure with CR. However, this is the first ER analysis of blinatumomab including a larger adult r/r ALL patient population across 3 studies, including MT103–211, with a SOC arm for comparison. The appropriateness of the recommended blinatumomab regimen was evaluated based on the results of ER analyses to support justification of the tested dosing regimen for blinatumomab in treatment of r/r adult ALL. A statistically significant ER relationship for efficacy outcomes and no relationship for safety outcomes would indicate the optimal appropriateness.

2 | METHODS

2.1 | Clinical data

This ER analysis pooled data from 3 clinical studies (ClinicalTrials.gov, NCT01466179, NCT02000427, and NCT02013167) in 646 adult patients with ALL. Data from studies MT103–211,[10] 20120216[11] and 00103311[12] were included in this analysis. In these 3 trials, patients received a cIV of blinatumomab at an initial dose of

9 µg/day for the 1st week, followed by 28 µg/day for weeks 2–4 of the first cycle (C1 WK2) and for all subsequent 4-week cycles. There were 2-week blinatumomab-free periods between cycles. PK of blinatumomab were assessed in the first 2 treatment cycles for determination of blinatumomab concentration at steady state (C_{ss}) following 9 µg/day (week 1 of cycle 1: C1 WK1) and 28 µg/day cIV dosing for weeks 2–4 of cycle 1 or cycle 2. Patients randomized to receive SOC chemotherapy in the phase 3 study were assigned to one of the following chemotherapy regimens per the investigator's choice: (i) fludarabine, cytarabine arabinoside and granulocyte colony-stimulating factor; (ii) high-dose ara-C; (iii) high-dose methotrexate-based combination regimen; or (iv) clofarabine or clofarabine-based regimens.[14] Additional details of each clinical study used for the current analyses are reported elsewhere.[10-12] All studies were sponsored by Amgen Inc. and conducted in accordance with principles for human experimentation as defined in the Declaration of Helsinki and the International Conference on Harmonisation's Good Clinical Practice guidelines. The study protocols were approved by the respective institutional review boards. Informed consent was obtained from each patient after being told the potential risks and benefits, as well as the investigational nature of the study.

CR was defined as having ≤5% blasts in the bone marrow, no evidence of disease and full recovery of peripheral blood counts: platelets >100 000/µL, and absolute neutrophil count >1000/µL.

Occurrence of CR and duration of OS were efficacy endpoints in the studies, and hence selected as the efficacy endpoints in these analyses. NEs and CRS were previously identified as adverse events of interest.[10-12,15] CRS was based on the reported terms, which were coded to the preferred term of 'cytokine release syndrome' or 'cytokine storm'. NEs were defined using a specific search strategy that included the Medical Dictionary for Regulatory Activities high-level group terms of cranial nerve disorders; 'deliria', including 'confusion'; 'disturbances in thinking and perception'; 'encephalopathies'; 'mental impairment disorders'; 'movement disorders', including 'Parkinsonism'; neurological disorders not elsewhere classified; neuromuscular disorders; personality disorders and disturbances in behaviour; psychiatric disorders not elsewhere classified; and seizures, including subtypes. NEs of any grade were included.

2.2 | Sampling for blinatumomab C_{ss}

Serum samples for determination of blinatumomab concentrations were collected at steady state following the 9 or 28 µg/day dose. In Study MT103–211, PK samples were taken during week 1 of cycle 1 (C1 WK1) following the 9 µg/day dose, and during weeks 2–4 of cycle 1 (C1 WK2) and cycle 2 following the 28 µg/day dose, with a median of 6 PK samples collected per patient. In Study 20120216, PK samples were collected during C1 WK1 following the 9 µg/day dose and during C1 WK2 and cycle 2 following the 28 µg/day dose, with a median of 2 PK samples collected per patient. In Study 00103311, PK samples were collected during C1 WK1 following the 9 µg/day dose and during week 3 of cycle 1 following the 28 µg/day dose in all patients

who received blinatumomab, with a median of 2 PK samples collected per patient. Blinatumomab serum concentrations were assessed using a bioassay reported elsewhere.[16] This assay had a lower limit of quantitation of 50 pg/mL and % coefficient of variation of ≤20%.

2.3 | Software

Descriptive PK data analysis and ER analyses were performed using SAS version 9.4 on Microsoft Windows. Graphical and all other statistical analyses were performed with TIBCO Spotfire S+ version 8.0 or above (TIBCO Software Inc., Palo Alto, CA, USA) and R version 3.0.3.

2.4 | ER modelling

As an exploratory analysis, CR, duration of OS, occurrence of first CRS or NE within an individual where C_{ss} was available were tabulated by quartiles of C_{ss}. Baseline covariates were summarized for each quartile of the C_{ss} group for cycle 1 and cycle 2 to investigate similarity. Details of CR, OS, CRS and NEs, and baseline covariates in the SOC arm have been reported previously.[12]

In the ER analysis, efficacy endpoints included CR and duration of OS. Safety endpoints included CRS and NEs. OS time was calculated from time of randomization until death due to any cause. Patients still alive were censored at the date last known to be alive. If the date last known to be alive was after the date that triggered the analysis, the patient was censored at the analysis trigger date. The analyses for CRS and NEs were split by week 1 vs week 2 and beyond because most CRSs (59 of 76, 78%) and NEs (212 of 406, 52%) occurred during the first week of cycle 1 which corresponded to the lower dose level (9 µg/day). Since the blinatumomab dose was different in week 1 from the rest of treatment, separate analyses were performed to focus on events occurring in week 1 and events occurring anytime during the study.

Blinatumomab C_{ss} was selected as the exposure metric to explore associations with efficacy or safety events. Since blinatumomab is administered by cIV infusion and the PK not time-dependent, the C_{ss} is the relevant exposure variable. For the analyses relating blinatumomab C_{ss} to occurrence of CR, CRS or NEs, the time-averaged blinatumomab C_{ss} at a given dose corresponding to the nearest time period of the CRS or NE in the treatment cycle was used to relate to the CRS or NE. For example, the week 1, cycle 1 C_{ss} was used for events occurring during week 1 of cycle 1, while the week 2, cycle 1 C_{ss} was used for events occurring during week 2 of cycle 1 if the doses in weeks 1 and 2 were different. Where there were more than 1 C_{ss} available within the cycle for the same dose level, the average of C_{ss} values was used. To allow for a pooled ER analysis of patients receiving blinatumomab or SOC chemotherapy, patients receiving SOC chemotherapy were assigned a blinatumomab C_{ss} of 0.0001 pg/mL so that they could be included when evaluating the effect of blinatumomab C_{ss} (log transformed) on efficacy and safety endpoints.

The occurrence and time-to-event analyses evaluated the association of blinatumomab C_{ss} and efficacy or safety events. To evaluate any potential confounding factors affecting the exposure–response relationship, multivariate analyses with a forward selection method were used to evaluate the effect of baseline covariates. For all analyses, the baseline covariates were age, weight, body surface area, sex, degree of bone marrow infiltration, blood counts (i.e. haemoglobin, platelets, peripheral blasts in blood, and CD3+ T cells[5]), prior allogeneic haematopoietic stem cell transplant (HSCT) and B-precursor ALL subtype (B-ALL with recurrent genetic abnormality, C-ALL, Pre-B-ALL and Pro-B-ALL) related to last relapse. Patients with missing baseline covariates were excluded from the analyses. CD molecule naming (i.e. CD19, CD3) conformed to the IUPHAR/BPS Guide to PHARMACOLOGY nomenclature classification.[5]

Occurrence analyses were conducted using univariate and multivariate logistic regression models, and the odds ratios (ORs) and respective 95% confidence intervals (95% CIs) were presented. Time-to-event analyses were conducted using Cox proportional hazards models, and the hazard ratios (HRs) and respective 95% CIs are presented. Effects were considered significant in the univariate forward-selection analysis for $P < .1$ and in the multivariate analysis for $P < .05$. A significance level of .1 was selected to provide a conservative criterion, especially when assessing exposure-safety analysis, such that even if a covariate had a significance level of .1, it would not be dropped, and its effect further assessed during the multivariate analysis. P values were not adjusted for multiplicity of comparisons, and therefore, should be interpreted with caution.

2.5 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[17] and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18.[5]

3 | RESULTS

3.1 | Patients

There were 225, 45 and 376 patients in each of MT103–211, 20120216 and 00103311, respectively, totalling 646 patients. Of the 646 patients in the analysis, 537 received blinatumomab and 109 received SOC chemotherapy. Blinatumomab C_{ss} was available from 342 patients receiving the 9 μg/day dose in cycle 1 week 1, from 407 patients receiving the 28 μg/day dose in cycle 1 week 2, and from 122 patients receiving the 28 μg/day dose in cycle 2. A summary of the efficacy/safety endpoints, categorical baseline covariates and continuous baseline covariates per exposure quartiles for the patients included in the analysis are presented in Tables 1–3. A trend was seen where patients with Pro-B ALL had lower exposure, whereas women had slightly higher exposures. In the lowest quartile, there appeared

to be higher blast counts (cycle 1), lower CD, and highest degree of bone marrow infiltration. Baseline CD19+ B cells could not be evaluated as a covariate in the analysis as it was only available in 32% of patients, all of whom were from Study MT103–211.

3.2 | Blinatumomab exposure and baseline covariates

A summary of mean (standard deviation) blinatumomab C_{ss} by dose and treatment cycle is provided in Table 4. Since blinatumomab at a given dose was constant over time during cIVs, individual C_{ss} values were calculated as the average of observed serum concentrations collected during the infusion at each dose (Table 4). Individual levels of C_{ss} in cycle 1 and cycle 2 for 28 μg/day were similar with the geometric mean of the individual ratios of cycle 2 exposure to cycle 1 exposure for those patients who had exposures in both cycles was 1.07, although the group mean/range in cycle 2 was greater than in cycle 1 due to survival bias because many patients with low exposure dropped out during cycle 1. The exploratory analyses shown in Tables 1–3 suggest that the distribution of baseline covariates (categorical and continuous), occurrence of CRS and occurrence of NEs appear to be similar across the exposure (C_{ss}) quartiles, while duration of occurrence of CR and OS appears to increase with increasing exposure with plateauing at the highest 2 quartiles of blinatumomab exposure. The apparent association between increasing duration of OS with increasing exposure is also seen graphically when the OS curves are stratified by quartiles of exposure (Figure 1).

3.3 | Exposure–occurrence of CR analysis

CR was observed in 178 (33%) of the 537 patients who received blinatumomab and in 21 (19%) of the 109 patients who received SOC chemotherapy across the 3 studies included in the analysis. Following stepwise inclusion of covariate effects identified in the univariate analysis, lower peripheral blasts in blood, lower degree of bone marrow infiltration and no prior allogeneic HSCT were associated with a greater probability of CR ($P < .05$) in the multivariate analysis (Table 5). Upon inclusion of treatment (blinatumomab vs SOC) or blinatumomab C_{ss} (following the 28 μg/day dose), in addition to the covariates identified as significant and listed above, both treatment with blinatumomab (OR [95% CI]: 1.969 [1.11–3.494]) and higher blinatumomab C_{ss} (OR [95% CI]: 1.073 [1.033–1.114]) were significantly associated with a greater probability of CR ($P < .05$). To confirm the effect of blinatumomab C_{ss} in patients receiving blinatumomab, the multivariate analysis was updated to exclude the patients receiving SOC chemotherapy alone. Upon exclusion of patients receiving SOC chemotherapy, blinatumomab exposure was still significantly associated with occurrence of CR (OR [95% CI]: 1.664 [1.194–2.319]). Thus, after adjusting for significant disease-related baseline covariates, blinatumomab treatment and higher blinatumomab C_{ss} were associated with a higher occurrence of a CR event ($P < 0.05$) when the patients treated with SOC chemotherapy or blinatumomab were

TABLE 1 Distribution of categorical baseline covariates (*n*) by quartiles of exposure

Categorical baseline covariate	Category	Quartiles of exposure following 9 μg/day dose (cycle 1, week 1)			
		Q1 (n = 83) < 88 pg/mL	Q2 (n = 85) ≥ 88 and < 133 pg/mL	Q3 (n = 88) ≥ 133 and < 227.5 pg/mL	Q4 (n = 86) ≥ 227.5 pg/mL
Sex	Female	32	34	40	36
	Male	51	51	48	50
Prior allogeneic HSCT status	No	54	58	58	54
	Yes	28	27	30	32
B-precursor ALL subtype related to last relapse	B-ALL with recurrent genetic abnormality	6	2	4	3
	C-ALL	16	23	22	8
	Pre-B-ALL	29	37	36	49
	Pro-B-ALL	12	11	8	3

Categorical baseline covariate	Characteristics	Quartiles of exposure following 28 μg day⁻¹ dose (cycle 1, weeks 2–4)			
		Q1 (n = 102) < 274.2 pg/mL	Q2 (n = 101) ≥ 274.2 and < 479 pg/mL	Q3 (n = 102) ≥ 479 and < 787.5 pg/mL	Q4 (n = 102) ≥ 787.5 pg/mL
Sex	Female	35	32	44	51
	Male	67	69	58	51
Prior allogeneic HSCT status	No	66	68	61	71
	Yes	36	33	41	31
B-precursor ALL subtype related to last relapse	B-ALL with recurrent genetic abnormality	8	3	2	7
	C-ALL	18	15	24	16
	Pre-B-ALL	40	48	37	45
	Pro-B-ALL	11	9	15	5

Categorical baseline covariate	Characteristics	Quartiles of exposure following 28 μg/day dose (cycle 2)			
		Q1 (n = 31) < 446.5 pg/mL	Q2 (n = 30) ≥ 446.5 and < 634.3 pg/mL	Q3 (n = 30) ≥ 634.3 and < 1000.6 pg/mL	Q4 (n = 31) ≥ 1000.6 pg/mL
Sex	Female	5	7	14	21
	Male	26	23	16	10
Prior allogeneic HSCT status	No	21	23	17	21
	Yes	10	7	13	10
B-precursor ALL subtype related to last relapse	B-ALL with recurrent genetic abnormality	0	0	0	0
	C-ALL	4	5	7	7
	Pre-B-ALL	10	13	13	8
	Pro-B-ALL	1	0	1	3

ALL, acute lymphoblastic leukaemia; B-ALL, B-precursor ALL; C-ALL, common type of ALL; HSCT, hematopoietic stem cell transplant; Q, quartile.

TABLE 2 Distribution of continuous baseline covariates [mean (range)] by quartiles of exposure

Continuous baseline covariate	Quartiles of exposure following 9 μg/day dose (cycle 1, week 1)			
	Q1 (n = 83) < 88 pg/mL	Q2 (n = 85) ≥ 88 and < 133 pg/mL	Q3 (n = 88) ≥ 133 and < 227.5 pg/mL	Q4 (n = 86) ≥ 227.5 pg/mL
Weight (kg)	72.8 (42.9–110)	72.4 (45–129)	74.2 (43.8–134)	74 (40.7–148.7)
Age (years)	40 (19–77)	39 (18–80)	44 (18–76)	44 (19–79)
BSA (m²)	1.824 (0.21–2.297)	1.84 (1.401–2.49)	1.845 (1.36–2.498)	1.841 (1.315–2.7)
Degree of bone marrow infiltration (%)	74.4 (3–100)	64 (1–99)	56.3 (2–99)	59 (2–99)
Peripheral blast counts (%)	17.3 (0–100)	16.5 (0–124.7)	8.8 (0–93.6)	9.8 (0–84.3)
Haemoglobin (g/L)	100 (69–167)	99.1 (68–143)	100 (66–143)	103.4 (63–142)
Platelets (×10⁹/L)	67.6 (2–457)	66.9 (3–496)	81.7 (7–318)	77.5 (8–355)
CD3+ T cells (%)	48.8 (0.7–97.6)	52.4 (0.9–99.4)	63.6 (3.6–99.2)	59.4 (0.5–98.7)
Continuous baseline covariate	Quartiles of exposure following 28 μg/day dose (cycle 1, weeks 2–4)			
	Q1 (n = 102) < 274.2 pg/mL	Q2 (n = 101) ≥ 274.2 and < 479 pg/mL	Q3 (n = 102) ≥ 479 and < 787.5 pg/mL	Q4 (n = 102) ≥ 787.5 pg/mL
Weight (kg)	77 (40.7–148.7)	72.7 (42.5–129)	71.3 (42.9–130.1)	72.9 (39–134)
Age (years)	38 (18–70)	41 (19–77)	42 (18–79)	45 (18–80)
BSA (m²)	1.915 (1.315–2.7)	1.813 (0.21–2.49)	1.821 (1.35–2.422)	1.836 (1.312–2.378)
Degree of bone marrow infiltration (%)	77.9 (2–100)	65.3 (3–99)	61 (3–100)	60.8 (2–99)
Peripheral blast counts (%)	27.7 (0–143.8)	10.6 (0–100)	9.2 (0–95)	8.1 (0–115)
Haemoglobin (g/L)	101 (71–139)	100.1 (48–167)	100.4 (66–141)	100.9 (63–142)
Platelets (×10⁹/L)	67.5 (2–570)	81 (10–496)	75.9 (6–300)	72.8 (3–355)
CD3+ T cells (%)	38.7 (0.2–99.1)	55.9 (1.2–96.3)	58.6 (0.3–99.4)	60.1 (0.5–98.7)
Continuous baseline covariate	Quartiles of exposure following 28 μg/day dose (cycle 2)			
	Q1 (n = 31) < 446.5 pg/mL	Q2 (n = 30) ≥ 446.5 and < 634.3 pg/mL	Q3 (n = 30) ≥ 634.3 and < 1000.6 pg/mL	Q4 (n = 31) ≥ 1000.6 pg/mL
Weight (kg)	80.2 (46.5–127.5)	77.7 (48–148.7)	71.7 (46.8–126.5)	74.9 (46.7–134)
Age (years)	38 (19–76)	39 (19–72)	40 (18–72)	52 (19–77)
BSA (m²)	1.931 (1.473–2.475)	1.927 (1.489–2.7)	1.832 (1.412–2.354)	1.807 (1.427–2.285)
Degree of bone marrow infiltration (%)	67.4 (11–96)	55.7 (2–98)	53.1 (4–98)	58.3 (3–99)
Peripheral blast counts (%)	12.8 (0–80)	12.5 (0–90)	8.2 (0–72)	10.3 (0–95)
Haemoglobin (g/L)	103.4 (78–138)	100 (75–128)	100.6 (81–141)	100.7 (73–130)
Platelets (×10⁹/L)	91.7 (13–490)	92.7 (6–417)	86.5 (10–496)	66 (3–355)
CD3+ T cells (%)	50.4 (1.2–96.3)	58.2 (3.6–97.2)	52.2 (0.9–97.5)	58.8 (1.8–98.7)

BSA, body surface area; Q, quartile.

pooled. Additionally, when evaluating effects in blinatumomab-treated patients alone, blinatumomab exposure was found to be significantly associated with CR ($P < 0.05$), suggesting the range of blinatumomab exposures following 28 μg/day was associated with a higher probability of CR.

3.4 | Exposure–duration of OS analysis

A total of 529 (82%; 420 treated with blinatumomab and 109 treated with SOC chemotherapy) of 646 patients had blinatumomab C_{ss} available for the analysis of duration of OS. The individual C_{ss} closest to the OS event was used if available; otherwise, the C_{ss} from cycle 1 while receiving 28 μg/day (weeks 2–4) was used. Patients who did not have a C_{ss} following the 28 μg/day dose were not included in the analysis. Among the 537 patients receiving blinatumomab, the median duration of OS was 216 (range: 180–244) days. For the 109 patients receiving

SOC chemotherapy, the median duration of OS was 124 (range: 93–179) days. Treatment with blinatumomab (HR [95% CI]: 0.755 [0.588–0.970]) and higher blinatumomab C_{ss} (HR [95% CI]: 0.967 [0.952–0.983]) were found to be significantly associated with a lower HR for OS ($P < 0.1$) in the univariate analysis.

Following stepwise inclusion of the covariate effects identified in the univariate analysis, lower peripheral blasts in blood, lower degree of bone marrow infiltration and higher platelets were associated with a lower hazard for OS ($P < .05$) in the multivariate analysis. Upon inclusion of treatment (blinatumomab vs SOC chemotherapy), in addition to the covariates identified, treatment with blinatumomab (HR [95% CI]: 0.69 [0.511–0.93]) was significantly associated with a lower hazard of OS ($P < .05$; Table 6). Upon inclusion of blinatumomab C_{ss}, in addition to the covariates identified, a higher blinatumomab C_{ss} was significantly associated (HR [95% CI]: 0.954 [0.936–0.973]) with a lower hazard of OS ($P < 0.05$) and degree of bone marrow infiltration was no longer significant ($P = 0.1907$). To confirm the effect of

TABLE 3 Summary of CR, OS, CRS and neurological events by quartiles of exposure (closest to event) in patients treated with blinatumomab

CR				
Quartile of exposure[a]	Total n	Event	Censored	
Q1 (< 276.3 pg/mL)	102	18	84	
Q2 (≥ 276.3 and < 494 pg/mL)	103	40	63	
Q3 (≥ 494 and < 795 pg/mL)	103	54	49	
Q4 (≥ 795 pg/mL)	103	51	52	
OS				
Quartile of exposure[a]	Total n	Event	Censored	Median (95% CI) [days]
Q1 (< 265.5 pg/mL)	105	79	26	104 (68–169)
Q2 (≥ 265.5 and < 501 pg/mL)	104	68	36	230 (167–339)
Q3 (≥ 501 and < 811.1 pg/mL)	106	55	51	368 (256–473)
Q4 (≥ 811.1 pg/mL)	105	57	48	294 (242–444)
CRS				
Quartile of exposure[a]	Total n	Event	Censored	
Q1 (< 105 pg/mL)	91	81	10	
Q2 (≥ 105 and < 215 pg/mL)	93	75	18	
Q3 (≥ 215 and < 589.7 pg/mL)	93	78	15	
Q4 (≥ 589.7 pg/mL)	92	80	12	
Neurological events				
Quartile of exposure[a]	Total n	Event	Censored	
Q1 (< 120 pg/mL)	97	70	27	
Q2 (≥ 120 and < 241 pg/mL)	98	68	30	
Q3 (≥ 241 and < 539 pg/mL)	98	59	39	
Q4 (≥ 539 pg/mL)	98	65	33	

Note: Kaplan–Meier estimation method used to calculate the median event or censoring times for OS. Censoring based on the criterion as defined in the study statistical analysis plan.

CI, confidence interval; CR, complete remission; CRS, cytokine release syndrome; OS, overall survival; Q, quartile; N, number.

[a]Css closest to event used if available.

TABLE 4 Mean (standard deviation) [n] blinatumomab C_{ss} (pg/mL) summarized by dose and cycle

	Studies			
Dose and cycle	MT103–211	20120216	00103311	All
9 μg/day, cycle 1 (week 1)	246 (304.9) [178]	155 (106.4) [8]	211 (413) [156]	228 (355.6) [342]
28 μg/day, cycle 1	632 (510.2) [188]	673 (613.6) [28]	592 (553.4) [191]	616 (537.5) [407]
28 μg/day, cycle 2	755 (432.7) [101]	756 (564.7) [21]	NA[a]	755 (455.5) [122]

C_{ss}, steady-state concentration; NA, not applicable.

[a]No pharmacokinetic samples were collected in cycle 2 per study protocol.

blinatumomab C_{ss} in patients receiving blinatumomab, the multivariate analysis was updated to exclude the patients receiving SOC chemotherapy. Upon exclusion of these patients, a higher blinatumomab C_{ss} was still significantly associated (HR [95% CI]: 0.7 [0.595–0.824]) with a lower hazard of OS ($P < 0.0001$).

3.5 | Exposure–CRS analysis

In the exposure–CRS analysis dataset, CRS events occurred in 76 (14%) of the 537 patients who received blinatumomab and in none of the 109 patients who received SOC chemotherapy. Therefore,

CRS appears to be a unique event of blinatumomab immunotherapy compared to SOC chemotherapy. A majority of the CRS events (59 of 76; 78%) occurred during week 1 of cycle 1 while patients received the 9 μg/day dose. The other 17 CRS events occurred while patients received the 28 μg/day dose during the remainder of cycle 1 (weeks 2–4) or subsequent cycles. Thus, exposure–CRS event analyses were conducted using data from blinatumomab-treated patients. Since the blinatumomab dose was different in week 1 from the rest of treatment period, separate analyses were performed to focus on events occurring in week 1 and events occurring anytime during the study (including week 1).
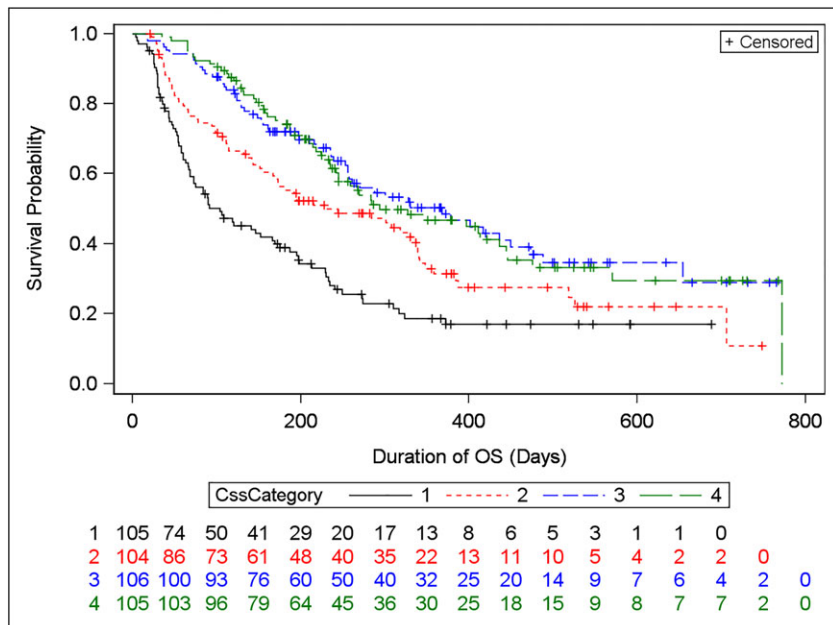
FIGURE 1 Kaplan–Meier survival curve across exposure quartiles in patients treated with blinatumomab. CssCategory is the category based on quartiles of C_{ss}. Black (CssCategory = 1), red (CssCategory = 2), blue (CssCategory = 3), and green (CssCategory = 4) lines represent, respectively, the overall survival Kaplan–Meier curves for the first (< 265.5 pg/mL), second (≥ 265.5 and < 501 pg/mL), third (≥ 501 and < 811.1 pg/mL) and fourth (≥ 811.1 pg/mL) exposure-based quartiles for patients treated with blinatumomab. C_{ss}, steady-state concentration; OS, overall survival. Numbers indicate the number of subjects at risk at times matching the tick values for the x-axis

TABLE 5 Multivariate logistic model for complete remission using a forward selection algorithm

Effect	Odds ratio (95% Wald confidence limits)	P value
Multivariate[a] (C_{ss} effect) in blinatumomab and SOC arm		
Peripheral blasts in blood (per %)	0.972 (0.957–0.988)	0.0007
Degree of bone marrow infiltration (per %)	0.983 (0.976–0.990)	< 0.0001
Prior allogeneic HSCT (No vs Yes)	1.755 (1.083–2.843)	0.0223
Blinatumomab exposure (C_{ss})[b]	1.073 (1.033–1.114)	0.0003
Multivariate[c] (C_{ss} effect) in blinatumomab-only arm		
Peripheral blasts in blood (per %)	0.977 (0.961–0.992)	0.0037
Degree of bone marrow infiltration (per %)	0.985 (0.977–0.993)	0.0003
Blinatumomab exposure (C_{ss})[b]	1.664 (1.194–2.319)	0.0026
Multivariate[d] (blinatumomab vs SOC)		
Peripheral blasts in blood (per %)	0.973 (0.957–0.989)	0.0008
Degree of bone marrow infiltration (per %)	0.981 (0.975–0.988)	< 0.0001
Prior allogeneic HSCT (No vs Yes)	1.927 (1.223–3.035)	0.0047
Blinatumomab treatment (blinatumomab vs SOC)	1.969 (1.11–3.494)	0.0205

C_{ss}, steady-state concentration; HSCT, hematopoietic stem cell transplant; SOC, standard of care.

[a]Of the 646 patients included in the analysis dataset, 415 were not missing any of the covariates or C_{ss} and were included in the multivariate analysis.

[b]C_{ss} closest to event used if available, or C_{ss} at weeks 2–4 of cycle 1 if unavailable; per log unit of C_{ss}.

[c]Of the 646 patients included in the analysis dataset, 325 were not missing any of the covariates or C_{ss} and were included in the multivariate analysis.

[d]Of the 646 patients included in the analysis dataset, 507 were not missing any of the covariates or C_{ss} and were included in the multivariate analysis.

In the univariate analyses for CRS events occurring in cycle 1 week 1, blinatumomab C_{ss} was not associated with the probability of CRS during cycle 1 week 1. Following stepwise inclusion of covariate factors identified in the univariate analysis, the multivariate analyses for cycle 1 week 1, a higher CD3+ T-cell count was associated (OR [95% CI]: 1.018 [1.007–1.028]; P < .001) with a higher probability of CRS events, while higher body weight was no longer associated with probability of CRS events (P = .2031).

Thus, following administration of blinatumomab, a higher CD3+ T-cell count at baseline was associated with a higher occurrence of CRS events. Blinatumomab C_{ss} was not associated with the occurrence of CRS events, suggesting that the variability in blinatumomab exposure following 9 or 28 μg/day dosing was not associated with the probability of a CRS event.

3.6 | Exposure–NEs analysis

In this exposure–safety analysis dataset, NEs (406) occurred in 353 (66%) of the 537 patients who received blinatumomab and in 53

TABLE 6 Multivariate Cox model for overall survival using a forward selection algorithm

Effect	Hazard ratio (95% CI)	P value
Multivariate[a] (C_{ss} effect) in blinatumomab and SOC arm		
Peripheral blasts in blood (per %)	1.013 (1.008–1.017)	< 0.0001
Platelets (per ×10³/mL)	0.994 (0.992–0.996)	< 0.0001
Blinatumomab exposure (C_{ss})[a]	0.954 (0.936–0.973)	< 0.0001
Multivariate[b] (C_{ss} effect) in blinatumomab-only arm		
Peripheral blasts in blood (per %)	1.011 (1.006–1.015)	< 0.0001
Platelets (per ×10³/mL)	0.996 (0.994–0.998)	< 0.0001
Blinatumomab C_{ss}[a]	0.700 (0.595–0.824)	< 0.0001
Multivariate[c] (blinatumomab treatment effect)		
Peripheral blasts in blood (per %)	1.008 (1.004–1.013)	0.0003
Degree of bone marrow infiltration (per %)	1.006 (1.001–1.01)	0.0132
Platelets (per ×10³/mL)	0.994 (0.992–0.997)	< 0.0001
Blinatumomab treatment (blinatumomab vs SOC)	0.69 (0.511–0.93)	0.015

CI, confidence interval; C_{ss}, steady-state concentration; SOC, standard of care.

[a]Of the 646 patients included in the analysis dataset, 409 were not missing any of the covariates or C_{ss} and were included in the multivariate analysis.

[b]Of the 646 patients included in the analysis dataset, 348 were not missing any of the covariates or C_{ss} and were included in the multivariate analysis.

[c]Of the 646 patients included in the analysis dataset, 466 were not missing any of the covariates or C_{ss} and were included in the multivariate analysis.

(49%) of the 109 patients who received SOC chemotherapy. Therefore, NEs were not uniquely related to treatment with blinatumomab compared to SOC chemotherapy. Approximately half of the NEs (212 of 406; 52%) occurred during week 1 of treatment while patients either received the 9 μg/day dose (185 of 353; 52%) or SOC chemotherapy (27 of 53; 51%). The other 194 NEs (194 of 406; 48%) occurred while patients received the 28 μg/day dose (168 of 353; 48%) or SOC chemotherapy (26 of 53; 49%) during the remaining treatment period. Thus, exposure–NEs analyses were conducted based on NEs from cycle 1 week 1 or NEs occurring anytime (including week 1) during the study. The C_{ss} used in this analysis was closest observed to the NE if nonmissing, or C1 WK2 C_{ss} if missing.

In the univariate analyses for NEs, blinatumomab treatment (vs SOC chemotherapy; OR [95% CI]: 1.596 [0.998–2.553]), a higher blinatumomab C_{ss} (OR [95% CI]: 1.034 [0.999–1.07]) and women (vs men; OR [95% CI]: 1.443 [1.035–2.013]) were associated with a higher probability of NEs (P < .1), and were selected for further evaluation in the multivariate analysis.

In the multivariate analysis, upon inclusion of treatment (blinatumomab vs SOC chemotherapy) or blinatumomab C_{ss}, in addition to sex (female), treatment with blinatumomab (OR [95% CI]: 1.609 [1.004–2.578]) was associated with a greater probability of NEs during cycle 1 week 1 (P < .05); however, blinatumomab C_{ss} was not (P = .0540). For NEs occurring anytime during the study, blinatumomab treatment (OR [95% CI]: 2.055 [1.352–3.124]) or blinatumomab C_{ss} (OR [95% CI]: 1.050 [1.020–1.081]) were associated with a higher probability of NEs (P < .05) after accounting for sex (female). To confirm the effect of blinatumomab C_{ss} in patients receiving blinatumomab, the multivariate analyses of NEs occurring during cycle 1 week 1 or anytime during the study were updated to exclude the patients treated with SOC chemotherapy. Upon exclusion of these patients, blinatumomab exposure was not associated with the

occurrence of NEs during cycle 1 week 1 (P = .3691) or anytime during the study (P = .1512).

Overall, blinatumomab treatment is associated with a higher probability of NEs compared to SOC chemotherapy treatment, and a higher blinatumomab C_{ss} is not associated with a higher probability of NEs in blinatumomab-treated patients.

4 | DISCUSSION

Most blinatumomab clinical trials were single-arm studies. This is because of challenges in the enrolment of a significant number of patients with r/r ALL due to the rareness of disease and the unique approach to blinatumomab dosing (cIV infusion for a month), which prevents a double-blind study design to compare with SOC (mainly short IV infusion for some hours). This paper reports the first analysis combining valuable data from multiple studies of r/r ALL patients receiving SOC or blinatumomab. The ER relationship is one of the key determinants of the safety and effectiveness of drugs, and clinical benefit is determined by weighing the favourable and unfavourable effects at a particular dose.[18] However, some limitations of an ER analysis may include: (i) patients in the randomized clinical trials being balanced across treatment arms, and not necessarily across exposure-based groups; (ii) presence of unrecognized confounders, such the lack of sufficient baseline CD19+ B-cell data for evaluation as a covariate; (iii) analysis of response for a specific endpoint at a given time point may not necessarily reflect the long-term effects of the drug.[19] Of note, a previous ER analysis demonstrated that lower proportions of CD19+ B cells at screening were significantly associated with achieving CR, a greater proportion in the occurrence of NEs, and shorter median time to NEs.[13] These limitations were considered during this ER analysis, and the results were interpreted after weighing in the

caveats in the analysis. However, given the lack of comparator arms in the 2 phase 2 studies included, other unknown confounding factors may have introduced an unidentified bias.

Blinatumomab PK was found to be stable with time (across cycles). Further, blinatumomab was given by cIV over the dosing interval, supporting the use of C_{ss} at the relevant dose for the occurrence and time-to-event analyses. To adjust for potential confounding effects, covariates that were univariately significant were considered during multivariate analysis, and C_{ss} was evaluated on top of significant baseline covariates, thus reducing the possibility of confounding effects on the ER relationship.

Exposure–efficacy analyses revealed a robust relationship multivariately between higher blinatumomab C_{ss} and higher CR, both with and without SOC included. Higher Css was also associated with higher CR in a previous analysis using Study MT103–211 alone and this relationship persisted when Studies 20120216 and 00103311 were incorporated into the current 3-study analyses.[13]

The duration of OS multivariately increased with increasing exposure, supporting the clinical benefit of blinatumomab in prolonging the duration of OS. This is consistent with the findings from the phase 3 study, which reported an HR for OS of 0.71 (95% CI: 0.55–0.93; P = .01) for blinatumomab treatment *vs* SOC treatment.[12]

Among the safety endpoints, CRS events, a known clinical safety endpoint for blinatumomab, occurred only in patients receiving blinatumomab; patients receiving SOC chemotherapy did not experience CRS events. Frequency of NEs, another previously known clinical safety endpoint, was higher with blinatumomab treatment. No relationship between the probability of CRS or NEs with the tested regimen was observed, after accounting for treatment effect. Thus, the blinatumomab step-dosing regimen was appropriate in the management of CRS and NEs.

In conclusion, the ER analysis indicated that blinatumomab exposure resulted in a greater probability of achieving CR and provided a longer OS than SOC chemotherapy. CRS occurred only during blinatumomab treatment, and the frequency of NEs was higher with blinatumomab treatment. Nevertheless, the recommended blinatumomab step-dosing regimen was appropriate in the management of CRS and NEs. In conclusion, the analysis demonstrates how ER analyses of phase 2 or 3 study data and their application can support the justification of an appropriate dosing regimen of a novel BiTE antibody construct, blinatumomab, in the treatment of adult r/r ALL.

COMPETING INTERESTS

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