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Brief Communication

Pooled biomarker analysis of the association of baseline T regulatory cells with response and T-cell recovery profiles with blinatumomab treatment

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Abstract

Introduction: Blinatumomab, a bispecific T-cell engager, requires the activity of CD3+ T-cells for tumor lysis. This pooled analysis aimed to re-examine baseline T regulatory cells (Tregs) as a biomarker of blinatumomab efficacy across multiple clinical trials and provide insights into peripheral T-cell dynamics during blinatumomab therapy in hematological malignancies.

Methods: Tregs and peripheral T-cells were enumerated by fluorescence-activated cell sorting and were statistically evaluated using the Wilcoxon rank-sum test and linear mixed effect modeling, respectively.

Results: Comparable baseline percentages of Tregs were observed in responders and non-responders of blinatumomab treatment ($N = 325$) in adults with leukemia or lymphoma. Peripheral T-cell count recovery occurred early during blinatumomab dosing, and before blinatumomab-free interval in patients ($N = 233$) from 4 clinical trials.

Conclusion: The pooled analysis revealed that baseline Treg levels do not serve as a predictive marker for blinatumomab response and that there is rapid peripheral T-cell recovery following blinatumomab dosing. These results suggest that patients with varying levels of Tregs can benefit from blinatumomab treatment and that blinatumomab-free intervals of 7 days may suffice in blinatumomab treatment regimens.

Key words: regulatory T-cells; blinatumomab; lymphoma; leukemia; biomarker; CD3+ T-cells.

Implications for practice

With the increasing use of blinatumomab as front-line consolidation therapy of B-cell acute lymphoblastic leukemia, an updated understanding of factors influencing clinical efficacy is critical. In this pooled analysis of clinical trials in patients with blinatumomab-treated lymphoma or leukemia, we observe that a high percentage of Tregs before treatment does not affect the clinical efficacy of blinatumomab therapy and Treg levels at baseline may not be a biomarker to predict response. Additionally, we propose that a blinatumomab-free interval of 7 days is sufficient for ensuring adequate T-cell numbers to drive anti-tumor activity, reduce treatment time and potentially increase efficacy of blinatumomab-based regimens.

Introduction

Assessment of biomarkers in cancer is gaining importance in predicting the efficacy of anti-cancer treatments and in informing rational combinations.[1,2] Prediction of efficacy is important to avoid unnecessary treatment and associated costs. Blinatumomab, a BiTE® (bispecific T-cell engager) molecule, engages CD3+ cytotoxic T-cells with CD19-expressing B-cells achieving targeted B-cell lysis. Blinatumomab is approved for the treatment of minimal residual disease (MRD)-positive B-precursor acute lymphoblastic leukemia (B-ALL), relapsed or refractory B- ALL, and for the treatment of B-ALL in the consolidation phase. Several biomarkers have been reported to predict outcomes in patients treated with blinatumomab, including higher baseline percentages of T regulatory cells (Tregs) and the magnitude of T-cell expansion (Supplementary Table S1).[3,4] Tregs are immunosuppressive cells that inhibit the function of effector T-cells, including CD3 + T-cells.[5,6] As CD3 + T cells are the primary effector cells that kill malignant B cells when activated by blinatumomab, the role of Tregs merits investigation in the context of blinatumomab treatment.[7] Previously, Duell et al. reported that the presence of ≥8.525% of peripheral Tregs at baseline was associated with a lack of response to blinatumomab in B-cell precursor acute lymphoblastic leukemia (B-ALL).[8] Here, we aimed to examine the association of Tregs at baseline with response to blinatumomab in 7 clinical trials of hematological malignancies in phases 1-3.[9–15]. Additionally, we a nalyzed

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peripheral T-cell redistribution profiles following blinatumomab treatment to optimize the dosing regimen towards shorter intervals between treatment cycles, without compromising treatment efficacy.

Methods

Blinatumomab is currently administered via continuous intravenous infusion (cIV), typically in cycles consisting of 4 weeks of infusion followed by a 2-week treatment-free interval. This regimen allows for the recovery of T-cell counts and minimizes potential adverse effects. Tregs were quantified from peripheral blood samples of patients during blinatumomab treatment cycle 1 (C1) using fluorescence-activated cell sorting. Blood samples were collected at baseline and at certain intervals within C1 and cells were stained with fluorescence-labeled antibodies for identifying T-cells. Tregs were defined as $CD4^{high}/CD25^{high}/CD127^{dim}$ in patients from clinical studies NCT03476239 (relapsed/refractory [r/r] B-ALL), NCT04521231 (r/r B-ALL), NCT02910063 (r/r

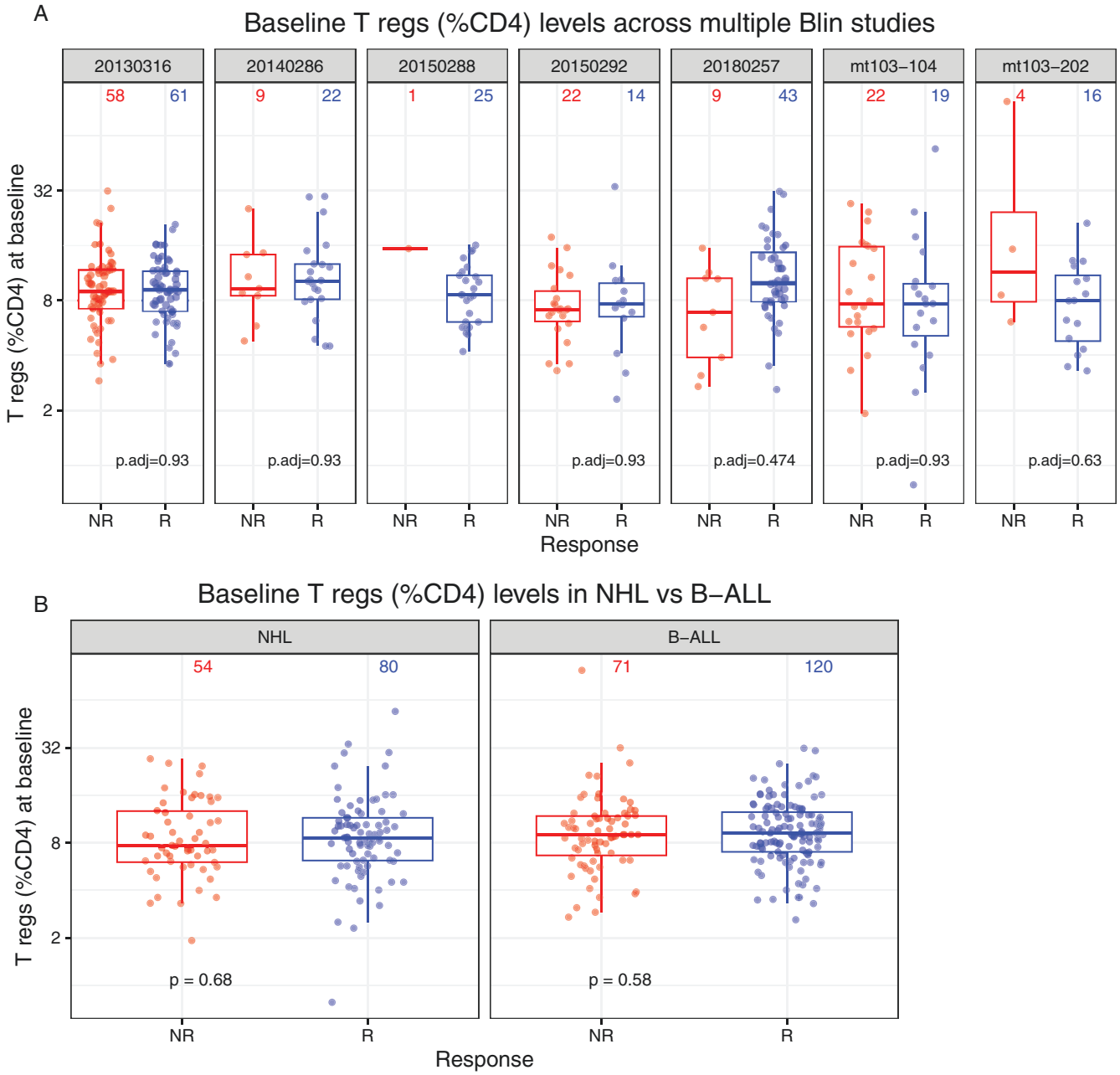

Figure 1. Association of baseline regulatory T-cells and response to blinatumomab in patients with hematological malignancies. Percentages of $CD4^{high}$/CD25high/$CD127^{dim}$ or $CD4^{high}/CD25^{high}/FOXP3^{+}$ regulatory T-cells in peripheral blood collected before blinatumomab therapy in patients with **(A)** r/r B-ALL, **(B)** r/r indolent NHL, **(C)** DLBCL, **(D)** r/r aggressive NHL, **(E)** r/r B-ALL, **(F)** relapsed NHL, and **(G)** MRD-positive B-ALL. Pooled analysis of responders and non-responders to blinatumomab treatment with respect to **(H)** Treg markers ($CD4^{high}/CD25^{high}/CD127^{dim}$ or $CD4^{high}/CD25^{high}/FOXP3^{+}$), and **(I)** hematological indication (NHL and B-ALL). Abbreviations: B-ALL, B-cell acute lymphoblastic leukemia; CR, complete remission; CRh, complete remission with partial count recovery; CRi, complete remission with incomplete count recovery without CRh, DLBCL, diffuse large B-cell lymphoma; MRD, measurable residual disease; NHL, non-Hodgkin's lymphoma; NR, non-responders; R, responders; PR, partial remission; r/r, relapsed/refractory; Treg, regulatory T-cell.

non-Hodgkin's lymphoma, NHL), NCT02961881 (r/r indolent NHL), and NCT03023878 (diffuse large B-cell lymphoma, DLBCL) or as $CD4^{high}/CD25^{high}/FOXP3^{+}$ in studies NCT00560794 (MRD-positive B-ALL) and NCT00274742 (relapsed NHL), and were expressed as a percentage of $CD4^{+}$ cells at baseline.[12] Labeled cells were analyzed on a BD FACSCanto™ cytometer (BD Biosciences). Absolute counts of peripheral Tregs and total T-cells were obtained by differential blood count. Two-sample Kolmogorov-Smirnov (KS) test provided a statistical evaluation of Treg levels across studies.[16] Wilcoxon rank-sum test was used to compare Tregs at baseline between responders and non-responders.[17] False determination rate-adjusted (FDR-adj) *P* values were reported.[18] The effect of blinatumomab on T-cell counts was modeled using a linear mixed-effects model (LMM) and implemented using the Imer function from the Ime4 (v1.1-34) library in R.[19] For comparisons, log2 transformed T-cell counts from different visits were modeled as a function of visit time (fixed effect) and subject (random effect) [log 2 of T-cell counts ~ Visit Times + (1 | Subject)]. Estimated means with ± 95% confidence interval values were calculated for longitudinal T-cell counts using emmeans (v1.8.9) and contrasts were determined between estimates for cycle 1 day 1(C1D1) at baseline and remaining visit times.

Results

First, we compared the distribution of Treg percentages across 7 phase 1-3 clinical trials of blinatumomab spanning hematological indications. The FDR-adj *P* values were below .05 for the distribution of Treg percentages only in study NCT02910063 (NHL) when considered against NCT02961881 (NHL), NCT03476239 (B-ALL), and NCT04521231 (B-ALL) (Supplementary Table S2). This difference could not be attributed to variation in hematological indication or staining methodology, as all 4 studies enumerated $CD4^{high}/CD25^{high}/CD127^{dim}$ Tregs. Further evaluation of baseline Treg percentages between responders ($n = 200$) and non-responders ($n = 125$) revealed no significant differences (adjusted *P* values,.47-.93) (Figure 1A-G and Supplementary Figure S1). Our combined analysis showed comparable baseline Treg percentages between responders and non-responders, irrespective of Treg staining methodologies (Figure 1H, $P[CD4^{high}/CD25^{high}/CD127^{dim}] = 0.19$ and $P[CD4^{high}/CD25^{high}/FOXP3^{+}] = 0.32$; *P*, raw *P* values) or the hematological indications studied (Figure 1I, P[NHL] = 0.68 and *P*[B-ALL] = 0.58; *P*, raw *P* values). Importantly, baseline Treg levels did not influence the degree of remission (partial or complete) across treatment indications or minimal residual disease response in blinatumomab-treated B-ALL (Figure 1A-G).

Further, total T-cell counts in peripheral blood were analyzed during C1 of blinatumomab treatment in patients ($N = 233$) with NHL ($n = 64$) and B-ALL ($n = 169$). In all studies, C1 consisted of a blinatumomab-treated period (range 26-56 days) followed by a blinatumomab-free period of 14 days, with blinatumomab administered as a continuous intravenous infusion (cIV), except for one phase 2 trial in r/r B-ALL (NCT04521231) in which adult patients were subcutaneously administered (SQ) blinatumomab followed by an 8-day treatment-free period (Figure 2D). Across all 4 studies, no significant difference was observed in total peripheral T-cell numbers at the end of C1 indicating that complete T-cell recovery had occurred by the end of the blinatumomab treatment cycle across blinatumomab dosages (range, 9-112 mg/day for cIV and in dosage regimen used for SQ)[20] and C1 duration (range 22-70 days) (Figure 2 and Supplementary Figure S2). Patients with B-ALL dosed with SQ blinatumomab demonstrated a significant increase from baseline in total T-cell counts by the end of C1 (adjusted $P < .001$), suggesting active T-cell proliferation after recovery, while on blinatumomab treatment (Figure 2D and Supplementary Table S3). In 2 clinical studies, peripheral T-cell count recovery was evident by day 8 of C1 in blinatumomab-treated patients with NHL (NCT02910063, $n = 36$) and B-ALL (NCT04521231, $n = 52$) (Figure 2A and D, and Supplementary Table S3).

Conclusions

Our study demonstrates that baseline Treg levels are not a consistent biomarker of blinatumomab response in hematological malignancies. Baseline Treg percentages, in this study, included the threshold of 8.525% previously proposed by Duell et al. to be a biomarker for lack of response

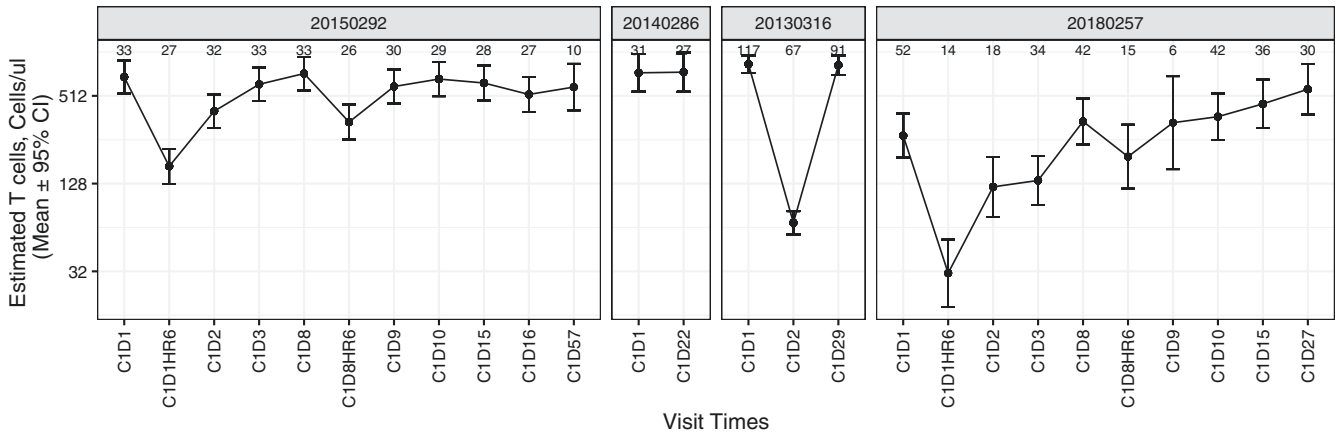

Figure 2. Recovery of peripheral CD3 + T-cells with blinatumomab treatment. Linear mixed-effects model-based estimates of T-cell counts during blinatumomab treatment cycle 1 in patients with (A) r/r aggressive NHL (B) r/r indolent NHL (C and D) r/r B-ALLB-ALL, B-cell acute lymphoblastic leukemia; CI, confidence interval; C1, cycle 1 of blinatumomab treatment; Dx, day × of cycle 1 of blinatumomab treatment; HR6, hour 6 of day × of cycle 1 of blinatumomab treatment; NHL, non-Hodgkin's lymphoma; r/r, relapsed/refractory.

to blinatumomab.[8] However, responders to blinatumomab treatment were observed both above and below this proposed value (median [inter quartile range] for non-responders vs responders, 8.6 [6.2-11.9] vs 9.05 [6.7-12.2]). Our findings include data from a large sample size (N = 325), from 7 clinical trials across hematological indications (B-ALL, NHL, and DLBCL), unlike the study by Duell et al. Furthermore, we observed that peripheral T-cell counts recovered to baseline early in C1 (within week 1 of treatment in most patients), across hematological indications and dosing regimens implying the presence of effector T-cell populations, which drive blinatumomab activity, in this period. Recovery of T-cell counts to baseline levels and the subsequent proliferation of peripheral CD3 + T-cells during blinatumomab treatment in many patients imply a beneficial state of readiness for sustained antitumor cytotoxicity.[5,6] T-cell count recovery that was revealed in this analysis is consistent with a previous report in r/r NHL, wherein activated T-cell numbers recovered to 50% of baseline after a mean (SD, range) of 3.13 (1.85, 0.82-9.10) days.[21] Interestingly, peripheral T-cells were observed to notably expand at the end of blinatumomab treatment cycle in r/r B-ALL patients receiving SQ blinatumomab. We propose that a shorter 7-day blinatumomab-free interval may be adequate to ensure the efficacy of blinatumomab-based treatment regimens.

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Author contributions

Yuliya Katlinskaya. and Bharat Panwar performed analyses and interpreted the data. Gerhard Zugmaier, Yi Zeng., Paul Gordon, Erik Rasmussen, Virginie Naegele, and Matthias Klinger developed the concept and designed the studies.

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Conflict of interest

Y.K., B.P., Y.Z., P.G., E.R., M.K., and V.N. are employed by Amgen and own Amgen stock. GZ is employed by Amgen and holds stock and reports issue of patents (20190300609, 20130323247, and 20110262440), and has patents pending (10696744, 10662243, 20190142846, 20190142846, 20170327581, 10130638, 9688760, 20170122947, 9486475, 20160208001, 9192665, 20150071928, 8840888, 20140228316, 20140227272, 20130287778, and 20130287774). MK holds patents WO2007068354 and WO2016184931. MK and VN report issue of patent WO2014122251.

Data availability

Qualified researchers may request data from Amgen clinical studies. Complete details are available at https://www.amgen.com/datasharing.

Supplementary material

Supplementary material is available at *The Oncologist* online.

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