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BRIEF COMMUNICATION

Relationship of T- and B-cell kinetics to clinical response in patients with relapsed/refractory non-Hodgkin lymphoma treated with blinatumomab

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Blinatumomab is a first-in-class immunotherapy based on the bispecific T-cell engager (BiTE®) immune-oncology platform, which redirects $CD3^+$ T cells to kill $CD19^+$ target cells. The objective of this analysis was to describe the correlation between B- and T-cell kinetics and response to blinatumomab in patients with relapsed or refractory (r/r) non-Hodgkin lymphoma (NHL). The clinical efficacy of treatment with blinatumomab in patients with r/r NHL was recently investigated in a phase 1 dose-escalation and expansion trial (NCT00274742) wherein 76 patients received blinatumomab by continuous intravenous infusion at various doses (0.5–90 $\mu g/m^2$/day). B-Cell depletion and expansion of $CD3^+$, $CD4^+$, and $CD8^+$ T cells was analyzed in patients stratified per clinical response (complete response [CR], n = 16; partial response [PR], stable disease [SD], or progressive disease [PD], n = 54) for at least 4 weeks (additional 4 weeks after clinical benefit) from the date of administration of blinatumomab until dose-limiting toxicity or PD. B-cell depletion kinetics were faster in patients who had a CR than in patients who did not have a complete response (PR, SD, or PD). T-cell expansion (T-cell counts exceeding the baseline level on day 22) was more pronounced in patients with CR than in patients without CR. T-cell expansion in patients with CR correlated with increased T-cell counts of both $CD4^+$ and $CD8^+$ T cells compared with patients without CR. Patients with r/r NHL who achieved a CR had faster B-cell depletion and increased expansion of $CD3^+$, $CD4^+$, and $CD8^+$ T cells than patients who did not achieve a CR.

Blinatumomab is a CD19/CD3 bispecific T-cell engager (BiTE®) molecule targeting CD19 on B cells and CD3ε on T cells, allowing targeted cell lysis. Clinical endpoints with blinatumomab have been reported in various clinical trials including adults and children with relapsed or refractory (r/r) B-cell precursor acute lymphoblastic leukemia (ALL) and B-cell precursor ALL with minimal residual disease (MRD) as well as r/r B-cell non-Hodgkin lymphoma (NHL) [1–6]. Changes in pharmacodynamic markers in adult patients with r/r or MRD^+ ALL and relationships between pharmacokinetics and pharmacodynamics with blinatumomab in adult patients with r/r NHL have also been reported in detail [7–9]. Such pharmacokinetic and pharmacodynamic data may help clinicians make decisions regarding dosing and evaluation of adverse events associated with

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blinatumomab, or may support predictions on the clinical outcome of patients.

Methods

Data for 76 patients with r/r NHL enrolled in a phase 1 open-label dose-escalation study of blinatumomab (NCT00274742) have been reported [1]. Patients received one of seven dosages of blinatumomab (0.5, 1.5, 5, 15, 30, 60, or 90 $\mu g/m^2$/day) as a continuous intravenous infusion for at least 4 weeks (or an additional 4 weeks after clinical benefit) unless there was dose-limiting toxicity or disease progression before that time. Dosing in different cohorts was administered as either flat, ramped, or stepped [7].

The study protocol was approved by an independent ethics committee at each institution. All patients provided consent to participate. Qualified researchers may request data from Amgen clinical studies. Complete details are available at: http://www.amgen.com/datasharing.

Results

Eleven patients had a complete response (CR) and an additional five patients had an unconfirmed complete response (CRu); 14 patients had a partial response (PR); 23 patients had stable disease (SD); 18 patients had progressive disease (PD); and five patients were not evaluated for clinical response [1]. Long-term follow-up on 38 patients at a single center has also been reported [10].

Detailed pharmacokinetic analysis revealed that the steady-state serum concentration of blinatumomab increased proportionally across all doses investigated in the study. A complete and sustained depletion of peripheral B cells was reported following dosing at doses ≥5 $\mu g/m^2$/day, which followed first-order kinetics and was dose dependent [7]. A transient decrease in T-cell counts within the first day of dosing was observed followed by return to baseline values within 2 weeks (typically 2–7 days) and, in some patients, T-cell expansion within weeks 2–4 [1].

The current study provides additional pharmacodynamic information observed in patients with r/r NHL in correlation to their clinical response (stratification based on clinical response categories CR and CRu in patients with complete response, n = 16; other response categories in patients without complete response, n = 55) by analyzing B-cell depletion, T-cell expansion, $CD3^+$ T cells, $CD4^+$ T cells, and $CD8^+$ T cells.

B-cell depletion kinetics are faster in patients who had a complete response (CR or CRu) than in patients without a complete response (PR, SD, or PD) (Figure 1). B-cell depletion occurs faster among patients with a complete response, reaching a first nadir in B-cell counts after 1.5 days of treatment compared with a slower decline in B-cell counts in patients without a complete response. However, after complete B-cell depletion is achieved within the first treatment week, depletion appears to be sustained in both groups throughout the remaining treatment (Figure 1).

With respect to $CD3^+$ T-cell kinetics, both patient groups had the typical T-cell redistribution kinetics with an initial drop in T-cell counts within the first days after start of treatment, then a nadir after 1.25 and 1.5 days, respectively, followed by return of T-cell counts to baseline levels within the first week (day 8) (Figure 2). In contrast, T-cell expansion (i.e., T-cell

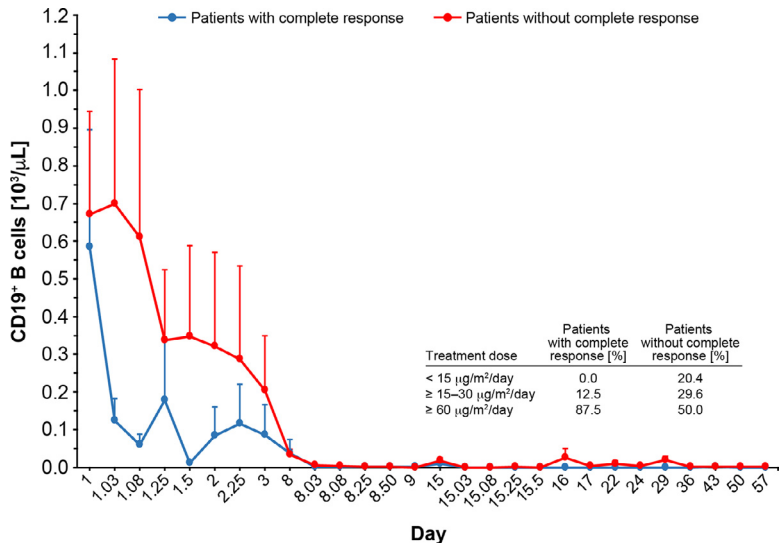

Figure 1. B-cell depletion kinetics by response group. Mean B-cell count ($10^3/\mu L$) ± SEM (y-axis) vs. study day (x-axis) for patients with a complete response (CR or CRu, n = 16) (*blue*) or without a complete response (PR, SD, or PD, n = 54; one patient was excluded because there was no B-cell depletion) (*red*). CR, complete response; CRu, unconfirmed complete response; PR, partial response; PD, progressive disease; SD, stable disease, SEM, standard error mean.

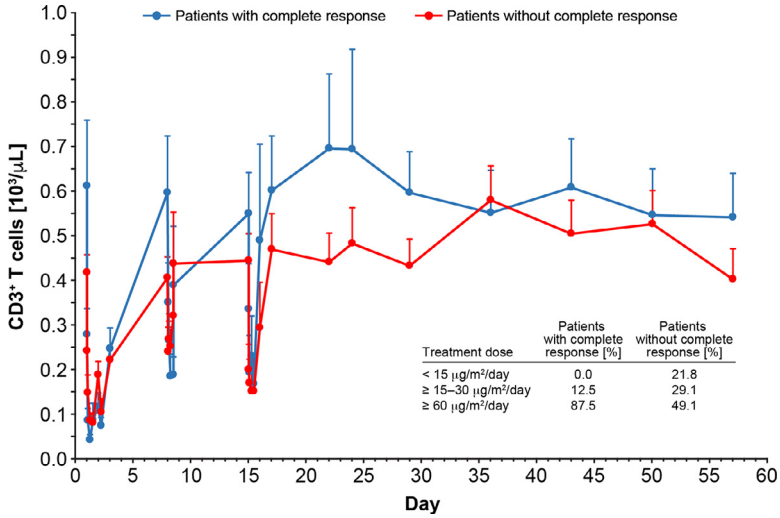

Figure 2. $CD3^+$ T-cell expansion kinetics by response group. Mean $CD3^+$ T-cell count ($10^3/\mu L$) ± SEM (*y*-axis) vs. study day (*x*-axis) for patients with a complete response (CR or CRu; $n = 16$) (*blue*) or without a complete response (PR, SD, or PD, $n = 55$) (*red*). CR, complete response; CRu, unconfirmed complete response; PR, partial response; PD, progressive disease; SD, stable disease, SEM, standard error mean.

counts exceeding the baseline level on day 22) was more pronounced in patients with a complete response than in patients without complete response (Figure 2). T-Cell expansion in patients with a complete response appears to reach maximum T-cell counts within the first 3–4 weeks of therapy, followed by a return of T cells to baseline levels by the end of study on day 57 (Figure 2).

Furthermore, T-cell expansion in patients with a complete response correlates with increased T-cell counts of both $CD4^+$ (Figure 3A) and $CD8^+$ T cells (Figure 3B) compared with patients without a complete response. As with $CD3^+$ T cells, after an initial drop in T-cell counts during redistribution in all patients after the start of blinatumomab treatment, T-cell expansion kinetics of $CD4^+$ and $CD8^+$ T cells was more pronounced in patients with complete response than in patients without complete response. T-cell expansion of $CD4^+$ and $CD8^+$ T cells also reached maximum counts within the first 3–4 weeks of treatment among patients with a complete response and return to baseline levels by the end of the study. We also investigated baseline levels of $CD3^+$ T cells and $CD4^+$ regulatory T cells ($Foxp3^+/CD25^+$) in patients with and without a complete response and found similar mean cell counts ± SEM for $CD3^+$ T cells (patients with CR: $0.612 \pm 0.147 \times 10^3/\mu L$; patients without CR: $0.418 \pm 0.040 \times 10^3/\mu L$) and $CD4^+$ regulatory T cells (patients with CR: $0.029 \pm 0.009 \times 10^3/\mu L$; patients without CR: $0.030 \pm 0.009 \times 10^3/\mu L$) in both groups. Together, the results of these exploratory analyses suggest that although all patients with r/r NHL who received blinatumomab had a rapid T-cell redistribution, those patients who achieved a complete response had faster B-cell depletion and increased expansion of $CD3^+$, $CD4^+$, and $CD8^+$ T cells than patients without a complete response.

Discussion

Decline of peripheral B cells during the first treatment week is a well-known early marker of blinatumomab activity and has been described extensively in blinatumomab studies of patients with r/r ALL, MRD^+ ALL, and r/r NHL [7–9]. However, a correlation between faster decline of B-cell counts and clinical response to blinatumomab treatment has been described only for patients with r/r ALL [11]. The present analysis is the first study suggesting a correlation of faster B-cell depletion kinetics within the first treatment days with clinical response in patients with r/r NHL.

A limitation of this exploratory study is the smaller number of patients who achieved a complete response ($n = 16$) compared with those who did not achieve a complete response ($n = 55$). Statistical analysis could not be performed because of the limited number of patients. Interpatient variability could also influence the interpretation of the results. However, the tables inserted in

Figures 1, 2, 3A, and 3B indicate that patients who received ≥60 $\mu g/m^2$/day blinatumomab had greater B-cell depletion, increased T-cell expansion, and better response compared with patients who received ≤30 $\mu g/m^2$/day blinatumomab. Nevertheless, the results of this study may lead to the assumption that increasing the B-cell depletion within the first days after treatment start, probably by increasing the starting dose of blinatumomab, could be beneficial for the clinical response of patients with r/r NHL. However, larger patient numbers would be required to analyze whether increasing the B-

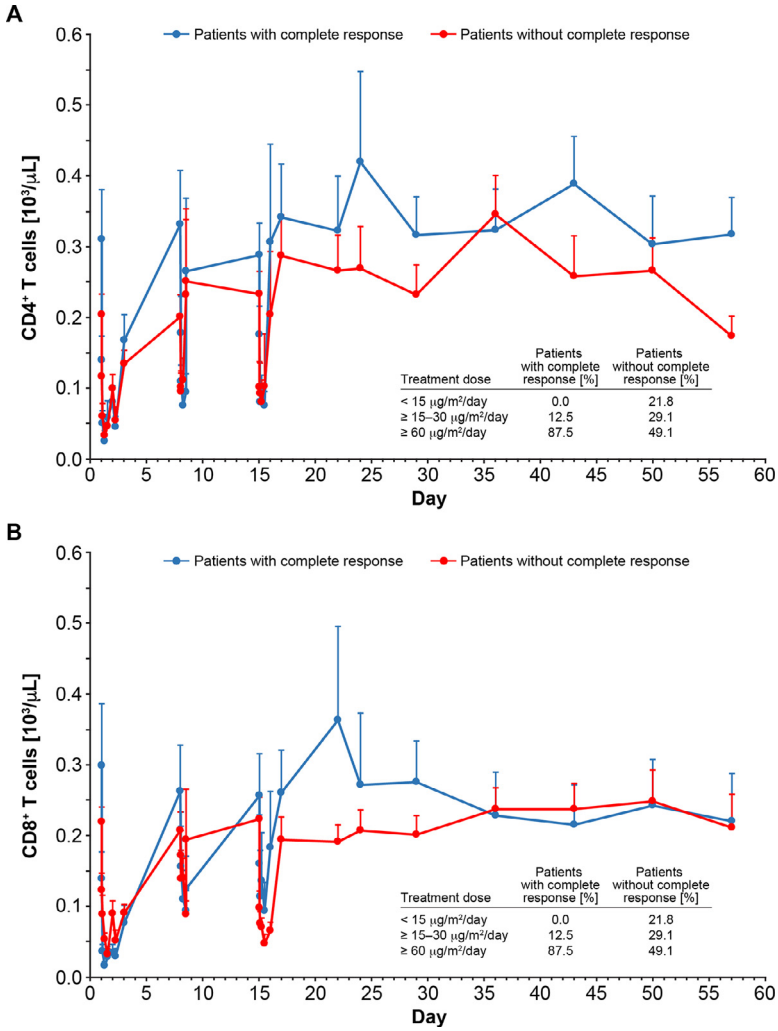

Figure 3. CD4$^+$ and CD8$^+$ T-cell expansion kinetics by response group. Mean CD4$^+$ **(A)** or CD8$^+$ **(B)** T-cell count ($10^3/\mu$L) ± SEM (*y*-axis) versus study day (*x*-axis) for patients with a complete response (CR or CRu, n = 16) (*blue*) or without a complete response (PR, SD, or PD, n = 55) (*red*). CR, complete response; CRu, unconfirmed complete response; PR, partial response; PD, progressive disease; SD, stable disease, SEM, standard error mean.

cell depletion in the first days after treatment start in patients with r/r NHL would significantly correlate with a better response.

T-cell redistribution of CD3$^+$ T cells could be observed in all analyzed patients with r/r NHL and is characterized by a rapid decline of peripheral T cells within the first hours or days after infusion start, followed by recovery to baseline levels by end of the first treatment week. This typical T-cell redistribution pattern has been described in all clinical trials with blinatumomab and seems to be independent of the formation of the cytolytic synapse. Disappearance of peripheral T cells within the first days of infusion is known to be mediated by increased T-cell adhesion to blood vessel endothelium involving an affinity shift in LFA-1 on the surface of T cells [12]. In addition, for patients with r/r NHL, no correlation of CD3$^+$ T-cell counts or CD4$^+$ regulatory T-cell counts at baseline with clinical response was determined here, whereas previously published data from Duell et al. [13] indicated regulatory T cells as a predictor of clinical outcome in patients with r/r ALL [13].

In general, blinatumomab-mediated pharmacodynamic effects such as T-cell expansion are most likely caused by activation of T cells after binding CD19$^+$ target B cells as consequence of the formation of the cytolytic synapse and the subsequently induced activation cascade. Such an activation cascade seems to be enhanced for patients with a complete response, especially within the 3–4 weeks after dose initiation, resulting in higher T-cell expansion compared with that of patients without a complete response.

Correlation of T-cell expansion with clinical response has been described in patients with r/r ALL [9]. Furthermore, T-cell expansion has been reported to prolong the long-term survival in patients with r/r ALL

but not in patients with MRD$^+$ ALL [11,14]. This illustrates the impact of the tumor load, indicating that a higher tumor load in patients with r/r ALL may provide a larger activation matrix leading to higher T-cell expansion compared with that in a disease setting with a lower tumor load as seen in patients with MRD$^+$ ALL. The accessibility of the tumor may also be relevant, indicating for the first time a correlation of T-cell expansion of CD3$^+$, CD4$^+$, and CD8$^+$ T cells with response in blinatumomab-treated patients with r/r NHL, an extramedullary disease with solid lesions. These results indicate that a BiTE®-induced pharmacodynamic change such as T-cell expansion may be relevant not only in hematological indications, but also in solid tumor indications, supporting its potential importance for the whole BiTE® platform.

The work described here is the first pharmacodynamic analysis describing T-cell expansion and B-cell depletion in correlation to response in patients with r/r NHL. Such pharmacodynamic markers may be used in the future to monitor and predict the clinical response of patients in the r/r NHL setting and may provide utility for BiTE® molecules targeting other indications.

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VN is employed by Amgen Research (Munich) GmbH, owns stock/equity in Amgen Inc., and holds patents. GZ is employed by Amgen Inc., owns stocks or options or other affiliations, and holds patents (10696744, 20170327581, 9688760, 9486475, 20140228316, and 20140227272). AV reports personal fees from Amgen, personal fees from Novartis, personal fees from Kite/Gilead, and personal fees from Roche outside the submitted work. RB owns stocks or options or other affiliations from Amgen, Cellex, Gemoab, and Catalym, outside the submitted work; in addition, RB has a patent for blinatumomab with royalties paid. PK is Vice President of BiTE® Technologies, has ownership interests (including patents) in Amgen, and received royalties related to blinatumomab. MK is employed by Amgen Research (Munich) GmbH, owns stock/equity in Amgen Inc., and holds patents in and receives royalties for blinatumomab. MEG has nothing to report.

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