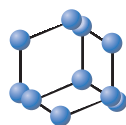


RESEARCH ARTICLE

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Pharmacokinetic and Pharmacodynamic Relationship of Blinatumomab in Patients with Non-Hodgkin Lymphoma



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Abstract: Background: Blinatumomab is a bispecific T-cell engager (BiTE[®]) antibody construct targeting CD3 ϵ on T cells and CD19 on B cells. We describe the relationship between pharmacokinetics (PK) of blinatumomab and pharmacodynamic (PD) changes in peripheral lymphocytes, serum cytokines, and tumor size in patients with non-Hodgkin lymphoma (NHL).

Methods: In a phase 1 study, 76 patients with relapsed/refractory NHL received blinatumomab by continuous intravenous infusion at various doses (0.5 to 90 $\mu\text{g}/\text{m}^2/\text{day}$). PD changes were analyzed with respect to dose, blinatumomab concentration at steady state (C_{ss}), and cumulative area under the concentration-versus-time curve (AUC_{cum}).

Results: B-cell depletion occurred within 48 hours at doses $\geq 5 \mu\text{g}/\text{m}^2/\text{day}$, followed first-order kinetics, and was blinatumomab exposure-dependent. Change in tumor size depended on systemic blinatumomab exposure and treatment duration and could be fitted to an E_{max} model, which predicted a 50% reduction in tumor size at AUC_{cum} of $\geq 1,340 \text{ h}\times\mu\text{g}/\text{L}$ and C_{ss} of $\geq 1,830 \text{ pg}/\text{mL}$, corresponding to a blinatumomab dose of 47 $\mu\text{g}/\text{m}^2/\text{day}$ for 28 days. The magnitude of transient cytokine elevation, observed within 1-2 days of infusion start, was dose-dependent, with less pronounced elevation at low starting doses.

Conclusion: B-lymphocyte depletion following blinatumomab infusion was exposure-dependent. Transient cytokine elevation increased with dose; it was less pronounced at low starting doses. Tumor response was a function of exposure, suggesting utility for the PK/PD relationship in dose selection for future studies, including NHL and other malignant settings.

Keywords: Blinatumomab, BiTE[®], non-Hodgkin lymphoma, exposure-response, pharmacokinetics, pharmacodynamics.

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1. INTRODUCTION

Non-Hodgkin lymphoma (NHL) is a cancer with worldwide prevalence and increasing incidence [1]. It is characterized by many histological subtypes that are mainly B-cell-derived, with malignant cells expressing common B-cell antigens, such as CD19, CD20, and CD22 [2, 3]. Although treatments with monoclonal antibodies have improved outcomes in NHL [4], many patients experience early relapse, refractory disease, or resistance to treatment [5, 6]. Blinatumomab is a bispecific T-cell engager (BiTE[®]) antibody construct that targets CD3 ϵ on T cells and CD19 on normal and malignant B cells, resulting in T-cell-mediated serial B-cell lysis and T-cell proliferation [7-9]. In a phase 1 proof-of-concept study, single-agent blinatumomab has shown antilymphoma activity in relapsed/refractory NHL [10, 11], and recent data from a phase 2 study have shown promising clinical activity of blinatumomab in diffused large B-cell

lymphoma (DLBCL) [12]. Blinatumomab has been approved for the treatment of relapsed/refractory acute lymphoblastic leukemia (ALL) [13-16].

Besides evaluations of appropriate doses, adverse events, and tolerability, the main objectives during early-phase development of cancer therapeutics include obtaining a comprehensive understanding of a molecule's mode of action, its pharmacokinetic (PK) and pharmacodynamic (PD) characteristics as well as their interrelationship(s), and how they may differ in various disease settings. Hence, PK/PD analyses provide important information for the development of phase 2 and phase 3 studies and represent a well-established tool that has gained increased attention by regulatory agencies [17-19]. An assessment of blinatumomab PK has been published [20]. Given the novel pharmacology and mode of action of blinatumomab, it is important to understand its PK/PD characteristics, and to assess how they may differ in NHL compared with other disease settings, such as ALL [9]. The objective of the current analysis was to evaluate PK/PD relationships in patients with relapsed/refractory NHL who

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received blinatumomab in the proof-of-concept phase 1 study. In particular, we sought to establish a quantitative relationship between blinatumomab concentrations and PD changes in peripheral lymphocytes, serum cytokines, and tumor size in lymph nodes using PK/PD modeling techniques. Furthermore, for any given novel therapeutic, selecting a dose that balances maximum clinical benefit against acceptable toxicity is critically important but continues to represent a significant challenge. Blinatumomab has a short serum half-life, mainly due to lack of an Fc γ portion compared with immunoglobulin G monoclonal antibodies [12]. Therefore, it is administered by continuous intravenous (cIV) infusion. The data from our analysis provide insight into the mode of action of blinatumomab and can ultimately contribute to selection of dosing regimens with a maximized benefit-risk ratio, thereby supporting the design of future studies in NHL, and possibly other malignant disease settings, including ALL.

2. MATERIALS AND METHODS

2.1. Study Design and Patients

The current analysis is based on the data collected in an open-label, multicenter, proof-of-concept phase 1 study in relapsed/refractory NHL (ClinicalTrials.gov, NCT00274742). The objectives of that proof-of-concept study were to investigate the adverse events associated with blinatumomab treatment in patients with relapsed/refractory NHL and to determine a dosing regimen for future studies in NHL. The phase 1 study explored various doses and administration schedules (flat and stepwise dosing; linear dose increases) in a dose escalation phase and in the follow-on extension phase (Supplemental Table 1). The study design, patient population, and treatment have been described in detail elsewhere [11]. Adult patients with histologically confirmed relapsed or refractory NHL were enrolled from February 2004 through August 2011. Blinatumomab doses of 0.5, 1.5, 5, 15, 30, 60, or 90 $\mu\text{g}/\text{m}^2/\text{day}$ were administered by cIV infusion for 4 weeks using a portable mini-pump. At the end of this 4-week treatment, an additional 4-week consolidation treatment could be administered to patients without disease progression or treatment-limiting adverse events. Approval of the study protocol was obtained from the independent ethics committees at the participating institutions. All patients provided written informed consent before any study-related procedures began.

The primary study endpoint was the overall frequency of adverse events. Secondary endpoints were blinatumomab concentrations in the serum, peripheral B- and T-cell counts, cytokine concentrations in the serum, formation of anti-blinatumomab antibodies, and overall response rate per Cheson criteria [21]. Final primary and key secondary endpoint data have been reported previously elsewhere [11].

Of the 76 patients treated during the study, 11 had one (n=9) or two (n=2) additional 4-week treatments due to clinical benefits. Time between initial and additional 4-week treatments varied (1.5 to 12 months). Data from additional treatment periods were included in the analyses where indicated. For the present analysis, only patients for whom samples were available were included.

2.2. PK and PD Sampling

Peripheral blood samples were collected at screening, prior to the start (baseline) of the treatment and every dose step, and at various time points during the blinatumomab infusion period. Intense samples were scheduled to be collected from 65 patients at 45 minutes and at 2, 6, 12, 24, and 48 hours after infusion initiation, and then once-weekly until the end of infusion. Relatively sparse samples were collected from 11 patients who received a stepwise dose of 5-15-60 $\mu\text{g}/\text{m}^2/\text{day}$, with sampling specifically undertaken at 48 hours after infusion initiation and when dose was escalated, and then once-weekly until the end of infusion.

2.3. Bioanalytical Methods

2.3.1. Blinatumomab Concentrations in Serum

A validated bioassay using CD69 activation was used to measure blinatumomab concentrations in serum. The assay is based on the principle that upon dual binding of blinatumomab to T cells and B cells, the CD69 activation marker is upregulated on T cells in a blinatumomab concentration-dependent manner. HPB-ALL cells (0.75 to 1.0×10^6 , human T-cell line; German Collection of Microorganisms and Cell Cultures [DSMZ], Braunschweig, Germany) were incubated with Raji cells (3 to 5×10^5 , human B-cell line; DSMZ) in RPMI medium (Invitrogen, Carlsbad, CA, USA) in the presence of blinatumomab serially diluted (200 ng/mL to 0.003 ng/mL) in human serum from healthy volunteers. After incubation, T cells were labeled with fluorescence-conjugated mouse anti-CD69 FITC monoclonal antibody (BD Biosciences, San Jose, CA, USA; No. 555530) and measured in a fluorescence-activated cell sorter (FACS) unit (FACSCalibur or FACSCanto II; BD Biosciences). Data were calculated using GraphPad Prism software (GraphPad Software, La Jolla, CA, USA). The lower limit of quantitation (LLOQ) was 100 pg/mL, and the limit of detection (LOD) was 3 pg/mL [9].

2.3.2. Immunogenicity

Anti-blinatumomab antibodies in patient serum were measured using a validated sandwich enzyme-linked immunosorbent assay (ELISA) or a validated assay based on electrochemiluminescence detection (Meso Scale Discovery, Rockville, MD, USA) using biotin- and ruthenium-conjugated blinatumomab. Both assays were cross-validated to ensure comparability.

For the ELISA, 100 μL /well blinatumomab antigen (0.25 $\mu\text{g}/\text{mL}$) was coated on a microtiter plate, incubated >12 hours at 2 to 8°C , washed in phosphate-buffered saline (PBS) with 0.1% Tween 20 (PBST; Sigma, St. Louis, MO, USA) and blocked (PBST/casein) for 1 to 4 hours at room temperature (RT). After another wash step (PBST), 100 μL of sample/control was added per well and incubated for 1 hour at RT. Polyclonal goat anti-blinatumomab antibodies (MedImmune, Gaithersburg, MD, USA) were used as a positive control. Biotinylated (Thermo Scientific; No. 21425) blinatumomab (0.34 mg/mL) was added ($1:1133$ dilution, 100 μL /well) and incubated for 1 hour at RT. Plates were washed (PBST), incubated with streptavidin-HRP ($1:5000$ dilution, 100 μL /well) for 1 hour at RT, and washed again

(PBST). TMB substrate was added (100 μ L/well) and the reaction stopped after 10 minutes at RT by addition of 2 N H_2SO_4 (100 μ L/well). Optical density (OD) at 450 nm was determined on a Power Wave x-select (BioTek Instruments Inc., Winooski, VT, USA) and the results calculated using KC4 software (BioTek Instruments Inc.). The presence of anti-blinatumomab antibodies was determined relative to the cutoff OD calculated from a panel of six individual blank human serum samples. This assay was designed and validated in accordance with the FDA guideline "Bioanalytical Method Validation" [22].

For the ECL assay, biotin- and ruthenium-conjugated blinatumomab were prepared using a kit from Thermo Scientific (No. 21425) and MSD SULFO-TAG (Meso Scale Discovery), respectively, following the manufacturers' instructions. Serum samples were diluted 1:10 in PBS and incubated with biotin- and ruthenium-conjugated blinatumomab (each at 0.5 μ g/mL) at RT for ≥ 1 hour. A streptavidin-coated 96-well microtiter plate (Meso Scale Discovery; cat. L15SA-1) was blocked with 5% bovine serum albumin (BSA; Paesel + Lorei GmbH & Co, Duisburg, Germany) in PBS at RT. Serum samples incubated with conjugated blinatumomab were then added to the plate and incubated for 0.5 to 2 hours. The plate was washed three times with PBST. Finally, 150 μ L of Reading Buffer (Meso Scale Discovery) was added to each well and the ECL signals were measured immediately using a Sector Imager 2400 analyzer (Meso Scale Discovery). ECL signals were normalized against a predose serum sample tested on the same microtiter plate in order to calculate the patient-specific floating cut point [23]. Each plate also contained a high QC and a low QC positive control (purified polyclonal goat anti-blinatumomab antibodies [Biogenes, Berlin, Germany]) as well as a negative control, to ensure plate validity. Potentially positive samples were retested in a confirmatory assay, in which a drug spike was introduced to block the signal, thereby ensuring drug specificity.

2.3.3. Cytokines

Concentrations of six cytokines in serum of patients (interleukin [IL]-2, IL-4, IL-6, IL-10, interferon [IFN]- γ , and tumor necrosis factor [TNF]- α), were measured using the FACS-based Human Th1/Th2 Cytometric Bead Array Kit (BD Biosciences; No. 551809) and system. IL-8 and IL-12 were measured by ELISA (Diaclone SAS, Besançon, France; No. 850.050.096). The assays were performed according to the manufacturers' protocols and internally validated. The LOD for cytokine determination was 20 pg/mL; the LLOQ was 125 pg/mL [9].

2.3.4. Peripheral Blood Lymphocytes

Lymphocytes were measured by FACS at various time points throughout treatment and quantified as described previously [9, 10, 13]. Peripheral blood mononuclear cells were isolated using Biocoll (Biochrom GmbH, Berlin, Germany) density gradient centrifugation followed by staining with fluorescence-conjugated monoclonal antibodies (anti-CD3 APC-H7, anti-CD56 PE-Cy7, anti-CD19 APC, anti-CD13 FITC, and anti-CD14 FITC) obtained from various commercial sources. Cell acquisition was performed on either FACSCalibur or FACSCanto II instruments (BD Biosciences),

with subsequent analysis using CellQuest Pro (BD Biosciences) or FACS Express (De Novo Software, Los Angeles, CA, USA) software. Absolute cell counts were obtained by combining FACS-based percentage data and absolute lymphocyte numbers from differential blood cell counts. Samples from 65 patients were analyzed by Amgen Research (Munich) GmbH. The remaining samples were analyzed by SYNLAB International GmbH (Augsburg, Germany) using the same methods but different suppliers of reagents, instruments, and software.

2.4. Determination of Sum of Longest Perpendicular Dimensions (SPD)

In the phase 1 study, tumor response, a secondary study endpoint, was determined per Cheson criteria [21], which are based on SPD. For each patient, SPD was determined across the six largest dominant lymph nodes and nodal masses using computerized tomography. SPD was measured at screening, at study days 29 and 57, and at the end of the study. For patients with incomplete treatment cycles or who withdrew early from the study, SPD was determined at unscheduled time points. Change from baseline (screening) in SPD was used as a PD endpoint.

2.5. PK/PD Data Analysis

2.5.1. Pharmacokinetics

Average individual steady-state concentration (C_{ss}) was calculated based on concentrations collected at ≥ 10 hours from infusion initiation. The systemic clearance (CL) of blinatumomab was calculated for each patient according to the equation $CL = R_o / C_{ss}$, where R_o was the dosing rate. The cumulative area under the concentration-versus-time curve (AUC_{cum}) was calculated as $dose_{cum} / CL$, where $dose_{cum}$ was the cumulative dose for an individual patient over a given 4-week treatment. Non-compartmental analysis was performed using Phoenix WinNonlin version 6.2.1 software (Certara, St. Louis, MO, USA). Dose proportionality of exposure was assessed by linear regression of mean C_{ss} versus dose. For patients with doses ≤ 5 μ g/m²/day, serum concentrations were generally below the LLOQ. Because these patients provided PD data, their C_{ss} were estimated from the relationship between exposure and dose.

2.5.2. Depletion of B-lymphocytes

The rate of B-cell depletion was determined at early time points (0 to 6 hours) after infusion initiation. The initial rate of depletion followed first-order kinetics; therefore, the depletion rate constant (λ) was estimated from linear regression of a semi-log plot of B-cell count versus time from baseline (hour 0) until 6 hours after infusion start, using three to four data points. The depletion rate constant was converted into percentage depletion per hour (B-cell-specific depletion rate, k_{dep}) using the equation $k_{dep} = (1 - e^{-\lambda}) \times 100$. The relationship between exposure to blinatumomab after first dose (C_{ss}) and B-cell-specific depletion rate was fitted to the simple E_{max} model $k_{dep} = (k_{dep,max} \times C) / (EC_{50} + C)$ using Phoenix WinNonlin version 6.2.1 (Certara USA, Inc.) [24], where $k_{dep,max}$ is the maximum specific depletion rate, C is the exposure (C_{ss}), and EC_{50} is the exposure that led to $1/2 k_{dep,max}$.

2.5.3. Change in Tumor Size

SPD at the end of each initial 4-week treatment was used to explore the relationship with corresponding AUC_{cum} over the treatment period or with C_{ss} at the highest dose tested in each patient. In patients who withdrew from study early, SPD measurement at the time closest to treatment end was used. The fold-change in SPD from screening to treatment end was used as the effect variable, and either AUC_{cum} or C_{ss} was used as the exposure variable. Data were fitted to a sigmoidal inhibitory E_{max} model, with baseline $E = E_0 - (E_{max} \times C^{\gamma}) / (EC_{50}^{\gamma} + C^{\gamma})$ using Phoenix WinNonlin version 6.2.1 (Certara USA, Inc.) [24], where E (effect) is defined as the change in SPD from screening as a function of exposure; E_0 is the baseline change in SPD without drug treatment; E_{max} is the maximum decrease in SPD relative to E_0 ; C is the exposure variable (AUC_{cum} or C_{ss}); EC_{50} is the exposure parameter that lead to $1/2 E_{max}$; and γ is the sigmoidicity factor. The model fit was optimized by refining the initial estimates and applying weighting factors. The quality of model fitting was assessed by random scatter of residuals, accuracy (percent coefficient of variation, CV%) of parameter estimates, and other available diagnostic tools in Phoenix WinNonlin version 6.2.1 (Certara).

2.5.4. Cytokines

The percentages of patients per dose level who had quantifiable levels (>LLOQ) of cytokines in the serum after the first blinatumomab dose along with the observed peak levels (C_{max}) of each cytokine during the first week of treatment at each first-dose level were assessed using descriptive statistics. The time courses of cytokine profiles in patients with and without dose steps were described graphically using GraphPad Prism software (GraphPad Software).

3. RESULTS

3.1. Patient Population

A total of 76 adult patients with relapsed or refractory NHL were enrolled and received treatment with blinatumomab in the phase 1 study. The median age was 65 years (range, 20–80); 75% of patients were male. Lymphoma histology included follicular lymphoma (n=28 patients), mantle cell lymphoma (n=24), diffuse large B-cell lymphoma (n=14), or other lymphomas (n=10). Complete patient demographics and baseline characteristics along with the primary analysis data are available elsewhere [11].

3.2. Pharmacokinetics

Following start of blinatumomab cIV infusion, steady-state levels of blinatumomab in serum were quickly reached (within 1 day), and exposure to blinatumomab was dose-proportional within the tested range. Mean (SD) values of blinatumomab C_{ss} at doses of 5, 15, 30, 60, and 90 $\mu\text{g}/\text{m}^2/\text{day}$ were 210 (85), 651 (307), 1210 (476), 2730 (985), and 3490 (904) pg/mL, respectively. The relationship between mean C_{ss} and dose was fitted to the linear regression equation $y = 42.5 \times x$, with a correlation coefficient of $R^2 = 0.98$ (Fig. 1). Mean C_{ss} at doses of 0.5 and 1.5 $\mu\text{g}/\text{m}^2/\text{day}$ were extrapolated for modeling purposes and were 21 and 63 pg/mL, respectively. PK was constant with time. The aver-

age CL (CV%) was 2.25 (52) L/h, and the average serum half-life (CV%) was 2.44 (66) hours.

3.3. Immunogenicity

From the 76 patients treated with blinatumomab, samples from 74 patients were available for immunogenicity analysis. Paired serum samples (including screening and end-of-study visit) were available from 62 (82%) of those patients. For 11 patients no end-of-study samples were available. For one patient, no screening sample was available, and the end-of-study sample was negative. None of the analyzed patients developed any detectable anti-blinatumomab antibodies during the duration of the study. Of the 11 patients who received additional treatment with blinatumomab, four patients had samples available for testing of anti-blinatumomab antibodies after the treatment. All four samples tested negative.

3.4. Pharmacodynamics

3.4.1. B-cell Depletion

Among all 76 patients, baseline B-cell levels before the initial 4-week treatment varied between zero and approximately 5,000 cells/ μL of peripheral blood. One patient who had 12,285 cells/ μL at baseline was excluded from the analysis because of an erratic profile. Despite variable baseline levels, once blinatumomab infusion started, B-cell counts declined quickly in the peripheral blood. Forty-nine patients had measurable B-cells before drug administration. Patients treated with blinatumomab at doses $\geq 5 \mu\text{g}/\text{m}^2/\text{day}$ showed complete B-cell depletion within 24 to 48 hours after treatment start (Fig. 2a); therefore, there was not a clear dose-response relationship. B-cell counts remained mostly undetectable during treatment and follow-up in all but five patients—one receiving 60 $\mu\text{g}/\text{m}^2/\text{day}$ and four receiving 15 $\mu\text{g}/\text{m}^2/\text{day}$ —who showed B-cell recovery during follow-up (≥ 4 weeks after end of treatment; Fig. 2a). Patients who received blinatumomab at doses of 0.5 or 1.5 $\mu\text{g}/\text{m}^2/\text{day}$ (n=9) did not show a complete depletion.

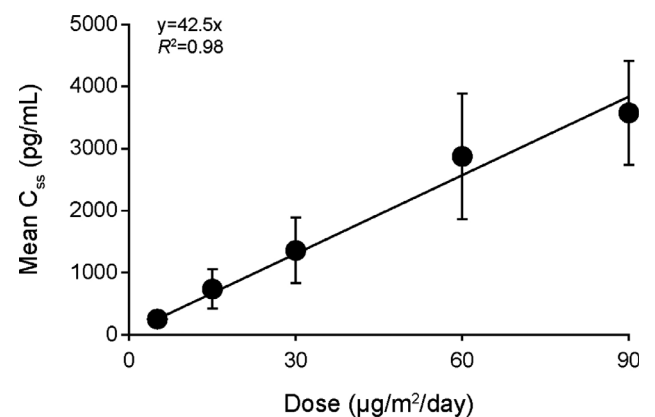


Fig. (1). Dose proportionality of blinatumomab steady state concentration (C_{ss}) across 67 patients with relapsed/refractory NHL who received blinatumomab at doses from 5 to 90 $\mu\text{g}/\text{m}^2/\text{day}$. Patients receiving dose steps and/or more than one treatment period provided more than one data point. Number of data points per dose was as follows: 5 $\mu\text{g}/\text{m}^2/\text{day}$, n=26; 15 $\mu\text{g}/\text{m}^2/\text{day}$, n=37; 30 $\mu\text{g}/\text{m}^2/\text{day}$, n=8; 60 $\mu\text{g}/\text{m}^2/\text{day}$, n=40; 90 $\mu\text{g}/\text{m}^2/\text{day}$, n=4.

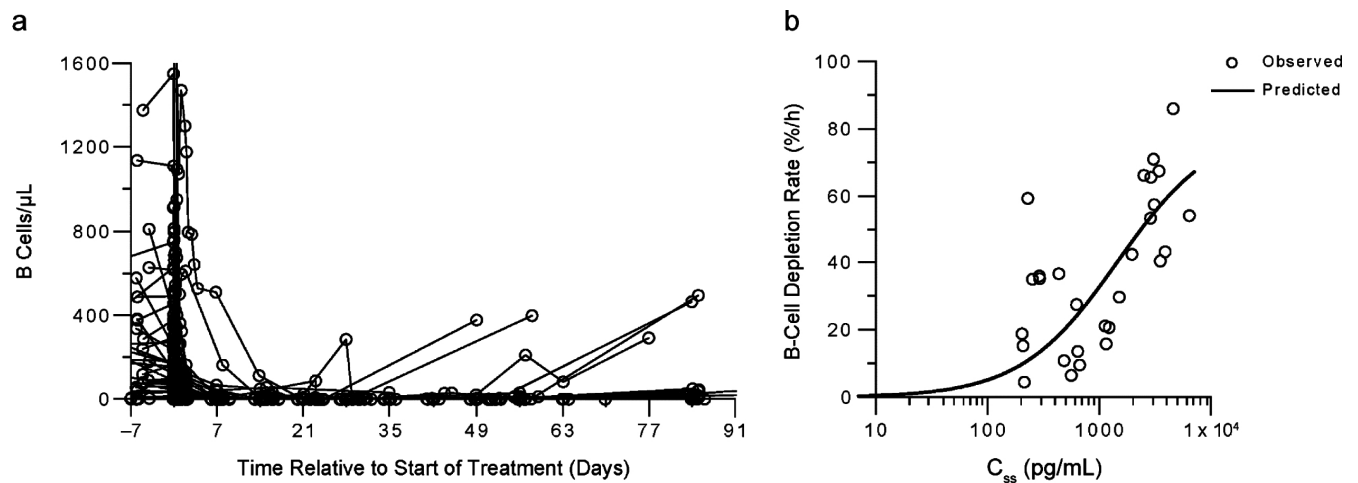


Fig. (2). Peripheral B-cell depletion in patients with relapsed/refractory NHL receiving blinatumomab at doses of $\geq 5 \mu\text{g}/\text{m}^2/\text{day}$. **a** Time profiles of Peripheral B-cell counts from 49 patients with detectable baseline B cells; the effect at different doses could not be distinguished. **b** Initial B-cell depletion rates as a function of C_{ss} after first dose ($n=28$) of first treatment period.

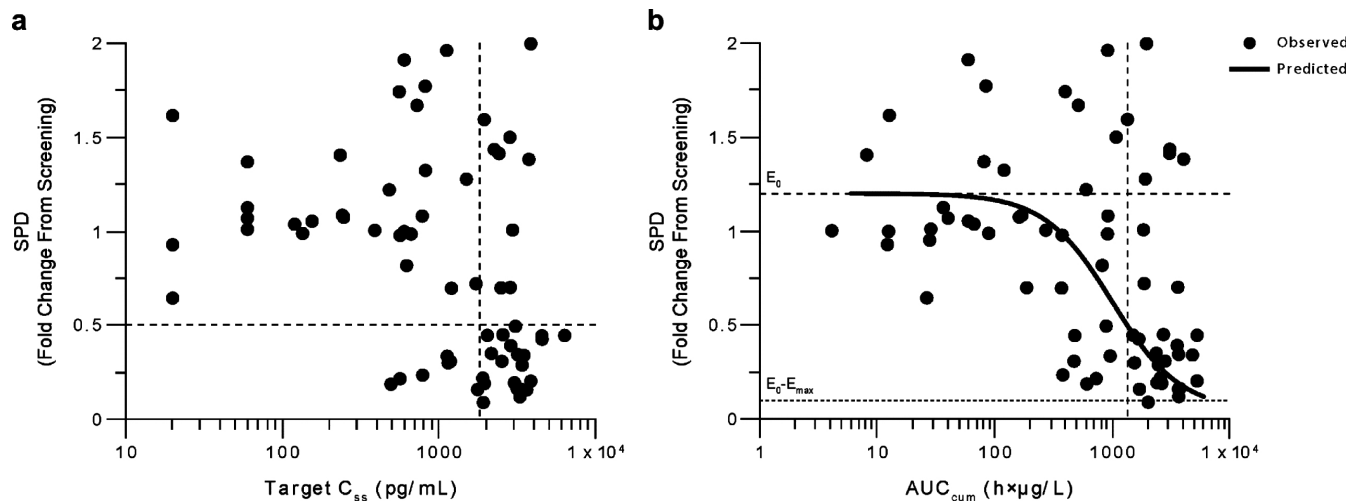


Fig. (3). Exposure–pharmacodynamic response relationship in patients with relapsed/refractory NHL. **a** Analysis using target C_{ss} and SPD ($n=66$). **b** Analysis using AUC_{cum} and SPD ($n=68$) with model fitting. The horizontal dotted line corresponds to a C_{ss} of 1,830 pg/mL leading to a $\geq 50\%$ decrease of SPD in 70% of patients. AUC_{cum} cumulative area under the concentration-versus-time curve, C_{ss} concentration at steady state, SPD sum of the products of the greatest diameters.

Of the 76 patients treated in the study, 28 (37%) had measurable B cells before and during treatment so that the B-cell depletion rate could be assessed. The other patients had either undetectable or very low (<10 cells/ μL) peripheral B-cell counts at baseline, or did not have sufficient data at time points between 0 and 6 hours. The estimated B-cell depletion rate constant from baseline (k_{dep}) ranged from 4.5% to 86% per hour. The relationship of C_{ss} after the first dose and k_{dep} was fitted to an E_{max} model (Fig. 2b). The estimated $k_{dep,max}$ was 81% per hour and the EC_{50} was 1,456 pg/mL. The quality of model fit was considered acceptable (CV% in parameter estimates $<30\%$). Residuals were randomly scattered around the x-axis.

3.4.2. Exposure–SPD Relationship

Of the 76 patients treated, data from five patients were excluded because of early blinatumomab discontinuation. For the remaining 71 patients, baseline SPD ranged from 2.3 to 261 cm^2 , with a mean (SD) of 45.5 (49.5) cm^2 . The

change in SPD from screening to end of treatment ranged from 0.1-fold (90% reduction) to 2.0-fold (100% increase) and was blinatumomab exposure–dependent (Fig. 3). Data from one patient who received stepwise blinatumomab dosing of 5–15–60 $\mu\text{g}/\text{m}^2/\text{day}$ ($AUC_{cum}=585 \text{ h}\times\mu\text{g}/\text{L}$, $C_{ss}=2,905$ pg/mL) were considered outliers and were excluded from the exposure–SPD analysis, because the SPD increased 3.2-fold from screening. Because patients were treated with different dose schedules (flat or stepwise dosing; Supplemental Table 1) and duration of treatment varied, two exposure metrics were used to describe the exposure–SPD relationship (ie, AUC_{cum} and C_{ss}). The C_{ss} –SPD relationship did not fit a PD model due to high data variability and confounded with the step-dosing regimen. However, a group of 26 patients had a C_{ss} of $\geq 1,830$ pg/mL, of whom 21 (70%) showed a reduction in tumor size by $\geq 50\%$ (Fig. 3a). Therefore, C_{ss} values of $\geq 1,830$ pg/mL were selected as target concentration range for antilymphoma activity. The AUC_{cum} –SPD relationship showed a greater correlation and was

Table 1. PD model parameters of AUC_{cum}–SPD relationship in patients with relapsed/refractory NHL.

Parameter estimate	All Patients (N = 68) ^a					
	E _o	E _{max}	E _o –E _{max}	EC ₅₀	γ	AUC _{cum} at 0.5-fold SPD Change
	1.20-fold (20% increase)	1.15	0.05-fold (95% decrease)	1,000 h×μg/L	1.50	1,340 h×μg/L
CV%	13%	32%	–	45%	60%	–

AUC_{cum} cumulative area under the concentration-versus-time curve, C_{ss} concentration at steady state, EC₅₀ median effective concentration, γ sigmoidicity factor, E_{max} maximum decrease in SPD relative to E_o, E_o baseline change in SPD from screening prior to blinatumomab treatment, NHL non-Hodgkin lymphoma, PD pharmacodynamics, SPD sum of the products of the largest diameters. CV% reflects accuracy in parameter estimates. ^aAll patients with evaluable samples.

fitted to a sigmoidal inhibitory E_{max} model (Fig. 3b). There were 68 patients with AUC_{cum} data for the model, and the model parameter estimates are shown in Table 1. The quality of fit was considered acceptable. Residuals or weighted residuals were randomly scattered around the x-axis. The AUC_{cum}–SPD model predicted that an AUC_{cum} of 1,340 h×μg/L would result in a 50% (0.5-fold) reduction of SPD from baseline. Without blinatumomab treatment, the model predicted a 20% (1.2-fold) increase of SPD from baseline. Therefore, a patient may achieve a 50% reduction of SPD at an AUC_{cum} of at least 1,340 h×μg/L and a C_{ss} of at least 1,830 pg/mL, which corresponds with a blinatumomab dose of at least 47 μg/m²/day administered for a duration (T) of 28 days using the PK dose proportionality equation C_{ss} (pg/mL)=42.5×dose (μg/m²/day) (Fig. 1) and the equation, time (day)=(AUC_{cum}×1000)/(C_{ss}×24).

3.4.3. Cytokine Elevation

Over a blinatumomab dose range of 0.5 to 90 μg/m²/day, the proportion of patients with quantifiable cytokine levels in serum after the start of blinatumomab infusion increased with dose (Fig. 4a). Cytokine elevation was transient and occurred predominantly after the start of blinatumomab infusion, peaked within 24 hours, and then declined within 2 days. At start of subsequent 4-week treatments (n=11 patients), cytokine elevation of a lower intensity was observed. Cytokines with quantifiable levels in serum included TNF-α, IL-2, IL-6, IL-10, and IFN-γ. The most prominent cytokines were IL-6, IL-10, and IFN-γ, which were elevated in ≥50% of patients who received doses of ≥30 μg/m²/day (n=51). TNF-α and IL-2 were quantifiable in 10% and 9% of patients, respectively; IL-4, IL-8, and IL-12 were not detectable in any of the patients analyzed.

Cytokine serum C_{max} generally increased with blinatumomab dose, particularly above 15 μg/m²/day (Fig. 4b). The blinatumomab target dose of 60 μg/m²/day was tested in both flat and stepwise dosing regimens. Stepwise dosing (5–15–60 μg/m²/day) showed less pronounced cytokine elevation after first dose and subsequent dose steps, compared with a flat dose of 60 μg/m²/day (Fig. 4c and 4d). There was a large interpatient variability in cytokine C_{max}; mean (SD) values after a first dose of 60 μg/m²/day were 2,460 (2,643), 4,409 (15,177), 446 (1,013), 171 (224), and 205 (370) pg/mL for IL-10, IL-6, IFN-γ, IL-2, and TNF-α, respectively.

4. DISCUSSION

In this phase 1 study, patients with relapsed/refractory NHL received blinatumomab for 4 or 8 weeks. As reported

previously [11], neurologic events were dose limiting, and 60 μg/m²/day was established as the maximum tolerated dose. Among patients treated who received the target dose of 60 μg/m²/day (n=35), the overall response rate was 69% across NHL subtypes [25–27]. In the present assessment, we explored the pharmacokinetic and pharmacodynamic relationships of blinatumomab in patients with NHL.

In this phase 1 study, the PK parameters of blinatumomab were stable over time while C_{ss} increased in a dose-proportional manner. PK data were consistent with those reported in patients with ALL [9]. Following blinatumomab cIV infusion, PD was characterized by peripheral B-cell depletion, cytokine elevation, and tumor reduction. The changes of these PD endpoints were dependent on blinatumomab exposure.

B-cell depletion was rapid, with B-cell levels dropping to zero within 48 hours in almost all patients receiving doses of ≥5 μg/m²/day, regardless of B-cell levels at baseline. The initial rate of depletion followed first-order kinetics, was exposure-dependent (C_{ss}), and could be fitted to an E_{max} model. This model can be used to predict how much time is required for a patient to have complete peripheral B-cell depletion at a given baseline count and a given dose. The depletion of B cells in patients with NHL enrolled in the present study was similar to that observed in studies of blinatumomab for the treatment of ALL [9] and is consistent with the *in vitro* pharmacology of the drug and its mode of action [8]. The variability among patients in B-cell count in the peripheral blood at baseline was high, with 17 of 67 patients treated at doses ≥5 μg/m²/day having had a zero B-cell count in blood before the first treatment period. This variability might be related to variations in disease state and previous anti-lymphoma therapies. The cIV infusion of blinatumomab is necessary for a comprehensive and sustained depletion of B cells throughout treatment and during the 4-week follow-up, because the B-cell depletion was not observed in earlier phase 1 studies in which blinatumomab was administered by repeated short-term IV infusions [28]. Peripheral CD19-positive B cells recovered slightly during follow-up in five patients; baseline lymphocyte counts and baseline tumor size were not different in these patients compared with other patients in the study. Sustained depletion of B cells, which would suppress immunoglobulin production, may also explain the absence of immunogenicity against blinatumomab in this study despite the murine origin of the molecule.

Despite complete B-cell depletion within 48 hours in peripheral blood at blinatumomab doses of ≥5 μg/m²/day, higher doses and longer treatment duration were required to

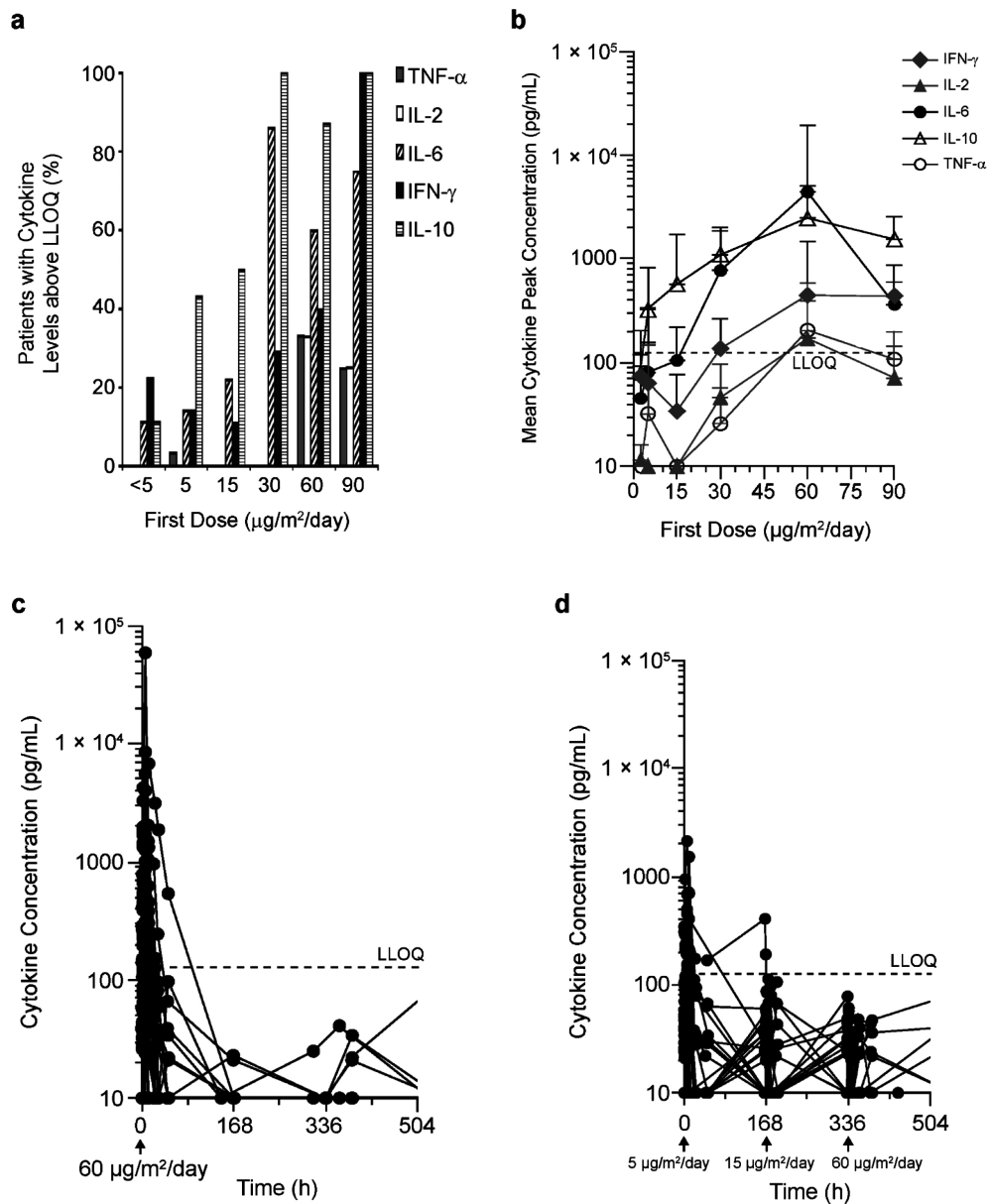


Fig. (4). **a**) Proportion of patients with serum cytokine levels above the lower limit of quantification (LLOQ) after blinatumomab infusion start. Number of patients per first dose level: <math><5\ \mu\text{g}/\text{m}^2/\text{day}</math>, $n=9$; $5\ \mu\text{g}/\text{m}^2/\text{day}</math>, $n=35$; $15\ \mu\text{g}/\text{m}^2/\text{day}</math>, $n=18$; $30\ \mu\text{g}/\text{m}^2/\text{day}</math>, $n=7$; $60\ \mu\text{g}/\text{m}^2/\text{day}</math>, $n=15$; $90\ \mu\text{g}/\text{m}^2/\text{day}</math>, $n=4$. **b**) Cytokine C_{max} (mean SD) in the first week of treatment as a function of blinatumomab dose. Number of patients per first dose level: <math><5\ \mu\text{g}/\text{m}^2/\text{day}</math>, $n=9$; $5\ \mu\text{g}/\text{m}^2/\text{day}</math>, $n=35$; $15\ \mu\text{g}/\text{m}^2/\text{day}</math>, $n=18$; $30\ \mu\text{g}/\text{m}^2/\text{day}</math>, $n=7$; $60\ \mu\text{g}/\text{m}^2/\text{day}</math>, $n=15$; $90\ \mu\text{g}/\text{m}^2/\text{day}</math>, $n=4$. Data below LLOQ were included as such into the analysis; data below LOD were set at $10\ \text{pg}/\text{mL}$ (ie, $1/2\text{LOD}$). **c**) Cytokine profiles after continuous IV blinatumomab doses of $60\ \mu\text{g}/\text{m}^2/\text{day}</math> throughout treatment ($n=9$); elevation of cytokines was transient and declined quickly during infusion. **d**) Cytokine time profiles after stepwise blinatumomab dosing of 5 – 15 – $60\ \mu\text{g}/\text{m}^2/\text{day}</math> ($n=21$); elevation of cytokines was transient and did not increase with increasing dose. C_{max} observed peak concentrations, *IFN* interferon, *IL* interleukin, *LLOQ* lower limit of quantitation, *TNF* tumor necrosis factor.$$$$$$$$$$$$

achieve better treatment response. Generally, antibody penetration of tissue is mainly driven by convection and porosity of the paracellular path [29, 30]. In NHL, the accessibility of blinatumomab to B cells within the tumor might be more limited than it is in peripheral blood and bone marrow (as in ALL, for example) because of the tight, dense structure of lymph nodes and extralymphatic lymphoma tissue. Likewise, the influx of T cells into lymphoma lesions may be limited and the expansion of intralesional T cells is expected to take

time before reaching a critical threshold translating into tumor shrinkage. These factors may contribute to the difference in dose and time required to achieve effects in tissues versus the blood.

Our observations suggest the involvement of two PD processes in B-cell depletion in NHL: one in the easily accessible blood compartment with immediate, intense B-cell killing associated with cytokine release; and a second, slower process in the deep compartments of lymphoid tissues

and extralymphatic lymphoma tissue. In ALL, blinatumomab doses between 15 and 30 $\mu\text{g}/\text{m}^2/\text{day}$ resulted in antileukemia activity [14, 15, 31]. The higher accessibility to the site of action in ALL (bone marrow), compared with NHL (lymph nodes), for both blinatumomab and T cells migrating from outside to inside the target tissue is a potential reason for the difference in dose requirements between the two B cell malignancies. In both cases, B-cell depletion in the peripheral blood has been identified as a prerequisite of clinical response and is an immediate PD marker. The observation that B-cell depletion rate in blood and extent of tumor reduction in tissues are both dependent on blinatumomab exposure, suggests an association between these two PD effects. Systems pharmacology models are currently being developed to explore a possible linkage of both compartments, using broader data sets from across various studies [32].

The exposure–SPD model indicated exposure-dependent reduction in tumor size, with a higher proportion of patients showing SPD reduction from baseline at higher doses. To achieve SPD reduction of $\geq 50\%$, the minimum reduction required to qualify as a clinical response according to Cheson criteria [21], the PK/PD model predicts that $C_{ss} \geq 1,830$ pg/mL and $AUC_{cum} \geq 1,340$ h \times $\mu\text{g}/\text{L}$ would be required, which is equivalent to a dose of ≥ 47 $\mu\text{g}/\text{m}^2/\text{day}$ for 28 days. In this phase 1 study, 60 $\mu\text{g}/\text{m}^2/\text{day}$ was identified as the maximum-tolerated dose [11]. Of the 26 patients who achieved the above thresholds for C_{ss} and AUC_{cum} , 20 had an SPD reduction from baseline of at least 50% and therefore, were considered partial or complete responders per Cheson criteria. Of note, the presented analysis only included response data after the initial 4-week treatment. However, some patients achieved their best response (*i.e.*, SPD reduction) only after 8 weeks of treatment or after the 4-week follow-up period. It might be speculated that despite efficient tumor cell eradication during the initial 4-week treatment, measurable tumor shrinkage might be delayed in NHL due to slower degradation of secondary tumor structures in the lymphoid microenvironment. Thus, tumor response rates might be underestimated. Based on the PK data from this analysis, PK/PD modeling can help in designing dosing regimens for future studies of blinatumomab in NHL. Furthermore, because relapsed/refractory NHL is more similar to bulky disease in solid tumors than to ALL, the PK/PD relationships reported here may also guide the evaluation of BiTE[®] antibody constructs in solid tumor settings.

The elevation of cytokines in serum was mainly a first-dose effect that occurred within the same time frame (48 hours) as peripheral B-cell depletion, and it was self-limiting under continued blinatumomab treatment. Despite high variability in cytokine levels, there was an obvious relationship between incidence and magnitude of cytokine elevation and initial blinatumomab dose; this was particularly apparent between doses of 15 and 30 $\mu\text{g}/\text{m}^2/\text{day}$. The differences between doses of 5 and 15 $\mu\text{g}/\text{m}^2/\text{day}$ were minor. Based on *in vitro* data [8, 17], it could be hypothesized that the transient cytokine elevation after the first dose augments the immune response and intensifies T-cell recruitment and tumor cell killing. However, considering the high variability of cytokine levels and the small sample size of the current analysis, this hypothesis could neither be confirmed nor rejected.

CONCLUSION

In conclusion, in this phase 1 study of blinatumomab for the treatment of relapsed/refractory NHL, PK parameters were consistent with the previously published data in ALL. PD data showed rapid, complete depletion of peripheral B-lymphocytes at blinatumomab doses ≥ 5 $\mu\text{g}/\text{m}^2/\text{day}$. Cytokine elevation was transient and dose-dependent. SPD decreased with increased exposure to blinatumomab, with the exposure–SPD model suggesting a minimum target dose of 47 $\mu\text{g}/\text{m}^2/\text{day}$ for 28 days to achieve activity in NHL. The observation that both B-cell depletion and tumor size reduction have been shown to be the functions of blinatumomab exposure indicates an association between these two PD effects.

LIST OF ABBREVIATIONS

ALL	=	Acute Lymphoblastic Leukemia
AUC	=	Area Under the Concentration-versus-time curve
AUC_{cum}	=	Cumulative AUC
BiTE [®]	=	Bispecific T-cell Engager
BSA	=	Bovine Serum Albumin
cIV	=	Continuous Intravenous Infusion
CL	=	Clearance
C_{ss}	=	Steady-state concentration
DLBCL	=	Diffuse Large B-cell Lymphoma
EC_{50}	=	Median effective concentration
E_o	=	Baseline change in SPD from screening prior to treatment
ECL	=	Electrochemiluminescence
ELISA	=	Enzyme-Linked Immunosorbent Assay
E_{max}	=	Maximum decrease in SPD relative to E_o
FACS	=	Fluorescence-Activated Cell Sorter
IFN	=	Interferon
IL	=	Interleukin
LLOQ	=	Lower Limit of Quantitation
LOD	=	Limit of Detection
NHL	=	Non-Hodgkin lymphoma
PBS	=	Phosphate-buffered saline
PBST	=	PBS Tween
PK	=	Pharmacokinetic
PD	=	Pharmacodynamic
RT	=	Room Temperature
SPD	=	Sum of the products of the largest diameters
TNF	=	Tumor necrosis factor

AUTHOR CONTRIBUTIONS

Designed research/study: Patrick Baeuerle. Performed research/study: Matthias Klinger, Andrea Kratzer, Peter Kufer. Collected or analyzed data: Youssef Hijazi, Matthias Klinger, Andrea Kratzer, Benjamin Wu, Andreas Wolf, Dirk Nagorsen, Min Zhu. Contributed to manuscript writing: Youssef Hijazi, Matthias Klinger, Benjamin Wu, Patrick Baeuerle, Peter Kufer, Andreas Wolf, Dirk Nagorsen, Min Zhu.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study will be conducted in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice (E6) and all applicable laws and regulations.

HUMAN AND ANIMAL RIGHTS

No Animals were used in the study. All research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation including the Declaration of Helsinki, June 1964, as modified by the 48th World Medical Association, Somerset West, Republic of South Africa, October 1996 (cf. Appendix 1).

CONSENT FOR PUBLICATION

All patients provided written informed consent before any study-related procedures began.

CONFLICT OF INTEREST

This work was supported by Amgen Inc. At the time this work was conducted, Youssef Hijazi and Patrick A. Baeuerle were employees of and shareholders in Amgen Research (Munich) GmbH, and Benjamin Wu and Min Zhu were employees of and shareholders in Amgen Inc. Matthias Klinger, Andrea Kratzer, Peter Kufer, and Andreas Wolf are employees of shareholders in Amgen Research (Munich) GmbH, and Dirk Nagorsen is an employee of and shareholder in Amgen Inc. Dirk Nagorsen is an inventor on blinatumomab patents. Matthias Klinger received royalties for patent US8007796B2. Peter Kufer receives royalties related to blinatumomab.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

REFERENCES

- [1] Ferlay J, Bray F, Pisani P, Parkin DM. Globocan 2000. Cancer Incidence, mortality and prevalence worldwide. IARC Press: Lyon, France 2001.
- [2] Armitage JO, Weisenburger DD. New approach to classifying non-Hodgkin's lymphomas: clinical features of the major histologic subtypes. Non-Hodgkin's lymphoma classification project. *J Clin Oncol* 1998; 16: 2780-95.
- [3] Harris NL, Nadler LM, Bhan AK. Immunohistologic characterization of two malignant lymphomas of germinal center type (centroblastic/centrocytic and centrocytic) with monoclonal antibodies. Follicular and diffuse lymphomas of small-cleaved-cell type are related but distinct entities. *Am J Pathol* 1984; 117: 262-72.
- [4] Cheson BD, Leonard JP. Monoclonal antibody therapy for B-cell non-Hodgkin's lymphoma. *N Engl J Med* 2008; 359: 613-26.
- [5] Fisher RI, LeBlanc M, Press OW, Maloney DG, Unger JM, Miller TP. New treatment options have changed the survival of patients with follicular lymphoma. *J Clin Oncol* 2005; 23: 8447-52.
- [6] Komrokji RS, Al Ali NH, Beg MS, *et al.* Outcome of diffuse large B-cell lymphoma in the United States has improved over time but racial disparities remain: review of SEER data. *Clin Lymphoma Myeloma Leuk* 2011; 11: 257-60.
- [7] Dreier T, Baeuerle PA, Fichtner I, *et al.* T cell costimulus-independent and very efficacious inhibition of tumor growth in mice bearing subcutaneous or leukemic human B cell lymphoma xenografts by a CD19-/CD3- bispecific single-chain antibody construct. *J Immunol* 2003; 170: 4397-402.
- [8] Hoffmann P, Hofmeister R, Brischwein K, *et al.* Serial killing of tumor cells by cytotoxic T cells redirected with a CD19-/CD3-bispecific single-chain antibody construct. *Int J Cancer* 2005; 115: 98-104.
- [9] Klinger M, Brandl C, Zugmaier G, *et al.* Immunopharmacologic response of patients with B-lineage acute lymphoblastic leukemia to continuous infusion of T cell-engaging CD19/CD3-bispecific BiTE antibody blinatumomab. *Blood* 2012; 119: 6226-33.
- [10] Bargou R, Leo E, Zugmaier G, *et al.* Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. *Science* 2008; 321: 974-7.
- [11] Goebeler ME, Knop S, Viardot A, *et al.* Bispecific T-cell engager (BiTE) antibody construct blinatumomab for the treatment of patients with relapsed/refractory non-hodgkin lymphoma: final results from a phase I study. *J Clin Oncol* 2016; 34: 1104-11.
- [12] Viardot A, Goebeler ME, Hess G, *et al.* Phase 2 study of the bispecific T-cell engager (BiTE) antibody blinatumomab in relapsed/refractory diffuse large B-cell lymphoma. *Blood* 2016; 127: 1410-6.
- [13] Topp MS, Kufer P, Gokbuget N, *et al.* Targeted therapy with the T-cell-engaging antibody blinatumomab of chemotherapy-refractory minimal residual disease in B-lineage acute lymphoblastic leukemia patients results in high response rate and prolonged leukemia-free survival. *J Clin Oncol* 2011; 29: 2493-8.
- [14] Topp MS, Gokbuget N, Stein AS, *et al.* Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study. *Lancet Oncol* 2015; 16: 57-66.
- [15] Topp MS, Gokbuget N, Zugmaier G, *et al.* Phase II trial of the anti-CD19 bispecific T cell-engager blinatumomab shows hematologic and molecular remissions in patients with relapsed or refractory B-precursor acute lymphoblastic leukemia. *J Clin Oncol* 2014; 32: 4134-40.
- [16] BLINCYTO[®]. Full prescribing information. Thousand Oaks, CA: Amgen Inc. 2017.
- [17] Breimer DD, Danhof M. Relevance of the application of pharmacokinetic-pharmacodynamic modelling concepts in drug development. The "wooden shoe" paradigm. *Clin Pharmacokinet* 1997; 32: 259-67.
- [18] Derendorf H, Meibohm B. Modeling of pharmacokinetic/pharmacodynamic (PK/PD) relationships: concepts and perspectives. *Pharm Res* 1999; 16: 176-85.
- [19] Peck CC. Quantitative clinical pharmacology is transforming drug regulation. *J Pharmacokinet Pharmacodyn* 2010; 37: 617-28.
- [20] Zhu M, Wu B, Brandl C, *et al.* Blinatumomab, a bispecific T-cell engager (BiTE[®]) for CD-19 targeted cancer immunotherapy: clinical pharmacology and its implications. *Clin Pharmacokinet* 2016; 55: 1271-88.
- [21] Cheson BD, Horning SJ, Coiffier B, *et al.* Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. *J Clin Oncol* 1999; 17: 1244.
- [22] Guidance for Industry: Bioanalytical Method Validation: United States Food and Drug Administration. <http://www.fda.gov/ucmf/>

- groups/fdagov-public/@fdagov-drugs-gen/documents/document/ucm070107.pdf (Accessed May 2, 2018).
- [23] Shankar G, Devanarayan V, Amaravadi L, *et al.* Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. *J Pharm Biomed Anal* 2008; 48: 1267-81.
- [24] Gabrielsson J, Weiner D. Pharmacokinetic and pharmacodynamic data analysis: concepts and applications, 5th ed. Swedish Pharmaceutical Press: Stockholm 2017.
- [25] Pishko A, Nasta SD. The role of novel immunotherapies in non-Hodgkin lymphoma. *Transl Cancer Res* 2017; 6: 93-103.
- [26] Sanders S, Stewart DA. Targeting non-Hodgkin lymphoma with blinatumomab. *Expert Opin Biol Ther* 2017; 17: 1013-7.
- [27] Wilke AC, Gokbuget N. Clinical applications and safety evaluation of the new CD19 specific T-cell engager antibody construct blinatumomab. *Expert Opin Drug Saf* 2017; 16: 1191-202.
- [28] Nagorsen D, Kufer P, Baeuerle PA, Bargou R. Blinatumomab: a historical perspective. *Pharmacol Ther* 2012; 136: 334-42.
- [29] Baxter LT, Zhu H, Mackensen DG, Jain RK. Physiologically based pharmacokinetic model for specific and nonspecific monoclonal antibodies and fragments in normal tissues and human tumor xenografts in nude mice. *Cancer Res* 1994; 54: 1517-28.
- [30] Lobo ED, Hansen RJ, Balthasar JP. Antibody pharmacokinetics and pharmacodynamics. *J Pharm Sci* 2004; 93: 2645-68.
- [31] Topp MS, Gökbuget N, Stein AS, *et al.* Confirmatory open-label, single-arm, multicenter phase 2 study of the BiTE antibody blinatumomab in patients (pts) with relapsed/refractory B-precursor acute lymphoblastic leukemia (r/r ALL). *J Clin Oncol* 2014; 32: 7005.
- [32] Singh I, Yuraszeck T, Klinger M, *et al.* A systems pharmacology model to characterize the effect of blinatumomab in patients with adult B-precursor acute lymphoblastic leukemia (B-ALL). *Clin Pharmacol Ther* 2014; 95: BP-007.