Translation and Clinical Development of Bispecific T-cell Engaging Antibodies for Cancer Treatment

T Yuraszeck[1], S Kasichayanula[1] and JE Benjamin[2]

Bispecific T-cell Engagers (BiTE®) antibody constructs enable a polyclonal T-cell response to cell-surface tumor-associated antigens, bypassing the narrow specificities of T-cell receptors and the need for antigen presentation through the major histocompatibility complex pathways. Blinatumomab, a CD19xCD3 BiTE® antibody construct, received accelerated approval for the treatment of relapsed/refractory Philadelphia chromosome negative acute lymphoblastic leukemia. Herein we review the pharmacology, safety, and efficacy observed in studies of blinatumomab and other BiTE® antibody constructs. Quantitative systems pharmacology is envisioned as a means to optimize dosing decisions for trials in which BiTE® antibody constructs are administered as monotherapy or in combination with other immunotherapies.

The biological mechanisms that permit cancer-cell escape from immune-mediated elimination has been given increasingly intense attention over the last 20 years (**Figure 1**). Recently, therapeutic modulation of some these mechanisms has yielded notable clinical successes, particularly in cancers that are less sensitive to standard chemotherapeutic approaches. As with standard chemotherapy, new immunotherapeutic agents are evaluated first as monotherapy and then in combination. Combination immunotherapy has been heralded as the next wave of cancer treatment strategies.[1,2] It is hoped that combination approaches will provide the possibility of cure in both early- and late-stage cancer.

The intentional deployment of the immune system to treat cancer was pioneered by William Coley in the late 19th century. Coley, a surgeon, observed regression of sarcoma in a patient who had developed erysipelas and then developed a "toxin" derived from the causative agent, *Streptococcus pyogenes* (**Figure 1**).[3,4] In the early 20th century, Paul Ehrlich suggested that the immune system distinguished between self and nonself. Ehrlich also proposed that the immune system could guard against the spontaneous development of cancer.[5] An immune surveillance theory was expanded by Burnet and Thomas.[6,7] Several decades later, data from animals and humans emerged linking cellular immune deficiency syndromes, either inborn or acquired, with increased rates of cancers.[8] More recently, Schreiber and colleagues have conceptualized the bidirectional interactions between neoplasia and the immune system as "immunoediting" and consists of three phases, elimination, equilibrium, and escape.[9]

A key early event in this process is the attraction of immune cells into the tumor microenvironment. Presence of tumor-infiltrating-lymphocytes (TIL) has frequently been linked to prognosis, but given the functional heterogeneity of lymphocytes, the correlation is rarely straightforward.[10] However, it is important to highlight three seminal observations. First, infiltrating T cells may have specificity for tumor antigens.[11] Second, specificity may be limited to private (i.e., nonshared) antigens resulting from novel mutations.[12] Third, the functional competence of infiltrating lymphocytes may be compromised.[11,13] The latter two observations have strongly influenced recent advances in immunotherapy.

MECHANISMS AND OUTCOMES FOR DRUGS THAT HARNESS T CELLS IN THE IMMUNE SYSTEM TO FIGHT CANCER

Cancer is characterized by the accumulation of a variable number of mutations that lead to the loss of normal cellular regulatory processes. Some mutations result in the expression of neoantigens that can be seen by the immune system as nonself, with the resulting generation of a T-cell response. For CD8-positive (usually cytotoxic) T cells, this response typically involves the recognition of distinct peptides bound to major histocompatibility class I

[1]Clinical Pharmacology, Modeling, and Simulation, Amgen Inc., Thousand Oaks, California, USA; [2]Global Development, Oncology, Amgen Inc., Thousand Oaks, California, USA. Correspondence: JE Benjamin (jbenjami@amgen.com)

Received 27 December 2016; accepted 4 February 2017; advance online publication 9 February 2017. doi:10.1002/cpt.651

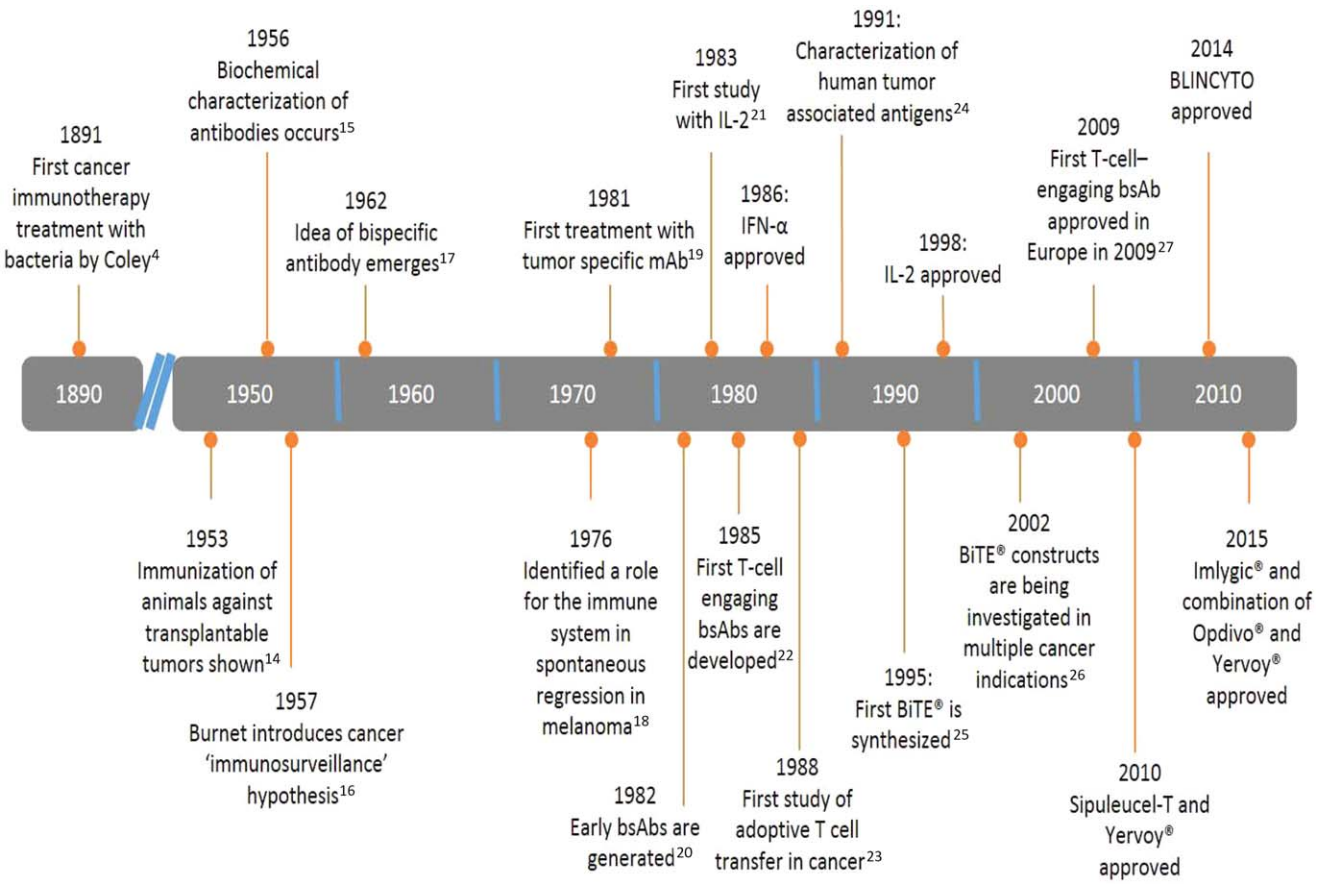

Figure 1 From Coley Toxins to the Approval of Bispecific T-Cell Engaging Antibody Constructs for Cancer Immunotherapy.

(MHC I) molecules on the cell surface. In order for the immune response to lead to effective killing of cancer cells, a series of stepwise events must be initiated and allowed to proceed and expand iteratively. This process is called the cancer-immunity cycle and was elegantly described by Chen and Mellman.[28] Another feature of cancer cells is maturational arrest, resulting in a conserved phenotype marked by expression of normal (i.e., nonmutated, or self) proteins. Typically, these type of lineage or stage-specific antigens will not be recognized by the immune system.

Immunotherapies may be classified according to their dependence on antigen specificities within the preexisting T-cell receptor repertoire or whether they introduce new specificities. The former include the anti-CTLA4 (cytotoxic T-lymphocyte-associated protein 4) and anti-PD1 (programmed cell death protein 1) checkpoint inhibitors (ipilimumab, nivolumab, and pembrolizumab), each of which interferes with physiologic attenuation of T-cell activation. Tumor vaccines attempt to amplify existing specificities by mimicking an infectious process. In contrast, other immunotherapies introduce new specificities. As currently practiced, the new specificities target lineage-specific antigens expressed with near uniformity by the cancer of interest. These new specificities may be introduced by stable genetic modification, such as occurs with chimeric antigen receptor (CAR)-T cells. Alternatively, new specificities may be conferred transiently, such as occurs with bispecific T-cell engaging (BiTE®) antibody constructs, a particular form of bispecific antibody, many of which are in development for cancer treatment (**Table 1**).

MECHANISM OF ACTION AND KEY CHARACTERISTICS OF BITE® ANTIBODY CONSTRUCTS

BiTE® antibody constructs are relatively small fusion proteins with a molecular weight of 50–60 kDa that flexibly link two single chain antibody variable fragments (scFv), simultaneously binding the invariant CD3ϵ component of T-cell receptors and any highly expressed structure on the surface of target cells, such as the CD19 receptor that is expressed on all B-cell lineage-derived leukemias and most lymphomas, including non-Hodgkin lymphoma (NHL) (**Figure 2**). Forcing T cells and target cells into proximity results in T-cell activation, proliferation, and T-cell-induced target cell lysis. Because these effects are accomplished without the need to engage typical immune system activation mechanisms that depend on the presentation of specific peptide antigens by MHC I molecules expressed on target cells and recognition of those antigens by their corresponding T-cell receptors, BiTE® antibody constructs engage any and all T cells.[29]

Common methods employed by cancer cells to evade detection and elimination by cytotoxic T cells include loss of MHC I molecules, impairment of cytotoxic activity of specific T-cell clones, resistance to cytotoxicity, or creation of an immune suppressive

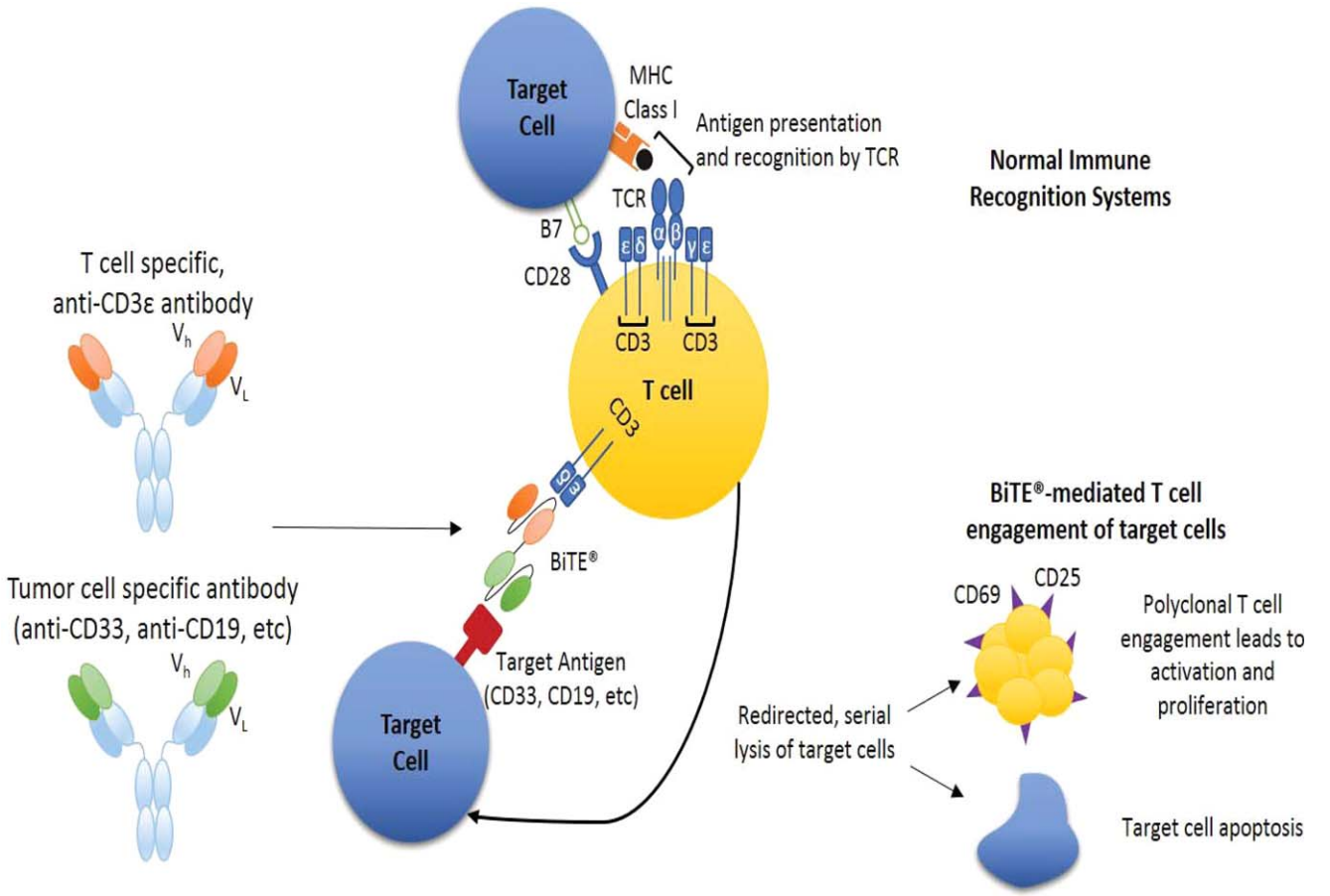

Figure 2 BiTE® antibody constructs promote T-cell mediated target killing without the need for standard T-cell recognition mechanisms.

microenvironment. As already stated, BiTE® antibody constructs bypass MHC I, but could be susceptible to other immune-evasive strategies. Therefore, BiTE® activity was tested against cell lines engineered to overexpress proteins known to contribute to tumor cell evasion: PD-L1, indoleamine-2,3-deoxygenase type 1, Bcl-2, serpin PI-9, TGF-β, IL-10, or adenosine. Subtle decreases in redirected target cell lysis were observed, but increasing BiTE® antibody construct concentrations mitigated those effects, and even when tested in combination complete resistance to BiTE® therapy was not observed.[30] These properties make BiTE® antibody constructs appealing modalities for cancer treatment, and indeed, blinatumomab gained accelerated approved by the US Food and Drug Administration (FDA) in 2014 for the treatment of relapsed and/or refractory (R/R) Philadelphia chromosome negative acute lymphoblastic leukemia (ALL).[31]

Preclinical pharmacokinetics

In preclinical species, these relatively small (blinatumomab is 55 kDa) fusion proteins have a terminal phase half-life of only a few hours.[32,33] Full-length monoclonal antibodies typically have a 21-day half-life, owing to neonatal Fc receptor (FcR_n)-mediated recycling. Fc- and albumin-fusion proteins bind FcR_n, are internalized, and then recycled back to the cell membrane, whereas similarly internalized non-FcR_n bound proteins are degraded. As BiTE® antibody constructs lack the Fc portion responsible for FcR_n binding, they are not expected to undergo FcR_n recycling and this likely contributes to the very short half-lives associated with these molecules.

Preclinical pharmacology

The preclinical properties of blinatumomab, the first and only approved BiTE® antibody construct, have been well characterized and are representative of the general characteristics of BiTE® antibody constructs. Blinatumomab targets CD3ϵ on T cells and the cell surface receptor CD19, which is expressed on B cells at all stages of development, both normal and cancerous, and is a reliable B-cell biomarker.[34,35] The affinity of blinatumomab for CD3 and CD19 is low; K_d values for each arm are in the range of 10^{-7} M and 10^{-9} M, respectively.[36,37] Blinatumomab receptor occupancy on T cells is also relatively low, mimicking the low-affinity interactions between naturally occurring T-cell receptors and peptides presented by MHC I molecules and providing optimal conditions for T-cell cytotoxicity. Indeed, blinatumomab is a highly potent molecule with cytotoxic effects observed at exposures as low as 10 pg/ml (1.8×10^{-13}M).[38] These effects were achieved with previously unstimulated peripheral blood lymphocytes (PBLs) and without the need for costimulation. Video-assisted microscopy demonstrated that T cells could

engage in serial target cell lysis in the presence of blinatumomab, rapidly binding and killing multiple target cells.[39] Cytotoxicity was specific to $CD19^+$ cells, mediated by both $CD4^+$ T cells and $CD8^+$ T cells, and observed at effector to target cell ratios as low as 2:1.[38] This was markedly different than the experience with previous CD3xCD19 bispecific molecules, where costimulation, high effector to target cell ratios, and exposures nearly 10,000 times higher were required.[40] Blinatumomab-enabled engagement between T cells and target cells also results in T-cell activation, proliferation, and the transient increase in the levels of the cytokines IL-6, IFN-γ, TNF-α, IL-2, IL-4, and IL-10. Each of these phenomenon are strictly target-dependent and only occur in the presence of blinatumomab.[37]

Blinatumomab also demonstrated activity in xenograft mouse models, inhibiting tumor growth and prolonging survival in a dose-dependent fashion. Because blinatumomab is not crossreactive with murine CD3, human T cells were administered as effector cells for redirected target cell lysis, but costimulatory agents were not required.[32] Several other BiTE® antibody constructs are in various stages of clinical development, including AMG 211/MEDI-565, AMG 212, AMG 420, and AMG 330. AMG 110 was previously developed for epithelial tumors. They share blinatumomab's characteristics, i.e., high potency, T-cell activation and proliferation as a result of target engagement, target cell lysis, and BiTE®-induced cytokine release.[41] Of these, AMG 330 and AMG 420 target myeloid and plasma cell antigens. AMG 211/MEDI-565 is a BiTE® molecule with dual specificity for CD3 and carcinoembryonic antigen (CEA), and is being tested in patients with gastrointestinal adenocarcinoma. For solid tumor indications such as these, eradication of cancer cells requires T-cell infiltration and BiTE® antibody construct distribution into the tumors, with potentially more difficult pharmacodynamic assessments. AMG 110/MT 110, among the first BiTE® molecules developed, targets EpCAM (epithelial cell adhesion molecule), a protein widely expressed on adenocarcinoma.[42] Mice were inoculated subcutaneously with various tumor cell lines and then injected intravenously with ^{89}Zr-AMG 110 and imaged with positron emission tomography (PET). These studies showed significant tumor uptake that was sustained for several days despite the short plasma half-life, and correlated with EpCAM expression levels on the cell lines, although EpCAM receptors are highly expressed and were not saturable even at doses up to 500 μg.[43]

Dexamethasone

Glucocorticoids are used in clinical oncology to treat lymphoid malignancies and to manage the side effects associated with chemotherapy and radiation in other cancer types.[44] The impact of glucocorticoids on side effects such as nausea, vomiting, and bone pain is mainly a result of its antiinflammatory properties, which were first recognized in the 1940s.[45] The mechanism of action by which glucocorticoids alleviate side effects associated with inflammation is through their immunosuppressive properties, including a decrease in cytokine levels.

Since cytokine release associated with engagement of T cells and target cells by BiTE® antibody constructs presents a safety concern, premedication with glucocorticoids such as dexamethasone was considered for clinical application of BiTE® antibody constructs. *In vitro* experiments were conducted to determine the impact of dexamethasone pretreatment on cytokine secretion, cytotoxicity, and proliferation. In fact, dexamethasone at clinically relevant concentrations could blunt the increase in cytokine levels with minimal impact on proliferation or killing of ALL cells.[46] This provided a rationale for coadministering blinatumomab with dexamethasone in clinical trials, a practice that has continued with other BiTE® antibody constructs.[47,48] However, it is not clear if the differential sensitivity of effector functions to dexamethasone can be generalized across the BiTE® platform.

First-in-human (FIH) dose selection

In March 2006, the superagonistic anti-CD28 antibody TGN1412 caused massive cytokine storm and multiorgan failure in six healthy human volunteers in an FIH study, events that were not predicted by preclinical safety testing and which occurred despite the application of an additional 16-fold safety factor to the maximum recommended starting dose based on the No Adverse Effect Level (NOAEL). The cause of this translational disconnect was likely species differences in the expression of CD28 on $CD4^+$ T cells[49]; the lack of CD28 expression in the toxicology species invalidated the toxicology results that were the basis for the starting dose selection. Following this incident, the European Medicines Agency (EMA) issued a guideline that emphasized the consideration of all available pharmacological data, including *in vivo*, *ex vivo*, and *in vitro* data to determine the Minimal Anticipated Biological Effect Level (MABEL), which should be the basis for the starting dose for so-called high-risk medicinal products.[50]

Because BiTE® antibody constructs stimulate cytokine release, are active at low concentrations *in vivo* and *in vitro*, and have steep dose–response curves, MABEL approaches have been used for starting dose selection for all BiTE® antibody constructs, including blinatumomab, AMG 211/MEDI-565, and AMG 330. In the case of AMG 211, there was no pharmacologically relevant animal species in which to conduct toxicology studies, as rodents do not express CEA and AMG 211 does not bind CD3 in cynomolgus monkeys. Tumor lysis was the most sensitive marker of AMG 211 activity; the EC_{20} value associated with this experiment was used to select a starting dose of 52 μg/d.

For AMG 330, the *de novo* expression of T-cell activation marker CD69 on T cells was defined to be the most sensitive marker of activity; PKPD modeling calculated a starting dose designed to provide exposures equivalent to the EC_{50} value from this experiment, the MABEL concentration, assuming that the *in vitro* pharmacodynamic data, generated in acute myeloid leukemia (AML) cell lines, was directly translatable to humans. This resulted in a starting dose of 0.5 μg/d, significantly lower than the maximum recommended starting dose of 30 μg/d. The EC_{50} value was used as the basis for the MABEL because of a combined consideration of the *in vitro, in vivo,* and *ex vivo* data, including the toxicology results. Recognizing that safety is the primary concern for starting dose selection, it is also desirable to minimize the number of patients subjected to less than

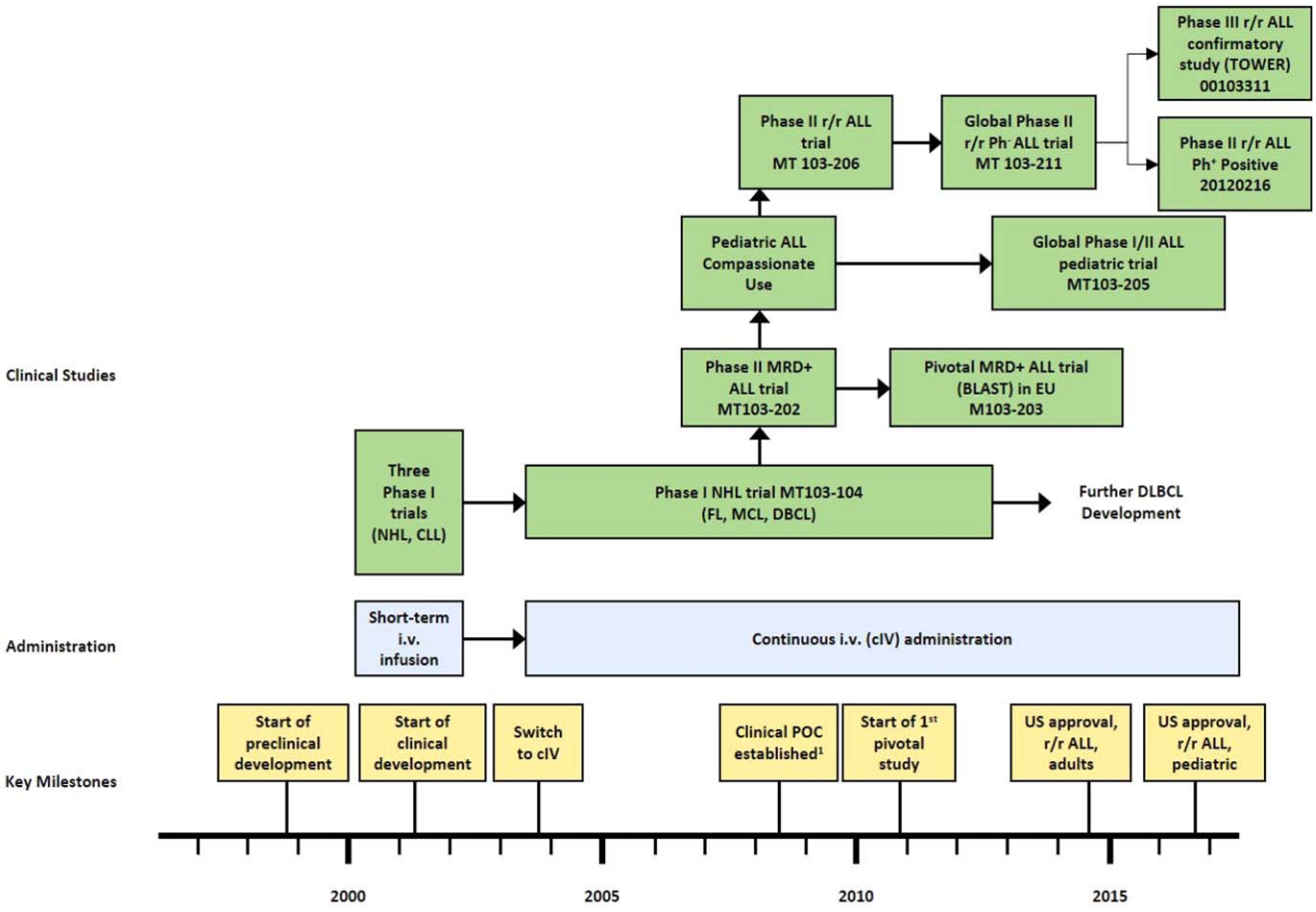

Figure 3 Overview of clinical studies conducted to support blinatumomab approval.

efficacious doses, and to proceed rapidly to efficacious doses from which the patients might benefit.

CLINICAL EXPERIENCE WITH BLINATUMOMAB

The initial phase I clinical trials of blinatumomab, launched in 2001, enrolled subjects with R/R indolent NHL and chronic lymphocytic leukemia (CLL), all CD19 expressing malignancies of mature B cells.[51] Short intravenous infusions of blinatumomab were administered in doses ranging from 0.75–13 μg/m^2, over 2–4 hours, 1–3 times per week. These initial studies were terminated early due to toxicity, particularly neurotoxicity. The pathophysiology of neurologic events remains unestablished, but is observed with other CD19-targeting immunotoxins and CAR-T cells consistent with on-target toxicity.[52,53] Evidence of activity was observed in some subjects, including upregulation of T-cell activation markers, release of cytokines, and transient reduction in peripheral blood B cells. These studies confirmed the short terminal half-life of the molecule, ~2 h, as observed in preclinical investigation.[51]

Continuous intravenous infusion (cIV) was tested in a subsequent phase 1 trial (MT103-104), again among subjects with indolent NHL (**Figure 3**, **Table 2**),[54,55] to mitigate toxicity and maintain exposures needed to sustain responses; it was hypothesized that intermittent dosing led to high C_{max} and that the drug-free period potentiated T-cell cytokine release. The dose–exposure relationship was linear from 5–90 μg/m^2/d, stable plasma blinatumomab levels were achieved rapidly with continuous infusion, and rapid clearance consistent with previous observations was observed.[56] The major dose-limiting toxicity (DLT) was neurologic, as described above, with 73% of patients experiencing a neurologic event and 21% experiencing a Grade 3 neurologic event. Discontinuation of the infusion, along with corticosteroid treatment, resulted in resolution of the neurologic abnormalities. The maximum tolerated dose (MTD) was 60 μg/m^2/d (d). The infusion duration ranged from 4–8 weeks.

The study was expanded to examine dosing strategies that would minimize adverse events and permit a greater proportion of subjects to reach MTD. Step dosing of blinatumomab was implemented with patients receiving 1) a flat dose of 60 μg/m^2/d, 2) 5 μg/m^2/d from Day 1 to Day 7, then 60 μg/m^2/d, or 3) 5 μg/m^2/d from Day 1 to Day 7, 15 μg/m^2/d from Day 8 to Day 14, then 60 μg/m^2/d (**Table 2**). This expansion also tested various schedules of corticosteroids as prophylaxis for neurologic. Patients received either a corticosteroid chosen by the investigator with the dose and duration of treatment also determined by the investigator, or treatment with oral dexamethasone starting before treatment with blinatumomab and continuing for 3 days, and repeated for 3 days after each escalation of the dose. Although the

sample size was small, these strategies reduced neurologic adverse events (AEs).[55] Pharmacodynamic evidence of T-cell activation and $CD19^+$ cell depletion were observed in multiple subjects, and serum cytokine levels were elevated in a majority of subjects, although in various combinations.[54] Complete peripheral blood B-cell clearance was observed in all subjects treated at doses $\geq$5 $\mu g/m^2/d$, but clinical responses were not observed below doses of 15 $\mu g/m^2/d$, with efficacy increasing with dose.

Subjects with R/R diffuse large B-cell lymphoma (DLBCL) were treated in the expansion cohort of the phase I trial (MT103-104). The response rates (36% CR/Cru) and duration of response among subjects with DLBCL compared favorably with other monotherapies currently approved or undergoing evaluation for this indication.[57,58] Therefore, a phase II successor trial restricted to R/R DLBCL was initiated and the results have recently been reported.[59] One objective of the study was to further compare step vs. flat dosing as a means to reach the target dose; step dosing was more effective at mitigating adverse events. Additionally, body surface area (BSA)-based dosing was abandoned for fixed dosing because fixed dosing has practical advantages such as better compliance and reduced risk of medical errors and the data from previous trials supported this decision; steady-state concentrations of blinatumomab were not affected by body weight nor BSA and interindividual variability in steady-state concentrations was not reduced after normalizing for BSA. Population pharmacokinetic modeling subsequently confirmed these analyses.[56] Clinical development of blinatumomab in DLBCL is ongoing.

Blinatumomab has also been tested in precursor-B cell acute lymphoblastic leukemia (B-ALL), an aggressive disease of immature B cells and with universal CD19 expression at diagnosis (**Figure 3**, **Table 2**).[34] The adjusted incidence of B-ALL is 17.3 per 1,000,000 person-years.[60] In the US, the annual incidence is ~6,600, with 60% of incident cases occurring in children. Treatment of pediatric ALL is widely regarded as a success story in hematology, with high response rates to frontline multiagent chemotherapy, prolonged consolidation and maintenance phases, resulting in 10-year survival around 90%.[61] The sequelae of therapy are substantial, and new protocols are focused on reducing toxicity by risk-adapted deintensification. In adults with ALL, frontline chemotherapy results in complete response (CR) rates of 80–90%. However, ~50% of adult patients relapse.[62,63] Response to salvage therapies are poor.[64]

The strongest predictor of relapse following frontline therapy (typically after three cycles of chemotherapy) is the presence of minimal residual disease (MRD). MRD may be detected by quantitative real-time polymerase chain reaction (Q-PCR) of clonotypic antigen receptor rearrangements, next-generation sequencing, or multiparametric flow cytometry. The prognosis of MRD^+ ALL is widely accepted to be poor without additional treatment.[65,66] MRD that persists after multiagent, frontline chemotherapy implies the persistence of leukemic cells resistant to standard cytotoxic agents. MRD levels appear to predict time to relapse.[67]

Response rates to salvage chemotherapy are ~40%, 21%, and 11% for patients in first, second, or later relapse, respectively.[50] Among patients in first relapse, a longer duration of remission is associated with a higher probability of response.[67–69] Allogeneic hematopoietic stem cell transplantation (HSCT) is the best-established treatment following relapse, with efficacy attributed to both conditioning regimens and immunologically mediated graft vs. leukemia effect. However, the outcome of HSCT is poor for patients not in remission at the time of transplant, and thus is typically only offered to patients achieving remission to last-administered therapy. Since a minority of patients with relapsed ALL respond, and those who respond may not stay in remission long enough to reach HSCT, there is a need to move HSCT earlier in the treatment course. Persistent or relapsed MRD for patients in CR1 (first complete remission) is widely seen as an indication for HSCT, although HSCT outcomes are inferior compared to MRD-negative CR1.

MT103-202 was a phase II trial testing blinatumomab for adult subjects with ALL with either persistent or relapsed MRD (**Figure 3**, **Table 2**). Blinatumomab was administered at a dose of 15 $\mu g/m^2/d$ to 21 subjects for 1–4 cycles. Each cycle consisted of 4 weeks of infusion followed by a 2-week treatment-free interval. The treatment period of 4 weeks was selected based on T-cell kinetics after blinatumomab administration in the phase I studies. T cells responding to infection expand, then contract; recovery after contraction was the basis for the selection of the dosing holiday.[70] The primary endpoint, MRD-negativity, was achieved in 16 of 20 (80%) evaluable subjects (one subject experienced a Grade 3 seizure and was not evaluable).[71] At a median follow-up of 33 months, hematologic relapse-free survival was 60%.[72] Of note, 11 subjects on this trial did not receive subsequent allogeneic HSCT and six subjects remain alive in continuous remission. Four subjects relapsed, including two subjects with blasts that lacked CD19 expression and two with extramedullary relapse. Six subjects remain alive in continued remission. A larger phase II trial of blinatumomab for MRD-positive ALL has recently been presented in abstract form and showed a complete molecular remission rate of 80%.[73]

Based on the encouraging results in NHL and MRD-positive ALL, blinatumomab was tested in two single-arm phase II trials (MT103-206 and MT103-211) in patients with R/R Philadelphia chromosome-negative ALL (**Table 2**).[70,74,75] Three dosing regimens were tested in the first 18 subjects enrolled into MT103-206: a flat dose of 15 $\mu g/m^2/d$, step dosing from 5 $\mu g/m^2/d \times 7$ d to 15 $\mu g/m^2/d$, and step dosing from 5–15–30 $\mu g/m^2/d$, all for a total infusion duration of 28 days. Dose selection was based on results from previous studies with blinatumomab (MT103-104 and MT103-202) with step dosing implemented to mitigate adverse events. The 5–15 $\mu g/m^2/d$ schedule had the lowest incidence of AEs and serious AEs (SAEs) and was chosen for an 18-subject expansion cohort. The combined CR and CRh (complete response with partial hematopoietic recovery) rate among all 36 subjects rate was 69%, including 100% response rates in those in first relapse. Among responders, the MRD-negativity ($<10^{-4}$) rate was 88%. The median relapse-free survival was 7.6 months. Relapses occurred in 10 subjects, including three who lost expression of CD19 and four who were extramedullary.

A confirmatory phase II study, MT103-211, enrolled 189 subjects with R/R precursor B-ALL. Patients with late first relapse (>12m) were excluded, due to a relatively high response rate to salvage cytotoxic chemotherapy (**Figure 3**, **Table 2**).[59] Subjects received dexamethasone premedication within 1 h of blinatumomab initiation and at each step-dose escalation. A prephase of dexamethasone was required of subjects with greater than 50% marrow blasts or greater than 15,000/μl circulating blasts. Blinatumomab was administered at a dose of 9 μg/d for the first week of the first cycle and then 28 μg/d for the remainder of the 28-day infusion period and for subsequent cycles. The dose selection was based on safety and efficacy results from MT103-104, MT103-202, and MT103-206. Fixed dosing was used in this study, although BSA-based dosing was used in previous studies, because fixed dosing is more convenient and the previously mentioned analysis of data from those studies suggested that blinatumomab concentrations were unrelated to body weight or BSA. A treatment-free interval of 2 weeks completed the 6-week cycle. The primary endpoint was the rate of CR/CRh. Subjects achieving CR/CRh/CRi (complete response with incomplete recovery of peripheral blood counts) could receive an additional three cycles of blinatumomab.

The rates of CR and CRh were 33 and 10%, respectively, in MT103-211. Prespecified analyses did not show statistically meaningful differences in response rates according to baseline features such as age, number of prior therapies, or prior allogeneic HSCT. However, baseline bone marrow blast percentage was predictive of response. Blast percentage ≥50% was associated with a CR/CRh rate of 29%, vs. 73% for blasts <50%. An *ad hoc* analysis did not show an impact of prephase dexamethasone on response. Among responders, the MRD-negative rate ($<10^{-4}$) was 82% and was associated with median recurrence-free survival (RFS) of 6.9 months. Responders who remained MRD-positive experienced median 2.3m RFS.

Blinatumomab was granted an accelerated approval for R/R Philadelphia chromosome-negative ALL. As a condition for an accelerated approval, the FDA requested a confirmatory, randomized control trial of blinatumomab in R/R ALL. The primary endpoint of the randomized trial, 00103311 (**Figure 3**), was overall survival (OS). Findings from the initial analysis have recently been reported after a Data Monitoring Committee closed the trial early, with an OS advantage for subjects randomized to blinatumomab vs. standard of care chemotherapy.[76]

Blinatumomab has also received regulatory approval for use in pediatric patients with Philadelphia chromosome-negative R/R B-cell precursor acute lymphoblastic leukemia based on the results of a two-part phase I / phase II study; the first part (phase I) was designed to be a dose-finding study and the second part (phase II) was a single-arm study to assess safety and efficacy of the recommended dose of blinatumomab in pediatric patients (**Figure 3**, **Table 2**). The MT103-205 trial enrolled 49 pediatric patients from <2 years to 17 years including eight infants in phase I of the study. Four BSA-based dose regimens were tested on the basis of results in adults: 5, 15, 30, and 15–30 $\mu g/m^2/d$. Stepwise dosing of 5 $\mu g/m^2/d$ for 7 days, then 15 $\mu g/m^2/d$ for weeks 2–4 of cycle 1, and 15 $\mu g/m^2/d$ for subsequent cycles, was selected for phase II because of the positive efficacy signal and favorable safety profile; there were no incidences of cytokine release syndrome or Grade 3 central nervous system (CNS)-related events for this dosing regimen in the phase I/II study.[77] A total of 44 pediatric patients from <2–17 years, including two infants, were enrolled in phase II of the study. The safety profile was consistent with other blinatumomab studies, and 39% of subjects achieved CR within two cycles of blinatumomab. The relapse-free survival time was 4.4 months, and median overall survival was 7.5 months.

Blinatumomab has demonstrated efficacy in relapsed and MRD-positive, pediatric and adult, ALL. Responses are typically MRD-negative. The greatest efficacy is in situations with lower leukemic burden. Other pretreatment factors do not appear predictive of response. The limitation by tumor bulk suggests most obviously sensitivity to effector: target ratio, although other mechanisms are plausible. The relationship between leukemic burden and T-effector functions, including exhaustion, has not been clarified. Conversely, even in the lowest ranges of MRD, ~20% of patients do not respond. The mechanisms for this resistance are unknown but may reflect leukemia and/or T-cell-specific factors.

CLINICAL PHARMACOLOGY OF BLINATUMOMAB

The pharmacokinetic properties of BiTE® antibody constructs share many characteristics with small molecules, including a short half-life and linear pharmacokinetics (PK); blinatumomab was linear over a dose range from 5–90 $\mu g/m^2/d$.[56] Unlike most therapeutic proteins, but like many small molecules, BiTE® antibody constructs do not undergo target-mediated disposition. The major route of elimination for BiTE® antibody constructs such as blinatumomab is via rapid catabolism into simple amino acids; without the Fc domain these constructs lack the protection against catabolism and intracellular degradation by mediating recycling to the cell surface.[78] Without FcRn-mediated recycling to prevent rapid clearance, the mean elimination half-life of blinatumomab was predictably short, at 2.11 h, and was consistent with the short half-lives observed for AMG 211 and AMG 110. The lack of an Fc region may also contribute to the <1% incidence of neutralizing antibodies observed in clinical studies with blinatumomab, although it is expected that B-cell depletion is the major reason. The mean volume of distribution was similar to plasma volume (4.52 L) and mean clearance was 2.72 L/hr. The variability in PK was high, with average steady-state concentrations fluctuating as much as 100% between individuals.[56]

The drug–drug interaction (DDI) potential for BiTE® antibody constructs such as blinatumomab is low. BiTE® antibody constructs are unlikely to be victims of DDI as they do not undergo CYP-mediated clearance, although they may perpetrate DDI indirectly. Although BiTE® antibody constructs do not directly affect CYP enzyme activities, transient cytokine elevation, especially of IL-6, has been observed in clinical trials with blinatumomab and in preclinical studies with blinatumomab and other BiTE® antibody constructs, and the suppression of CYP enzymes in response to cytokine elevation has been well documented.[79] A dedicated DDI study in this population would be

Table 1 Selected bispecific antibodies in clinical development as anticancer agents

Format	Molecule	Targets	Phase	Indication
BiTE®	Blinatumomab (Blincyto)	CD19 x CD3	Approved	ALL
BiTE®	AMG 211/MT 211	CEA x CD3	Ph I	Gastrointestinal cancer
BiTE®	AMG 110/MT 110	EPCAM x CD3	NA	Solid tumors
BiTE®	MEDI-565/MT 111	CEA x CD3	Ph I	Gastrointestinal adenocarcinoma
BiTE®	AMG 330	CD33 x CD3	Ph I	AML
BITE	AMG 420	BCMA x CD3	Ph I	Multiple myeloma
BiTE®	AMG-212/BAY-2010112/ MT-112	PSMA (FOLH1)/CD3	Ph I	Prostate cancer
TrioMab	Catumaxomab (Removab)	EPCAM x CD3	Approved	Malignant ascites
TrioMab	Ertumuxomab	HER2 x CD3	Ph II	Breast cancer
DART	PF-06671008	P-cadherin x CD3	Ph I	Advanced solid tumors
DART	MGD006	CD123 x CD3	Ph I	r/r AML; MDS
DART	MGD007	gpA33 x CD3	Ph I	r/r metastatic colorectal carcinoma
DART	MGD009	B7-H3 x CD3	Ph I	B7-H3-expressing solid tumors
DART	MGD011/ JNJ-64052781	CD19 x CD3	Ph I	B-cell malignancies
CrossMab	RG7892/RO6958688	CEA x CD3	Ph I	Locally advanced or metastatic solid tumors
CrossMab	RG7221/RO5520985	Anti-ANG2/anti-VEGFA	Ph I	Advanced solid tumors
BsAb	REGN1979	CD20 x CD3	Ph I	B-cell malignancies
BsAb	BTCT4465A/ RG7828	CD20 x CD3	Ph I	NHL and CLL
BsAb	MEHD7945a	EGFR x HER3	Ph II	Head and neck cancers; mCRC
DVD-Ig	OMP-305B83	DLL x VEGF	Ph I	Advanced solid tumors
orthoFab-IgG	LY3164530	MET x EGFR	Ph I	Advanced or metastatic cancer
Tetravalent BsAb	RG7386	FAP x DR5	Ph I	Solid tumors
Tetravalent BsAb	MM-141	IGF-IR and ErbB3	Ph II	Metastatic pancreatic cancer
TandAb	AFM13	CD30 x CD16a	Ph II	Hodgkin's lymphoma
TandAb	AFM11	CD19 x CD3	Ph I	NHL[4]

BiTE®, bispecific T-cell engaging antibody; DART, dual-affinity retargeting; BsAb, bispecific antibody; TandAb, tetravalent bispecific tandem diabody; mCRC, metastatic colorectal cancer; NHL, non-Hodgkin's Lymphoma; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome ; CLL, chronic lymphocytic leukemia; r/r, relapsed/ refractory.

impractical, however, so a physiologically based pharmacokinetic (PBPK) model was developed to evaluate the impact of IL-6 elevation on CYP suppression after blinatumomab administration.[80] The predicted suppression of hepatic CYP450 activities was <30%, and IL-6-mediated changes in exposure to sensitive substrates of CYP3A4, CYP1A2, and CYP2C9 were <2-fold and lasted <1 week.[80]

Renal impairment studies are not typically conducted for large molecules, but renal excretion of blinatumomab was assessed because its molecular weight is below the renal filtration cutoff of 69 kDa.[81] Although clinically meaningful renal elimination is less likely to be observed for proteins with a molecular weight of 69 kDa, since the process of glomerular filtration is dependent on other physicochemical characteristics such as molecular charge, vulnerability to renal enzymes, and aggregation, urinary excretion of blinatumomab was evaluated and only 0.2% was excreted unchanged, supporting a limited role for the kidneys in the clearance of blinatumomab.[56] Creatinine clearance was a significant covariate in the population PK model and a ~21% decrease in blinatumomab clearance was observed in patients with mild renal impairment, but clearance values in patients with mild and moderate renal impairment were within the range of patients with normal renal function, and these differences were not considered clinically meaningful with respect to efficacy or safety; as a result, no dose adjustment is recommended for these patients.[56] Blinatumomab was not studied in patients with severe renal impairment.

The approved dose and schedule of blinatumomab in R/R ALL, i.e., a 4 week on / 2 week off schedule and stepwise dosing in cycle 1 with 9 μg/d cIV for 1 week followed by 28 μg/d cIV for 3 weeks and 28 μg/d cIV for 4 weeks in subsequent cycles,

Table 2 Overview of blinatumomab clinical studies

Study	Ph	Indication/objectives	Dose tested	Key results
MT103-104[54,55] (n = 76)	I	Adults with relapsed NHL MTD, PK, PD, anti-tumor activity	Flat dosing: 0.5, 1.5, 5, 15, 30, 60 and 90 ug/m^2/d cIV over 4–8 weeks Step dosing: 5/60 and 5/15/60 ug/m^2/day Different steroid doses tested at 15 ug/m^2/d	OR: 69%; CR: 37% PK was linear with cIV infusion up to 90 ug/m^2/d DLT: neurotoxicity MTD: 60 ug/m^2/d
MT103-202[86] (n = 21)	II	Adults with MRD+ ALL Efficacy (MRD response rate), safety, PK, and PD	Flat dosing: 15 μg/m^2/d[a], cIV; 4 weeks on, 2 weeks off; 1-4 cycles	MRD response: 80% Hematologic RFS (33 mo): 60% C_{ss} increased dose-dependently and remained stable over time
MT103-203[87,88] (n = 116)	II	Adults with MRD+ ALL PK/QTc, efficacy, safety	5/15/30 ug/m^2/d[b] cIV for 4 weeks followed by 2 weeks off drug per cycle	MRD response: 78% OS: 36.5 mo RFS: 19.9 mo
MT103-206[70] (n = 36)	II	Adults with R/R ALL PK/QTc, efficacy, safety	Flat dosing: 15 ug/m2/d; 4 wk on, 2 wk off Step dosing: 5-15 ug/m^2/d, 5-15-30 ug/m^2/d, 4 wk on, 2 wk off	CR and CRh: 69% MRD- rate among responders: 88% Median RFS: 7.6 months 5-15 ug/m^2/d schedule had the lowest incidence of AEs and SAEs
MT103-211[74,75] (n = 225)	II	Adults with r/r ALL PK/PD, efficacy, safety	Step dosing: 9 - 28 ug/d for 4 weeks per cycle	CR: 33%; CRh: 10% MRD- negative rate: 82% Median RFS: 6.9 months.
MT103-208[59] (n = 25)	II	Adults with r/r NHL; Adults with DLBCL PK/PD, efficacy, safety	Flat dosing: 112 ug/d cIV for 4 weeks per cycle Step dosing: 9 - 28 -112 ug/d for 4 weeks per cycle	ORR: 43% CR: 19%
MT103-205[77] (n = 36)	I/II	Pediatric patients with R/R ALL	Phase I: Flat dosing: 5, 15, 30 15/30 ug/m^2/d cIV Step dosing: 15/30 ug/m^2/d cIV Phase II: 5/15 ug/m^2/d cIV	MTD: 15 ug/m^2/d PK linear across dosage levels and consistent among age groups. Recommended blinatumomab dosage for children with R/R ALL was 5 - 15ug/m^2/d CR: 39%; MRD response: 52%

OR, overall response; DLT, dose limiting toxicity; MTD, maximum tolerated dose; ORR, overall response rate; CR, complete response; CRh, complete response with partial hematopoietic recovery; RFS, relapse free survival; Css, steady-state concentration; AE, adverse event; SAE, serious adverse event; R/R, relapsed and/or refractory.
[a]Intrapatient dose escalation to 30 μg/m^2/d was permitted for patients with stable disease who had not responded after 1 cycle at 15 μg/m^2/d.

was based on the PKPD relationships and an assessment of the observed efficacy and safety data. Blinatumomab and other BiTE® antibody constructs in clinical development are administered to patients by cIV infusion because of the short plasma half-life; although short intravenous infusions of blinatumomab were effective in preclinical species, this approach was abandoned after initially unsuccessful clinical trials in patients. Although PK interindividual variability was high and increasing concentrations of blinatumomab were associated with increased redistribution of T cells upon initiation of dosing or elevation of the dose, increased initial B-cell depletion rate, and peak cytokine levels, the data suggest that increasing the dose in R/R ALL patients beyond the marketed dose of 28 μg/d would not be an effective treatment for nonresponders, for whom other cellular resistance mechanisms may be responsible for failure to respond to blinatumomab treatment.[40] The average steady-state concentration ranged from ~500–700 pg/ml after blinatumomab at a cIV dose of 28 μg/d, concentrations that exceed the *in vitro* IC_{90} value of 470 pg/ml for B-cell suppression in NALM-6 CD19 human B-cell precursor leukemia cell line incubated with healthy donor T cells and treated with blinatumomab (data on file, Amgen, Thousand Oaks, CA). There was a 1–2 log range in potency that is likely due to variability in the activity of T cells from different donors, but blinatumomab exposures still exceeded the *in vitro* EC_{50} value for even the least sensitive patients (500 pg/ml).[36] The interindividual variability in the pharmacodynamic data from the clinical trials was also high, and other factors such as baseline T-cell counts, disease burden, baseline B-cell counts, and other patient disease characteristics (e.g., length of first remission, number of prior relapses), may have an important influence on the variability in these measures and, more important, clinical outcomes. A quantitative systems pharmacology model developed to describe the action of blinatumomab in patients with ALL supports this hypothesis; the model indicated that the dose–response for nonresponders may be relatively flat.[82,83] Moreover, this dose and schedule was considered appropriate from a safety perspective; although PK was highly variable the safety profile was manageable with the implementation of stepwise dosing and initiation of treatment with a lower dose before escalation to the target dose reduced the magnitude of cytokine elevation. The 2-week drug-free period was considered appropriate because continuous B-cell suppression was maintained and clinically

meaningful benefits to R/R ALL patients were demonstrated with this regimen, and the safety and tolerability profile of this regimen was favorable. The PK of blinatumomab was not affected by demographic characteristics (body weight, body surface area, sex, age), disease type (ALL, NHL), or baseline disease characteristics (Eastern Cooperative Oncology Group (ECOG) performance status, T-cell count, B-cell count). These observations coupled with analyses that showed similar exposures after fixed-dose and BSA-based dosing regimens for blinatumomab supported fixed dosing in adults.[56]

The totality of the pharmacokinetic, pharmacodynamic, and safety and efficacy data supported the selected dose and schedule in R/R ALL patients. It should be noted that the dosing regimen for NHL is different from that of ALL,[7] as effective dose levels were determined based on EC_{90} ranges and drug concentrations at the sites of action (i.e., in blood, lymph nodes, or tumor tissues) and on safety profiles.

FUTURE PERSPECTIVES

Unlike many cancer immunotherapies, BiTE® antibody constructs do not require endogenous T-cell receptor (TCR) specificity for a cancer-specific MHC peptide complex, an advantage over other modalities, given the analytical challenges associated with characterizing the polymorphisms of TCR and the MHC. BiTE® antibody constructs can redirect the specificity of any T-cell via engagement of the invariant CD3ϵ chain that is expressed on all T cells. Individual T cells can engage in serial lysis of targets. Blinatumomab, which targets the B-cell antigen CD19, is the first BiTE® antibody construct to gain regulatory approval, based on a response rate of 42.9% in subjects with R/R ALL patients. There is a great need to understand the mechanisms of nonresponse. Unfavorable effector: target ratios appear to be one predictor of primary resistance and may be amenable to debulking with cytotoxic chemotherapy. High antigen load may also contribute to T-cell exhaustion, justifying investigations into concurrent PD-1 or PD-L1/2 blockade.[84,85] Dose and schedule of combinations will be challenging to determine empirically, but may be aided by quantitative systems pharmacology models that capture the pathophysiology of the disease and the mechanisms of action of each agent. Likewise, PB/PK modeling will continue to be a useful tool to address the potential for cytokine-mediated drug–drug interactions that may be more apparent when BiTE® antibody constructs are administered in combination regimens and particularly with small molecule anticancer agents.

For solid tumor malignancies, efficacy of BiTE® antibody constructs will require penetration of the tumor by the BiTE® antibody construct and by the T cells. Preclinical models have demonstrated the distribution of BiTE® antibody constructs into the tumor, as was expected based on the small size of BiTE® antibody constructs relative to full-length antibody. However, as has been discussed, the density and type of T cells in the tumor tissue are less predictable. Additionally, preexisting T cells in the tumor may exhibit signs of exhaustion. Whether BiTE® antibody constructs are sufficient to enhance trafficking of additional functional T cells to a tumor is of great interest and may mitigate one potential mechanism of nonresponse in solid tumor indications.

The theory of cancer immune surveillance provided a conceptual framework for observations and experimentation that now have resulted in new therapies and new hope for cancer patients. Immunotherapeutic agents must also be viewed as tools to further probe the intricacies of tumor cell clearance and escape. Careful observation of patient responses, including robust collection of biomarker data, must be fed back into the conceptual models that inform immunology research, as well as into the pharmacologic models that guide dosing decisions. These iterative processes will lead to rational and potent combinations of agents that hopefully will meet the needs of cancer patients.

ACKNOWLEDGMENT

The first two authors contributed equally to this work.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

1. Khalil, D.N., Smith, E.L., Brentjens, R.J. & Wolchok, J.D. The future of cancer treatment: immunomodulation, CARs and combination immunotherapy. *Nat. Rev. Clin. Oncol.* **13**, 273–290 (2016).
2. Morrissey, K.M., Yuraszeck, T.M., Li, C.C., Zhang, Y. & Kasichayanula, S. Immunotherapy and novel combinations in oncology: current landscape, challenges, and opportunities. *Clin. Transl. Sci.* **9**, 89–104 (2016).
3. Coley, W.B. The treatment of malignant tumors by repeated inoculations of erysipelas. With a report of ten original cases. 1893. *Clin. Orthopaed. Relat. Res.* **262**, 3–11 (1991).
4. McCarthy, E.F. The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. *Iowa Orthopaed. J.* **26**, 154–158 (2006).
5. Ehrlich, P. Ueber den jetzigen Stand der Karzinomforschung. *Ned. Tijdschr. Geneeskd* **5**, 273–290 (1909).
6. Burnet, F.M. Immunological surveillance in neoplasia. *Transpl. Rev.* **7**, 3–25 (1971).
7. Thomas, L. On immunosurveillance in human cancer. *Yale J. Biol. Med.* **55**, 329–333 (1982).
8. Dunn, G.P., Bruce, A.T., Ikeda, H., Old, L.J. & Schreiber, R.D. Cancer immunoediting: from immunosurveillance to tumor escape. *Nat. Immunol.* **3**, 991–998 (2002).
9. Ikeda, H., Old, L.J. & Schreiber, R.D. The roles of IFN gamma in protection against tumor development and cancer immunoediting. *Cytokine Growth Factor Rev.* **13**, 95–109 (2002).
10. Fridman, W.H., Pages, F., Sautes-Fridman, C. & Galon, J. The immune contexture in human tumours: impact on clinical outcome. *Nat. Rev. Cancer* **12**, 298–306 (2012).
11. Kawakami, Y. *et al*. Identification of a human melanoma antigen recognized by tumor-infiltrating lymphocytes associated with in vivo tumor rejection. *Proc. Natl. Acad. Sci. U. S. A.* **91**, 6458–6462 (1994).
12. Schumacher, T.N. & Schreiber, R.D. Neoantigens in cancer immunotherapy. *Science* **348**, 69–74 (2015).
13. Ahmadzadeh, M. *et al*. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. *Blood* **114**, 1537–1544 (2009).
14. Foley, E.J. *Cancer Res.* **13**, 835–837 (1953).
15. Nobel prize in physiology or medicine 1972. <www.nobelprize.org/nobel_prizes/medicine/laureates/1972/press.html> Accessed November 11, 2015.
16. Burnet, M. *Br. Med. J.* **1**, 779–786 (1957).
17. Fudenberg, H.H., et al. *J. Exp. Med.* **119**, 151–166 (1964).

18. . . .
19. Miller, R.A., et al. *N. Engl. J. Med.* **306**, 517–522, (1981).
20. Chames, P., et al. *mAbs.* **1**, 539–547 (2009).
21. Bindon, C., et al. *Br. J. Cancer.* **47**, 123–133 (1983).
22. Bohlen, H., et al. *Blood* **82**, 1803–1812 (1993).
23. Rosenberg, S.A., et al. *N. Engl. J. Med.* **319**, 1676–1680 (1988).
24. van der Bruggen, P., et al. *Science* **254**, 1643–1647 (1991).
25. Mack, M., et al. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 7021–7025 (1995).
26. Frankel, S.R., et al. *Curr. Opin. Chem. Biol.* **17**, 385–392 (2013).
27. Linke, R., et al. *mAbs.* **2**, 129–136 (2010).
28. Chen, D.S. & Mellman, I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* **39**, 1–10 (2013).
29. Offner, S., Hofmeister, R., Romaniuk, A., Kufer, P. & Baeuerle, P.A. Induction of regular cytolytic T cell synapses by bispecific single-chain antibody constructs on MHC class I-negative tumor cells. *Mol. Immunol.* **43**, 763–771 (2006).
30. Deisting, W., Raum, T., Kufer, P., Baeuerle, P.A. & Münz, M. Impact of diverse immune evasion mechanisms of cancer cells on T cells engaged by EpCAM/CD3-bispecific antibody construct AMG 110. *PLoS One* **10**, e0141669 (2015).
31. Przepiorka, D. *et al*. FDA approval: blinatumomab. *Clin. Cancer Res.* **21**, 4035–4039 (2015).
32. Dreier, T. *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.* **170**, 4397–4402 (2003).
33. Schlereth, B. *et al*. Potent inhibition of local and disseminated tumor growth in immunocompetent mouse models by a bispecific antibody construct specific for Murine CD3. *Cancer Immunol. Immunother.* **55**, 785–796 (2006).
34. Raponi, S. *et al*. Flow cytometric study of potential target antigens (CD19, CD20, CD22, CD33) for antibody-based immunotherapy in acute lymphoblastic leukemia: analysis of 552 cases. *Leukemia Lymphoma* **52**, 1098–1107 (2011).
35. Wang, K., Wei, G. & Liu, D. CD19: a biomarker for B cell development, lymphoma diagnosis and therapy. *Exp. Hematol. Oncol.* **1**, 36 (2012).
36. Dreier, T. *et al*. Extremely potent, rapid and costimulation-independent cytotoxic T-cell response against lymphoma cells catalyzed by a single-chain bispecific antibody. *Int. J. Cancer* **100**, 690–697 (2002).
37. Brischwein, K. *et al*. Strictly target cell-dependent activation of T cells by bispecific single-chain antibody constructs of the BiTE® class. *J. Immunother.* **30**, 798–807 (2007).
38. Loffler, A. *et al*. A recombinant bispecific single-chain antibody, CD19 x CD3, induces rapid and high lymphoma-directed cytotoxicity by unstimulated T lymphocytes. *Blood* **95**, 2098–2103 (2000).
39. Hoffmann, P. *et al*. Serial killing of tumor cells by cytotoxic T cells redirected with a CD19-/CD3-bispecific single-chain antibody construct. *Int. J. Cancer* **115**, 98–104 (2005).
40. Benjamin, J.E. & Stein, A.S. The role of blinatumomab in patients with relapsed/refractory acute lymphoblastic leukemia. *Ther. Adv. Hematol.* **7**, 142–156 (2016).
41. Friedrich, M. *et al*. Preclinical characterization of AMG 330, a CD3/CD33-bispecific T-cell-engaging antibody with potential for treatment of acute myelogenous leukemia. *Mol. Cancer Ther.* **13**, 1549–1557 (2014).
42. Brischwein, K. *et al*. MT110: a novel bispecific single-chain antibody construct with high efficacy in eradicating established tumors. *Mol. Immunol.* **43**, 1129–1143 (2006).
43. Warnders, F.J. *et al*. Biodistribution and PET imaging of labeled bispecific T cell-engaging antibody targeting EpCAM. *J. Nucl. Med.* **57**, 812–817 (2016).
44. Pui, C.H. & Jeha, S. New therapeutic strategies for the treatment of acute lymphoblastic leukaemia. *Nat. Rev. Drug Disc.* **6**, 149–165 (2007).
45. Lin, K.-T. & Wang, L.-H. New dimension of glucocorticoids in cancer treatment. *Steroids* **111**, 84–88 (2016).
46. Brandl, C. *et al*. The effect of dexamethasone on polyclonal T cell activation and redirected target cell lysis as induced by a CD19/CD3-bispecific single-chain antibody construct. *Cancer Immunol. Immunother.* **56**, 1551–1563 (2007).
47. Fiedler, W.M. *et al*. Phase I safety and pharmacology study of the EpCAM/CD3-bispecific BiTE® antibody MT110 in patients with metastatic colorectal, gastric, or lung cancer. *J. Clin. Oncol.* **28**, 2573–2573 (2010).
48. Pishvaian, M. *et al*. Phase 1 dose escalation study of MEDI-565, a bispecific T-cell engager that targets human carcinoembryonic antigen, in patients with advanced gastrointestinal adenocarcinomas. *Clin. Colorectal Cancer* **15**, 345–351 (2016).
49. Eastwood, D. *et al*. Monoclonal antibody TGN1412 trial failure explained by species differences in CD28 expression on CD4+ effector memory T-cells. *Br. J. Pharmacol.* **161**, 512–526 (2010).
50. European Medicines Agency (EMA), Committee for Medicinal Products for Human Use (CHMP). Guideline on Strategies to Identify and Mitigate Risks for First-in–Human Clinical Trials with Investigational Medicinal Products (July 2007).
51. Nagorsen, D., Kufer, P., Baeuerle, P.A. & Bargou, R. Blinatumomab: a historical perspective. *Pharmacol. Ther.* **136**, 334–342 (2012).
52. Stone, M.J. *et al*. A phase I study of bolus versus continuous infusion of the anti-CD19 immunotoxin, IgG-HD37-dgA, in patients with B-cell lymphoma. *Blood* **88**, 1188–1197 (1996).
53. Davila, M.L. *et al*. Efficacy and toxicity management of 19–28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci. Transl. Med.e* **6**, 224ra225 (2014).
54. Bargou, R. *et al*. Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. *Science* **321**, 974–977 (2008).
55. Goebeler, M.E. *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.* **34**, 1104–1111 (2016).
56. Zhu, M. *et al*. Blinatumomab, a bispecific T-cell engager (BiTE®((R))) for CD-19 targeted cancer immunotherapy: clinical pharmacology and its implications. *Clin. Pharmacokinet.* **55**, 1271–1288 (2016).
57. Pettengell, R. *et al*. Pixantrone dimaleate versus other chemotherapeutic agents as a single-agent salvage treatment in patients with relapsed or refractory aggressive non-Hodgkin lymphoma: a phase 3, multicentre, open-label, randomised trial. *Lancet Oncol.* **13**, 696–706 (2012).
58. Wang, M. *et al*. Oral lenalidomide with rituximab in relapsed or refractory diffuse large cell, follicular and transformed lymphoma: a phase II clinical trial. *Leukemia* **27**, 1902–1909 (2013).
59. Viardot, A. *et al*. Phase 2 study of the bispecific T-cell engager (BiTE®) antibody blinatumomab in relapsed/refractory diffuse large B-cell lymphoma. *Blood* **127**, 1410–1416 (2016).
60. Dores, G.M., Devesa, S.S., Curtis, R.E., Linet, M.S. & Morton, L.M. Acute leukemia incidence and patient survival among children and adults in the United States, 2001–2007. *Blood* **119**, 34–43 (2012).
61. Pui, C.H. *et al*. A revised definition for cure of childhood acute lymphoblastic leukemia. *Leukemia*, **28**, 2336–2343 (2014).
62. Hoelzer, D. *et al*. Improved outcome in adult B-cell acute lymphoblastic leukemia. *Blood* **87**, 495–508 (1996).
63. Larson, R.A. *et al*. A five-drug remission induction regimen with intensive consolidation for adults with acute lymphoblastic leukemia: cancer and leukemia group B study 8811. *Blood* **85**, 2025–2037 (1995).
64. Gokbuget, N. *et al*. International reference analysis of outcomes in adults with B-precursor Ph-negative relapsed/refractory acute lymphoblastic leukemia. *Haematologica* **101**, 1524–1533 (2016).
65. Coustan-Smith, E. *et al*. Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia. *Blood* **96**, 2691–2696 (2000).
66. Bruggemann, M. *et al*. Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. *Blood* **107**, 1116–1123 (2006).
67. Gokbuget, N. *et al*. Outcome of relapsed adult lymphoblastic leukemia depends on response to salvage chemotherapy, prognostic factors, and performance of stem cell transplantation. *Blood* **120**, 2032–2041 (2012).
68. Thomas, D.A. *et al*. Primary refractory and relapsed adult acute lymphoblastic leukemia: characteristics, treatment results, and prognosis with salvage therapy. *Cancer* **86**, 1216–1230 (1999).
69. Fielding, A.K. *et al*. Outcome of 609 adults after relapse of acute lymphoblastic leukemia (ALL); an MRC UKALL12/ECOG 2993 study. *Blood* **109**, 944–950 (2007).

70. Topp, M.S. *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.* **32**, 4134–4140 (2014).
71. Topp, M.S. *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.* **29**, 2493–2498 (2011).
72. Topp, M.S. *et al*. Long-term follow-up of hematologic relapse-free survival in a phase 2 study of blinatumomab in patients with MRD in B-lineage ALL. *Blood* **120**, 5185–5187 (2012).
73. Goekbuget, N. *et al*. An evaluation of molecular response in a phase 2 open-label, multicenter confirmatory study in patients (pts) with relapsed/refractory B-precursor acute lymphoblastic leukemia (r/r ALL) receiving treatment with the BiTE® antibody construct blinatumomab. *Blood* **124**, 3704–3704 (2014).
74. Topp, M.S. *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.* **16**, 57–66 (2015).
75. Topp, M.S., Gockbuget, N. & Stein, A.S. Correction to Lancet Oncol 2015; 16: 60, 61. Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multi-centre, single-arm, phase 2 study. *Lancet Oncol.* **16**, e158 (2015).
76. Topp, M.S. *et al*. Blinatumomab improved overall survival in patients with relapsed or refractory philadelphia negative b-cell precursor acute lymphoblastic leukemia in a randomized, open-label phase 3 study (Tower). European Hematology Association. Copenhagen, Denmark (June 10, 2016).
77. von Stackelberg, A. *et al*. Phase I/Phase II study of blinatumomab in pediatric patients with relapsed/refractory acute lymphoblastic leukemia. *J. Clin. Oncol.* **34**, 4381–4389 (2016).
78. Wu, B. & Sun, Y.N. Pharmacokinetics of Peptide-Fc fusion proteins. *J. Pharm. Sci.* **103**, 53–64 (2014).
79. Kenny, J.R. *et al*. Therapeutic protein drug-drug interactions: navigating the knowledge gaps-highlights from the 2012 AAPS NBC Roundtable and IQ Consortium/FDA workshop. *AAPS J* **15**, 933–940 (2013).
80. Xu, Y. *et al*. Physiologically based pharmacokinetic model to assess the influence of blinatumomab-mediated cytokine elevations on cytochrome P450 enzyme activity. *CPT Pharmacometrics Syst. Pharmacol.* **4**, 507–515 (2015).
81. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Guidance for Industry: Pharmacokinetics in Patients with Impaired Renal Function — Study Design, Data Analysis, and Impact on Dosing and Labeling (March 2010).
82. Singh, I. *et al*. A systems pharmacology model to characterize the effect of blinatumomab in patients with adult B precursor acute lymphoblastic leukemia. Poster presented at the ASCPT annual meeting, Atlanta, GA (March 2014).
83. Yuraszeck, T. *et al*. A population-based approach to systems pharmacology modeling of the effect of blinatumomab (blin) in adult B-precursor ALL patients. Poster presented at the ASCPT annual meeting, San Diego, CA (March 2016).
84. Kohnke, T., Krupka, C., Tischer, J., Knosel, T. & Subklewe, M. Increase of PD-L1 expressing B-precursor ALL cells in a patient resistant to the CD19/CD3-bispecific T cell engager antibody blinatumomab. *J. Hematol. Oncol.* **8**, 111 (2015).
85. Feucht, J. *et al*. T-cell responses against CD19+ pediatric acute lymphoblastic leukemia mediated by bispecific T-cell engager (BiTE®) are regulated contrarily by PD-L1 and CD80/CD86 on leukemic blasts. *Oncotarget* **7**, 76902–76919 (2016).
86. Klinger, M. *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* **119**, 6226–6233 (2012).
87. Goekbuget, N. *et al*. BLAST: a confirmatory, single-arm, phase 2 study of blinatumomab, a bispecific T-cell engager (BiTE®) antibody construct, in patients with minimal residual disease B-precursor acute lymphoblastic leukemia (ALL). *Blood* **124**, 379–379 (2014).
88. Gökbuget, N. *et al*. Long-term outcomes after blinatumomab treatment: follow-up of a phase 2 study in patients (Pts) with minimal residual disease (MRD) positive B-cell precursor acute lymphoblastic leukemia (ALL). *Blood* **126**, 680–680 (2015).