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New Drugs

# Bispecific antibodies in haematological malignancies

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ABSTRACT

Bispecific antibodies (bsAbs) combine the binding sites of two monoclonal antibodies in one molecule. The close proximity of a tumor specific antigen and an effector cell antigen results in a targeted activation of effector cells. The mechanism is similar to the chimeric antigen receptor (CAR) T-cells, recently approved in two haematologic cancers. CAR T-cells and bsAb represent the most powerful tools for major-histocompatibility complex (MHC) independent T-cell immune response against cancer. In contrast to CAR T-cells, bsAbs are "off the shelf" drugs. As a drawback, the efficacy is dependent on a prolonged application. More than 40 years of intensive research generate a plethora of bispecific constructs with a remarkable difference in manufacturability, stability, half-life time and receptor affinity. Blinatumomab was the first approved bsAb in relapsed and refractory acute lymphoblastic leukemia. By the mature experience of blinatumomab in more than 10 clinical trials over more than one decade, we learned some lessons on how to use this new principle. The efficacy is higher in patients with less tumor burden, suggesting the use as consolidation more than for initial debulking. Main resistance mechanisms are extramedullary relapses and the expression of the inhibitory PD-L1 molecule, suggesting the value of combination with checkpoint inhibitors. CD19 loss is infrequent after blinatumomab, preserving the option for alternative CD19-direct treatments. New bsAbs in lymphoma, myeloma and acute myeloid leukemia enter phase-I trials, together with many new constructs in solid cancer.

## Historical perspective

In the early 60s, Alfred Nisonoff – a pioneer in antibody engineering – worked, for the first time, on the idea of "preparing antibodies of mixed specifity" [1]. However, it took more than 20 years, along with the introduction of the hybridoma technique, to establish the first monoclonal bsAb, enabling T-cell recruitment by Staerz and Bevan in 1985 [2]. This discovery was the origin of a rapidly growing interest in these technologies, between 1985 and 1995, called the "bispecific explosion" [3]. At the end of the nineties, there was a plethora of different bsAb constructs. The first clinical trial in humans was performed in 1990 [4] using a coupled antibody with specificity to T-cell-receptor and glioma antigen in glioblastoma patients. The first bsAb in haematologic malignancies might be a clinical trial using a CD19 × CD3 antibody in Non-Hodgkin-lymphoma (NHL) in 1995 [5]. This antibody showed no clinical response, but the tumor necrosis factor alpha associated cytokine release syndrome (CRS) was recognized as a relevant side effect. In 1997, a Natural Killer (NK)-cell activating CD30x CD16 antibody shows some clinical responses in Hodgkin lymphoma [6]. In 1995, preclinical data of the first bispecific T-cell engager (BITE™) against CD3 and 17-1A was published [7], which was the ancestor of the CD19 × CD3 BITE blinatumomab [8].

In 2001, Blinatumomab entered a first-in-man study [9] in Germany and Sweden, based on short-term intravenous infusions at doses ranging from 0.75 to 13 µg/m [2]. These trials were terminated early due to the lack of clinical response and the occurrence of neurologic adverse events, cytokine release syndromes (CRS) and infections. In 2004, a phase-I dose escalation trial began with a continuous infusion, resulting in the first meaningful clinical responses at a dosage of 15 µg/m$^2$/day [10]. The observation of depletion of CD19 positive peripheral blood cells and the clearance of bone marrow at very low dose levels was the rationale for the use in leukemic disease. Between 2006 and 2008, heavily pretreated pediatric patients with acute lymphoblastic leukemia (ALL) received blinatumomab as a compassionate use program and showed responses [11]. These observations justified the further clinical development in ALL.

Ten years later, Blinatumomab was approved by the FDA and FMA for the treatment of relapsed and refractory B-cell precursor ALL. The FDA accelerated approval in 2014 was converted in a full approval in July 2017, including patients with Philadelphia-positive and pediatric ALL. Blinatumomab was not the first approved bsAb. In 2009, the trifunctional EPCAM × CD3 antibody Catumaxomab was approved by

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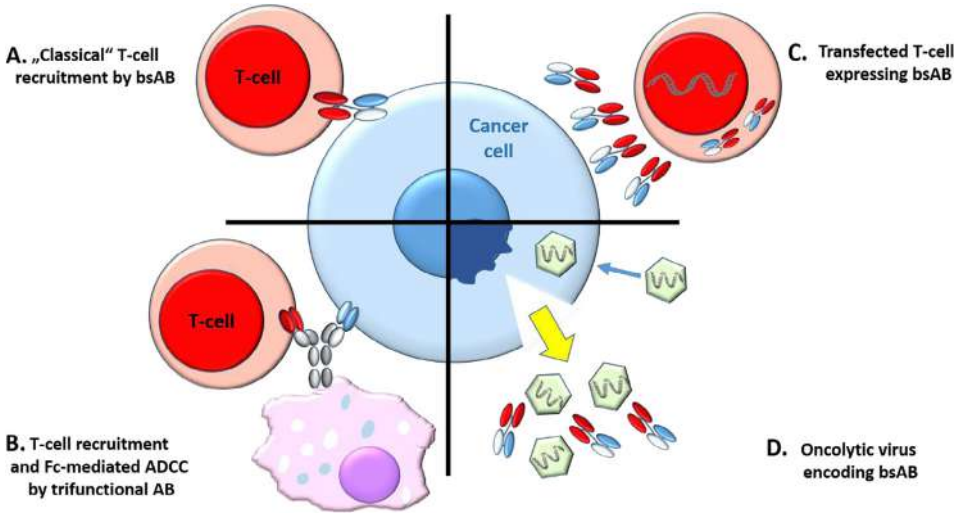


**Fig. 1.** Mode of action of bispecific antibodies and novel constructs (bsAb: bispecific antibodies; ADCC: antibody dependent cellular toxicity).

EMA for the local treatment of malignant ascites in solid tumors. However, the marketing authorization of Catumaxomab was withdrawn in July 2017 at the request of the manufacturer. Due to the success of blinatumomab and the recent developments in antibody engineering, there is a growing interest in bsAbs and novel construct possibly heralding a second "bispecific explosion" in the next years (see Fig. 1).

## Terminology

More than 30 years of development result in a pronounced diversity of different bispecific molecules in clinical and preclinical trials. In a recent review, there is an overview about the "zoo" of more than 100 bispecific constructs [12–14]. Most of them combine two or more variable regions of monoclonal antibodies in complexly engineered molecules - with differences in size, half-life, stability and receptor affinity. The first generation of bsAbs was chemically coupled. Most of the more recent developed antibodies are based on recombinant DNA technology.

A striking difference between bsAbs is the size of the molecule, which depends on the presence of the Fc part of a monoclonal antibody. Variable domain-only antibodies like BiTE™ (bispecific T-cell engager), DART™ (Dual-Affinity Re-Targeting) or TandAb™ (Tandem Antibodies) have short half-lives as they lack the Fc domain. For example, the molecular weight of blinatumomab is only 50 kDa resulting in a half-life of less than two hours. A major drawback, particularly in BITEs, is the need of a continuous infusion to maintain exposure. Full-size bsAb have a near-native antibody architecture including the Fc part, which enables comfortable dosing intervals. The Fc part of monoclonal antibodies can hinder the formation of the cytolytic synapsis by attracting macrophages. Therefore, the Fc function is mitigated by mutated Fc binding sites in some of the new constructs.

BsAbs have *per definitionem* two different specificities including two different variable regions of monoclonal antibodies. Constructs of tri- or multispecific antibodies (e.g. triabodies) combine more binding sites. BsAbs can be bi-, tri- or even tetravalent, if it has more than one binding site of one specificity per molecule to augment the binding capacity. BsAbs with a functioning Fc part, which can attract macrophages, are called "trifunctional" (e.g. Catumaxomab). An overview about this terminology is in Fig. 2.

In cancer, the most prominent function of bsAb is the recruitment of immunocompetent cells for redirected tumor lysis. The majority of bsAbs binds to the CD3/T-cell receptor complex to recruit T-cells. However, there are alternative constructs binding CD16 (NK-cells), CD64 (monocytes and macrophages) and CD89 (granulocytes). BsAbs

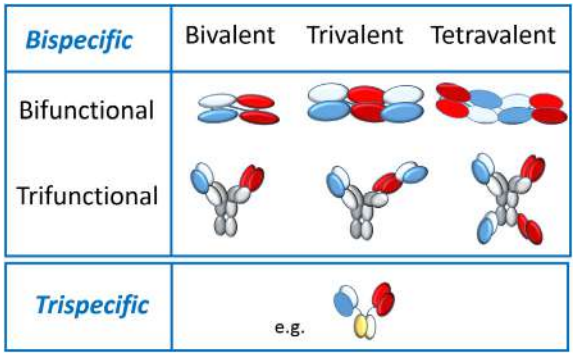


**Fig. 2.** Nomenclature of bi- or multispecific antibody constructs.

can also neutralize or activate receptors or their ligands (e.g. Crossmabs, DVD Ig). These constructs can be applied to cancer, but also to inflammatory or autoimmune disease (review in [15]). BsAbs can force the association of proteins or enzymes, which is the principle of emizicumab, recently approved by the FDA for the treatment of hemophilia A with acquired inhibitors.

Additional differences and characteristics of the "zoo" of bispecifics are explained by the challenge in manufacturing, e.g. to prevent the mispairing of heavy or light chains. There are several excellent technical reviews on this issue [12–16].

## Blinatumomab

Blinatumomab is the first FDA and EMA approved bispecific construct for the treatment of relapsed and refractory (r/r) ALL. It is a small (55 kDa) single chain peptide connecting two variable antibody fragments directed against CD3 and CD19 [10]. Blinatumomab induces the formation of a cytolytic synapsis and activates T-cells without costimulatory molecules. There is a continuous recharging of granzymes resulting in a continuous attack of tumor cells without anergy or T-cell apoptosis [17]. Blinatumomab leads to an expansion of CD8 positive T-cells, dominated by cytotoxic CD8+ T effector memory (TEM) [18].

A major drawback is the short half-life requiring a continuous intravenous infusion and a port system over several weeks. Patients with ALL receive up to 5 cycles of a 4-week infusion with an intermission of two weeks. Patients with Non-Hodgkin lymphoma (NHL) were treated in clinical trials over 8 weeks, followed by an additional cycle of 4 weeks in responding patients. On the other hand, the short half-life may have some advantages. Severe side effects are manageable by

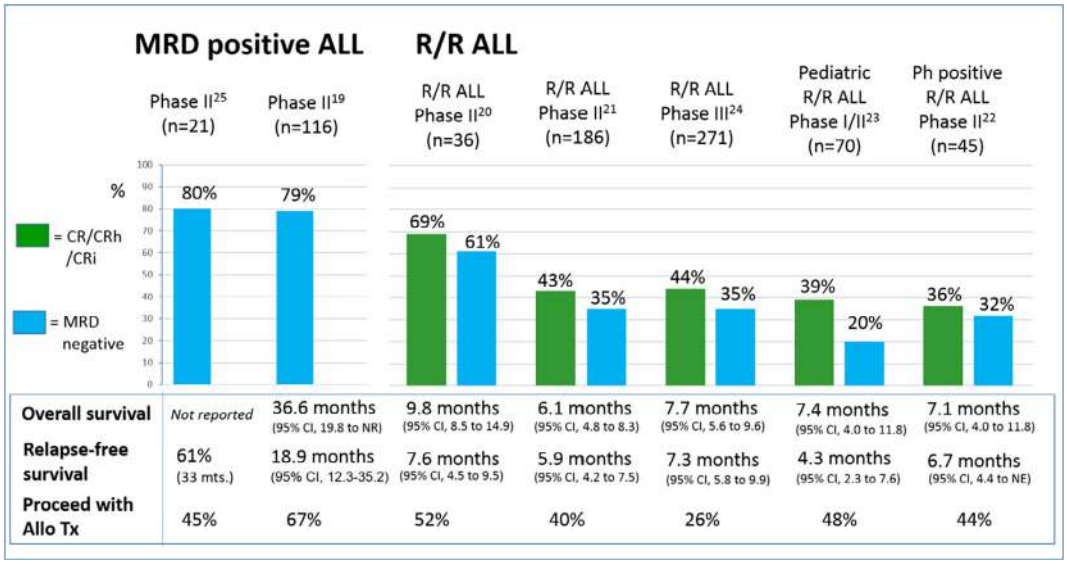


**Fig. 3.** Overview of results in clinical trials with blinatumomab in ALL (R/R: relapsed or refractory, CR: complete remission; CRh: complete remission with partial haematologic recovery, CRi: complete remission with incomplete recovery, Allo Tx: allogeneic stem cell transplantation, 95%-CI: 95% confidence interval).

stopping the infusion and are usually reversible within a few hours. The bioavailability of blinatumomab might allow a subcutaneous administration, which is currently being tested in a phase-Ib trial (NCT02961881).

## Blinatumomab in ALL

In ALL, several phase-II trials were realized in the setting of minimal residual disease positive (MRD+) ALL [18,19], in refractory and relapsed (r/r) Philadelphia (Ph) negative ALL [20,21], in refractory and relapsed Philadelphia positive (Ph+) ALL [22] and in pediatric ALL [23]. At the time of this review, blinatumomab is approved in r/r Ph-negative ALL (FDA and EMA), in Ph+ ALL (FDA) and in pediatric ALL (FDA). Additionally, there is a randomized phase-III trial in ALL comparing blinatumomab with "standard of care" chemotherapy [24]. Results are listed in Fig. 3.

Blinatumomab was started with 15 μg/m$^2$/d (or using a flat dose of 28 μg/d in the more recent generation of trials) in MRD+ ALL patients [18,19]. In r/r ALL, the stepwise dose escalation from 5 to 15 μg/m2/d (or flat dose from 9 to 28 μg/d) was associated with less adverse events and was recommended for further clinical trials [20]. A similar observation came from the pediatric ALL phase I/II trial, in which the immediate start with 15 μg/m/d or 30 μg/m$^2$/d was associated with dose limiting toxicities (DLT) and even with fatal DLTs [23].

In summary, blinatumomab is able to achieve a complete molecular remission in MRD positive ALL in 80% or 79% of cases [18,19]. In r/r ALL, the rate of CR/CRh/CRi (complete remission/with partial haematologic recovery/with incomplete haematologic recovery) was 43% or 44% [20,21]. In the first phase-II trial in patients with MRD+ ALL, the relapse free survival (RFS) of all patients was 61% after a median follow-up of 33 months [25]. The RFS was also 60% after 31 months in 11 patients who received no subsequent allogeneic haematopoietic stem cell transplantation (HSCT). In a confirmatory phase-II trial, the median RFS of 110 evaluable patients was 18.9 months without significant differences in patients without subsequent allogeneic HSCT [19]. In the first phase-II trial in r/r ALL, 10 out of 33 patients achieved long-term complete remissions, 3 out of 10 without additional HSCT [26]. Long-term remissions seem possible particularly in patients with MRD or low tumor burden. The correlation between tumor burden and response could be demonstrated in a larger phase-II trial with 189 patients: in patients with less than 50% bone-marrow blasts at baseline, CR/CRh/CRi occurred in 73% of patients, whereas it was 20% in patients with higher bone-marrow blasts [21]. Similar (but significant) results were obtained in the pediatric ALL trial with a difference between 56% and 33%, respectively [23].

In the randomized TOWER trial [24] for patients with r/r P h-negative ALL, the median overall survival was almost doubled in contrast to the "standard of care" treatment (7.7 vs. 4.0 months; HR 0.71, 95%-CI 0.55–0.93). However, less than 30% of patients were alive after 12 months in both treatment arms. The contrast between the high MRD clearance in the MRD setting and the long-term survival even in patients without allogeneic HSCT suggests that blinatumomab might work better as a consolidation than as an induction or debulking. Clinical trials replacing conventional chemotherapy with blinatumomab as consolidation in first line treatment of ALL are ongoing (e.g. NCT02003222). Furthermore, combination strategies may improve the results.

In a phase-II trial with 45 patients with Ph+ ALL relapsed or refractory to a second or later generation tyrosinkinase inhibitor (TKI) [22], the response rate was relatively low (36%). However, it is obvious to improve the results of the monotherapy by combining blinatumomab and a second generation TKI. There is a case report [27] of 12 patients with r/r or MRD+ PH+ ALL after allogeneic HSCT. The haematologic response rate of the combination with ponatinib, dasatinib or bosutinib was 50% (3 out of 6). The MRD rate in all 12 patients was 75% and the 1-year overall survival was 73%. These data suggest safety and efficacy of a combination of TKI and blinatumomab. Ongoing trials will test the combination of blinatumomab and ponatinib in the first-line treatment of Ph+ ALL (NCT03263572).

## Blinatumomab in Non-Hodgkin lymphoma

In Non-Hodgkin lymphoma (NHL), several dosages and dose steps were evaluated in a multi-cohort phase-I trial [28]. This trial included seven dose levels (range form 0.5–90 μg/m$^2$/day) as continuous intravenous infusion over 4 or 8 weeks using an implanted port and an ambulatory pump. 60 μg/m$^2$/day was established as the maximum tolerated dosage. To avoid treatment discontinuation due to side effects, a stepwise dose escalation beginning with 5 μg/m$^2$/day (flat dose 9 μg/day in subsequent trials) over one week, followed by 15 μg/m$^2$/day (flat dose 28 μg/day) over one week, followed by the target dose of 60 μg/m$^2$/day (flat dose 112 μg/day) for up to 6 weeks was established.

Among patients treated at the target dose of 60 μg/m$^2$/day (n = 35), the overall response rate was 69% across NHL subtypes: 80%

for follicular lymphoma (FL; n = 15), 72% for mantle cell lymphoma (MCL; n = 7) and 55% for diffuse large B-cell lymphoma (DLBCL; n = 11). The complete response rates were remarkably high (FL: 40%, MCL 43%, DLBCL 36%). At the data-cut of this manuscript, the median response duration was 404 days (95% CI, 207–1129 days). In 2015, researchers from Wuerzburg reported a follow-up [29] on study patients treated at their site. In 22 patients treated with the target dose, the median overall survival was 60 months, the median progression-free survival 16 months and treatment-free survival was 25 months.

A phase-II study [30] evaluated stepwise (9–28–112 µg/d with weekly dose increases; n = 23) or flat (112 µg/d; n = 2) dosing of blinatumomab by continuous infusion in patients with relapsed/refractory DLBCL. The flat dose cohort was stopped due to neurologic events in both patient and the stepwise dose escalation was used in an extension cohort. Among 21 evaluable patients, the overall response rate after one blinatumomab cycle was 43%, including CRs in 19%. Three patients had late CR in follow-up without another treatment. Eight out of 25 patients (32%) in this trial did not reach the target dose of 112 µg/d for at least one week, which is the precondition for response. The reason for early continuation was rapid tumor progression (n = 4) and discontinuation due to side effects (n = 4). Mitigation of side effects and early achievement of the target dose should be adressed in the next study generation to increase the response rate.

By analogy to the experience in ALL, more recent trials apply blinatumomab as an consolidation after debulking, e.g. in the first-line treatment of high-risk DLBCL (NCT03023878) or after HSCT (NCT03072771; NCT03298412).

## Neurotoxicity and cytokine release syndrome

Administration of blinatumomab is frequently associated with adverse events, leading to permanent discontinuation in a significant proportion of patients. The most relevant side effects are neurotoxicity and cytokine release syndrome (CRS).

The mechanism of neurological toxicity of blinatumomab is not well understood. However, neurotoxicity occurs in trials with alternative CD19-bsAbs and with CD19 directed CAR T-cells. The extent of neurologic events in blinatumomab is dose-dependent; neurotoxic side effects were dose limiting at the dose level of 90 µg/m$^2$in the phase-I trial in lymphoma [28]. Neurotoxicity is more frequent in lymphoma trials (22–26% Grade 3–4 adverse events) than in leukemia trials (9–19% Grade 3–4 adverse events; see Fig. 4a), most likely due to the fourfold higher dosage in lymphoma. Neurologic symptoms are usually reversible after stopping the infusion and susceptible to dexamethasone. Stepwise dose escalation and prophylactic use of dexamethasone can mitigate or prevent the symptoms. Neurologic events may be infrequent in bsAbs with other antigen than CD19. Interestingly, chimeric antigen receptor transfected T-cells (CAR T-cells) against CD19 can induce comparable neurotoxic side effects.

Neurological side effects may be caused by an inflammatory irritation at the neuroendothelium through activated T-cells that locally release neurotoxic cytokines and chemokines on their way into the CNS. Peripheral B-cell may have a protective role since they function as a CD19 positive target cell causing a cytokine release of the T-cells before their entry into the CNS. This is in line with the observation from the phase-I trial in NHL, that a lower ratio between B- and T-cells was associated with a lower incidence of neurologic events [28]. Three patients from this trial received the heparin-like agent Pentosanpolysulfate (PPS) and had no interruption due to neurologic side effects [28]. PPS is a P-selectin antagonist, can decrease the blinatumomab-induced adhesion of circulating T-cells to blood vessel endothelium, and therefore can interfere with T-cell migration from the blood into the brain. A potential value of PPS in neuromitigation has to be analyzed in future trials.

CRS occurs due to high-level immune activation in several modalities of cancer immunotherapy, particularly in CAR T-cells. Grade 3 or 4 CRS occurs more frequently in the leukemia trials (2–6%), whereas they are rare in MRD + ALL and lymphoma trials (0–2%; see Fig. 4b). Mild infusions reactions including fever and chills are frequent (76% in the phase-I lymphoma trial [28]) and transient during the continuous infusion. The majority of these events occurred within 72 h of treatment initiation or dose escalation. Stepwise dose escalation and prophylactic dexamethasone can mitigate the symptoms. In contrast to CAR T-cells, an interruption of the infusion is effective to stop severe side-effects within a short time. Tocilizumab, an interleukin-6 receptor antagonist, approved for treatment of severe or life-threatening cytokine release syndrome induced by CAR-T-cell therapy is also effective for the treatment of blinatumomab-induced CRS [31].

## Mechanisms of resistance in blinatumomab

In the first phase-II study in r/r ALL, three out of 10 relapsed patients had a secondary loss of CD19 and additional three patients had an extramedullary involvement [20]. With increasing data on relapsed patients, the secondary CD19 loss seems to be a rare event, between 3% and 8% of all relapsed patients [32,33]. In CAR T-cells therapies, the incidence of CD19 loss is obviously higher (review by [34]). The CD19 loss in blinatumomab is often an isolated event without changing the biology of the disease [35]. Comparable to the experience in CD19-directed CAR T-cells, the CD19 loss may be associated with a myeloid

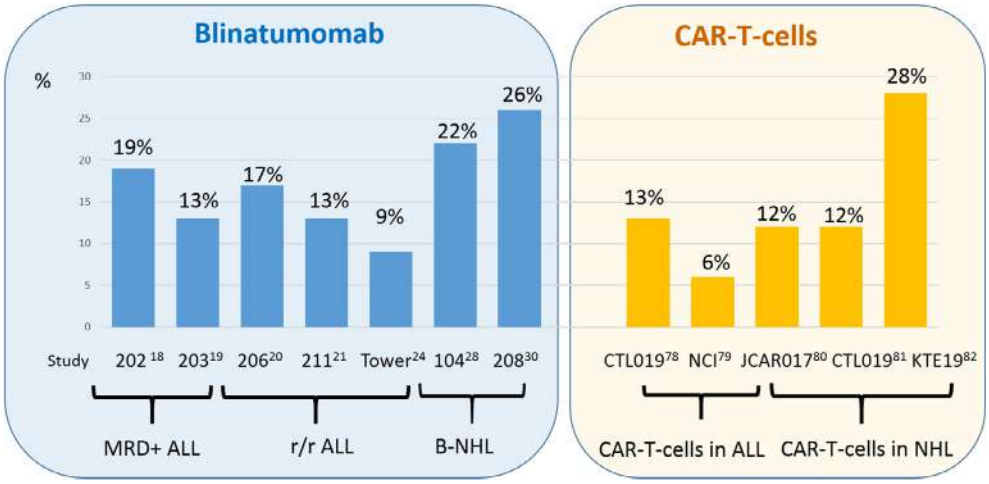


**Fig. 4a.** Overview about grade ≥ 3 neurotoxicity in blinatumomab trials; compared with results in CD19 directed CAR-T-cell trials (MRD: minimal residual disease; r/r relapsed/refractory).

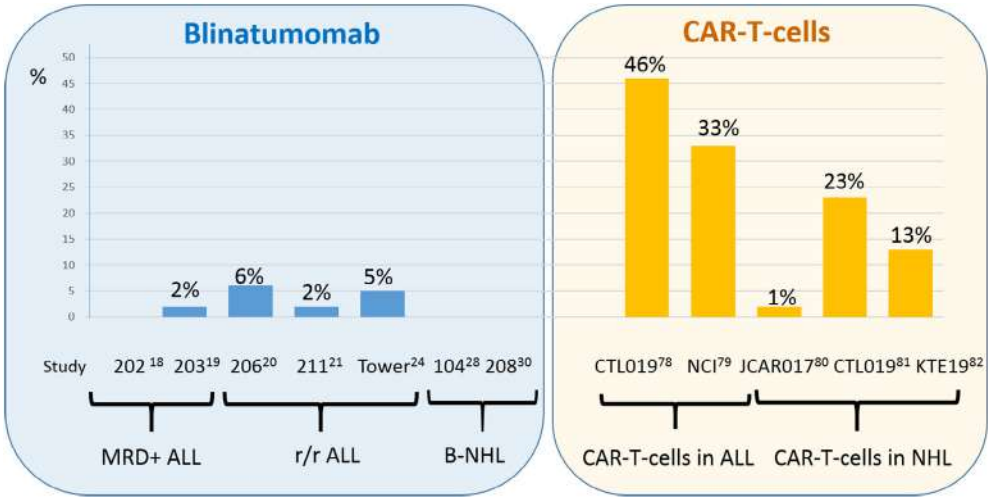


**Fig. 4b.** Overview about grade ≥ 3 Cytokine release syndrome in blinatumomab trials, compared with results in CD19 directed CAR-T-cell trials (MRD: minimal residual disease; r/r relapsed/refractory).

**Table 1**
BsAbs in clinical trials for haematologic malignancies.

| Specifity | Construct | Platform | Indication | clinicaltrials.gov identifier |
|---|---|---|---|---|
| CD19 × CD3 | Blinatumomab | BITE | ALL, NHL (phase I-III) | Multiple trials |
| | AFM11 | TandAb (Affimed) | ALL (Phase-I) | NCT02106091 NCT02848911 |
| | MGD011 | DART (Macrogenics) | NHL (Phase-I) | NCT02454270 |
| | | | NHL (Phase I) | |
| CD20 × CD3 | FBTA05 (Lymphomun) | Triomab (Trion/Fresenius) | NHL, after HSCT (Phase-I) | NCT01138579 |
| | CD20Bi | Chemically combined | NHL, after HSCT (Phase-I) | NCT00244946 |
| | RGN1979 | Heterodimeric (Regeneron) | Myeloma, after HSCT (Phase-I) | NCT00938626 |
| | RG6026 (RO7082859) | Knobs into hole (Roche/Genentech) Knobs into hole (Roche/ | NHL, CLL (Phase-I) | NCT02290951 |
| | RG7828 (BTCT4465A) | Genentech) | NHL (with Obinutuzumab, Phase-I) | NCT03075696 |
| | | | NHL, CLL (with Atezulizumab, Phase-I) | NCT02500407 |
| BCMA × CD3 | AMG 420 | BITE (Amgen) | Myeloma (Phase-I) | NCT02514239 |
| | PF-06863135 | (Pfizer) | Myeloma (Phase-I) | NCT03269136 |
| | JNJ64007957 | Duobody (Genmab) | Myeloma (Phase-I) | NCT03145181 |
| TCRH5 × CD3 | BFCR4350A (RG6106) | | Myeloma (Phase-I) | NCT03275103 |
| CD33 × CD3 | AMG330 | BiTE (Amgen) | AML (phase-I) | NCT02520427 |
| CD123 × CD3 | Flotetuzumab | DART (Macrogenics) | AML (phase-I) | NCT02152956 |
| | JNJ-63709178 | Duobody (Genmab/Janssen)) | AML (phase-I) | NCT02715011 |
| CD30 × CD16 | AFM13 | TandAb (Affimed) | Hodgkin (phase-I) | NCT02321592 |
| | | | Cutaneous CD30 + lymphoma (phase-I) | NCT03192202 |
| | | | Hodgkin (+ pembrolizumab, phase-I) | NCT02665650 |

shift with increased resistance particularly (1) in patients with KMT2A (formerly MLL) positive ALL [36,37] and (2) in patients with Philadelphia positive ALL [38] with regard to the hematopoietic stem cell involvement of these translocations. The low incidence of CD19 loss encouraged the use of alternative CD19 redirected cellular therapies in patients after blinatumomab failure.

Extramedullary involvement may be the most important reason for relapses. In a retrospective series of 65 patients [32], 41% of the blinatumomab-refractory patients progressed within an extramedullary lesion. Forty percent of the patients, who relapsed after initial response, had an extramedullary relapse. The history of extramedullary relapse was a strong prognostic factor for not achieving a complete remission and for an extramedullary relapse. The most common sites of extramedullary relapse in this report were lymph node, kidney and spleen whereas CNS relapses were infrequent possibly due to intrathecal prophylaxis usually continued during blinatumomab treatment.

A third potential mechanism for blinatumomab resistance may be the secondary overexpression of PD-L1 [39], a transmembrane molecule that plays a major role in cancer immunity. PD-L1 was overexpressed in primary ALL cells of non-responders compared to responders and particularly in tumor cells of five patients refractory to blinatumomab [39]. A 12-years old patient refractory to blinatumomab achieved a CR after the combination of the anti-PD-L1 antibody pembrolizumab and blinatumomab [40] Clinical trials with pembrolizumab and blinatumomab are ongoing in leukemia (NCT03160079) and lymphoma (NCT03340766). Further immunomodulating partners in combination trials are lenalidomide in NHL (NCT02568553), nivolumab ± Ipilimumab in ALL (NCT02879695) and ibrutinib in ALL (NCT02997761).

The relevance of suppressive effects of the microenvironment is demonstrated by the fact that a low pre-therapeutic count of regulatory T-cells (Tregs) can predict the outcome of blinatumomab in r/r ALL [41]. This observation may not only provide a biomarker for patients with a low chance for response, but had also clinical implications: decreasing Tregs before the administration of blinatumomab can augment the efficacy. Theoretically, a pre-treatment with cyclophosphamide and/or fludarabine or a simultaneous or sequential treatment with checkpointinhibitors may reduce Tregs.

Despite these potential mechanisms of resistance, the complete response rate in re-treatment of initially responding patients is comparable to the results of the first blinatumomab treatment (36%) [42]. This suggests that re-treatment with blinatumomab but also other CD19

directed treatments seem reasonable in selected patients.

## New constructs in NHL: CD19 and CD20

Based on the success of blinatumomab and CD19 directed CAR T-cells, alternative CD19 × CD3 antibodies have been developed with improved half-life and receptor affinity (overview in Table 1). AFM11 [43] is a bispecific but tetravalent construct (TandAb™), which has a half-life of 20 h. An additional advantage may be a higher CD3 affinity providing a lower effector to target ratio. AFM11 entered a phase-I program in lymphoma (NCT02106091) and ALL (NCT02848911).

Duvortuxizumab (MGD011) is a bispecific and bivalent CD19 and CD3 DART™ compound [44] with an extended half-life (340–460 h) by fixing a heavy chain compound to the molecule. An ongoing phase-I trial had to be terminated due to neurotoxicity.

Anti-CD20 bsAbs are a worthwhile alternative in mature B-cell neoplasia like NHL with the potential to avoid the neurologic side effects. There are at least two chemically coupled constructs in clinical trials. One of the first CD20 × CD3 bsAb was FBTA05 (Lymphomun™), a trifunctional chimeric rat/mouse antibody construct and therefore highly immunogenic [45]. FBTA05 was tested in a clinical trial after allogeneic stem cell transplant and donor lymphocyte infusion [46] (NCT01138579; no results published). There are reports about the compassionate use of FBTA05 in pediatric ALL [47] and NHL patients before and after allogeneic HSCT or in a pediatric patient with post-transplant lymphoma [48].

Another compound with a slightly different application is CD20Bi, which is co-cultured with T-cells from patients to activate T-cells and "arm" the T-cells with the CD20 antibodies. There are two reports [49,50] with twelve patients and three patients receiving CD20Bi prepared autologous activated T-cells without severe side effects, but with a slightly delayed engraftment. There are no ongoing clinical trials with both compounds.

RGN1979 is a full-length CD20x CD3 antibody with altered Fc binding function. In a phase-I trial [51,52], RGN1979 was administered weekly for four doses followed by a 4-weekly basis. The most common adverse events were infusion reactions, fever and chills, including CRS grade ≥ 3 in 6%. There were no neurologic events. At dose levels of 5–7 mg REGN1979, the preliminary overall response rate across different NHL subtypes was 45%. There is an additional report [53] on the combination of REGN1979 and a PD-L1 inhibitor (REGN2810) in 10 patients with NHL, demonstrating a higher cytokine release but only minor clinical responses.

There are several new CD20xCD3 bsAbs starting in clinical trials [54,55]. Two constructs - RG6026 (RO7082859) and RG7828 (BTCT4465A; Genentech, Roche) - are full-length antibody with two domains for CD20 and a single domain for CD3 ("2:1") enhancing the activity to CD20 in contrast to bivalent antibodies [55]. Both compounds are entering clinical trials, e.g. a phase-I trial with Obinutuzumab (with NCT03075696) or without atezolizumab in NHL and chronic lymphocytic leukemia (NCT02500407).

## New constructs in multiple myeloma

Since precursors of Multiple Myeloma (MM) have a B-cell origin, CD19-directed constructs like blinatumomab (NCT03173430) and even CD20-directed constructs (NCT00938626) are under clinical investigation. There is already a case report of a patient with coexistent MM and ALL who achieved a very good partial response (VGPR) of MM to blinatumomab, actually initiated for ALL treatment [56].

The B-cell maturation antigen (BCMA) is an attractive target in MM, since it is more selectively expressed on myeloma cells in contrast to other antigens (CD38, CD138). BCMA is also used as target in a recent CAR T-cell trial with encouraging data [57]. An alternative BCMA-bsAB, AMG420 (BI 836909), uses the BITE platform [58] (phase-I trial in "last-line" myeloma patients: NCT02514239). EM801 [59] is a trivalent "2:1" full-length antibody which binds bivalently to BCMA and monovalently to CD3ε. Further BCMA constructs in clinical trials are PF-06863135 (Pfizer, NCT03269136) and JNJ64007957 (Genmab, NCT03145181).

CD38 is an alternative target, which is highly expressed in myeloma cells. The "naked" CD38 antibody daratumumab is effective in r/r MM patients. There are several constructs under preclinical evaluation [60–62]. AMG 424 [60] (Amgen) is an anti-CD3/CD38 BITE construct containing modified Fc domain.

A second BITE construct is directed against the tumor-associated antigen Fc receptor-like protein 5 (TCRH5) which is overexpressed in myeloma cells: BFCR4350A (RG6106; N CT03275103).

## New constructs in acute myeloid leukemia

In acute myeloid leukemia (AML), several bsAbs have been developed (review by [63]). Potential targets are CD33, CD123, CLL1, CD45, CD46 and Anti-IL1RAP. Since neither of these targets are highly selective to leukemic cells, potential side effects like cytokine release syndrome are possible. The FDA placed a temporary hold on two of three ongoing clinical trials due to adverse effects.

The CD33 antigen was one of the first targets, which was therapeutically addressed by monoclonal antibodies. However, it is not expressed on all myeloid blasts. It can be expressed on activated T- or NK-cells and can interfere with the activation of effector cells. A phase-I trial with a CD3 × CD30 BITE™ (NCT02520427) is still ongoing.

CD123 (also known as Interleukin-3A receptor alpha chain) is an alternative antigen present in the majority of myeloid blasts, but lower in normal hematopoietic effector cells. In contrast to CD123, it is not present in effector cells. There are two constructs in clinical trials: the DART construct flotetuzumab [64] and the duobody JNJ-63709178. In a recent phase-I trial [64], 45 patients with AML and high-risk myelodysplastic syndrome (MDS) received flotetuzumab. The main side effects were infusion-related reactions and cytokine release syndrome (grade ≥ 3 13%). In 14 patients with a dose level of ≥500 ng/kg/day, six patients responded according to the IWG criteria (3 CR and 1 CRi).

## New constructs in Hodgkin lymphoma

In Hodgkin lymphoma, bispecific CD30 antibodies have a long history. In contrast to B-cell malignancies, antibody constructs activate compounds of the innate immune system [65]. The first CD30 × CD16 antibody was tested in patients in the late 90s with a moderate response rate (1 CR and 1 PR in out of 15 patients) [6]. In 2001, a bispecific CD30 × CD64 (recruiting monocytes and macrophages) showed one CR and four PRs in 10 patients without severe side effects [66]. Despite these early encouraging signals, development was stopped due to manufacturing issues. AFM13 is a tetravalent bsAb (TandAb™) activating NK cells. In a phase-I trial [67] in patients with r/r Hodgkin lymphoma, 3 out of 26 patients (11.5%) achieved a partial response and 13 patients (50%) a stable disease. In patients with a dose ≥ 1.5 mg/kg, the partial response was 23%. AFM13 was well tolerated with grade 3 adverse events in only 9% and without neurologic side effects. AFM13 will be tested in a larger ongoing phase-II trial in Hodgkin lymphoma (NCT02321592), in a trial with cutaneous CD30-positive lymphoma (NCT03192202) and in combination with pembrolizumab (NCT02665650).

## New developments

In contrast to the "living" and self-expanding CAR T-cells, bsAbs have the handicap of short persistence in the patient and the low target to effector ratio in heavily immunosuppressed patients. A merging of both principles may be the modification of immune or tumor cells to permanently express bispecific molecules [68]. There are at least four concepts, which are under preclinical evaluation: (a) modified oncolytic

viruses, (b). transfected T-cells, (c) transfected mesenchymal stem cells (MSC) and (d) non-viral cDNA vectors. In a, b and c, the bispecific molecule will be released locally in the cancer tissue.

One example is the genetic modification of an oncolytic virus to express a bispecific molecule during infection of a cancer cell. The virus preferentially infects cancer cells followed by releasing progeny virus into the environment. During the virus cycle, the virus produce BiTE molecules inducing a T-cell response in the cancer proximity. Examples are the Adenovirus EnAdenotucirev [69] expressing an EPCAM × CD3 BiTE or the adenovirus ICOVIR-15 K [70] expressing EGFR × CD3. Since bsAbs have a poor penetration in solid cancers, this principle seems to be particularly attractive in this field.

Transfected autologous T-cells expressing BiTEs or other bispecific constructs are the closest link between CAR T-cells and bsAbs. There are at least two constructs developed for haematologic malignancies. A CD3 × CD123-BiTE [71] - so called CD123-ENG T-cells – was preclinically tested in mouse models. Since targeting CD123 may affect hematopoietic stem cells, a suicide gene with CD20 was introduced to eliminate the 123-ENG T-cells by rituximab. From the same group, modified CD19-ENG T cells [72] were used in mouse models for human B-cell lymphoma expressing a CD19 × CD3 BiTE molecule.

MSCs have limited immunogenicity and maybe an attractive tool for an “off the shelf” adoptive cell therapy. MSCs usually accumulate next to tumors, and may influence the suppressive tumor environment. There are, for example, preclinical data on the use of an immortalized human MSC line which was genetically modified to express a CD33x CD3 construct [73], or on the use of umbilical cord-derived MSC secreting the CD19 × CD3 TandAb [74].

An alternative approach is the injection of non-viral cDNA minicircles encoding a CD20 × CD3 antibody construct [75]. After injection into mouse liver, there was a constant expression of a therapeutic level of anti-CD3/CD20 and with anticancer activity in a NHL xenograft mouse model.

Due to the scarcity of targetable antigens, there are attempts to construct compounds with more than one target specificity. For example, there is a preclinical report of a trispecific killer engager (so called TRIFLEX™ by Affimed), combining CD16 (NK-cell activation) with BCMA and CD200 [76]. More than for recruitment of immune cells, tri- and tetraspecific antibodies were used for receptor crosstalk, e.g. in HIV or in breast cancer [77].

## Future directions

Effective targeting of cancer cells, selection of the optimal tumor antigen, stimulation of effector cells without an overshooting immune response, overcoming the immunosuppressive environment, stability and comfortable application is still challenging after more than 40 years of research and development in bsAbs. With the first approvals of CAR T-cells [78–82], the contest of the best way of targeted immunotherapy is still open. Looking to the available response data of the recently approved CAR T-cells in aggressive NHL and pediatric ALL, there seems to be a competitive edge for CAR T-cells. However, the immediate availability, the ability to control side effects by stopping the drug and the absence of possible long-term effects in a non-gene therapeutic approach, are in favor for further development of bsAbs. Based on the success of this technology, there is the potential of breakthroughs, which might change the practice not only in haematologic and solid cancer, but also in a broad spectrum of internal diseases.

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