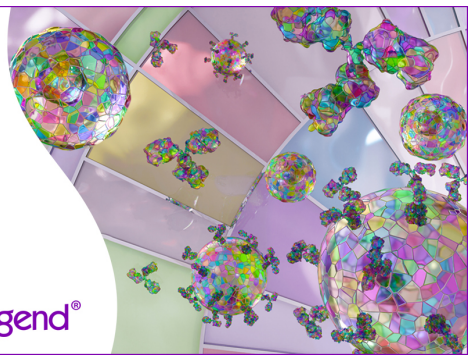

The Journal of Immunology
BRIEF REPORT | AUGUST 01 2019

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J Immunol (2019) 203 (3): 585–592.
https://doi.org/10.4049/jimmunol.1900496

Translating Immunology

The Journal of Immunology

T Cell–Activating Bispecific Antibodies in Cancer Therapy

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Effector lymphocytes are multifunctional cells of the immune system that promote cytolysis of pathogen-infected cells and nascent tumors. Tumors must learn to evade effectors and employ a wide variety of mechanisms to do so. Bispecific Abs (BsAbs) are an emerging cancer immunotherapy approach seeking to re-engage either T effectors or NK cells with malignant cells. Possessing specificity for effector cells on one end and a tumor Ag on the other, these molecules work by attracting effectors to the target cell to build an immunologic synapse and induce tumor cell killing. The BsAb blinatumomab, for example, has specificity for the T cell–activating cell surface protein CD3 and the B cell Ag CD19. The only BsAb with regulatory approval currently, blinatumomab is used in the treatment of relapsed or refractory B cell acute lymphoblastic leukemia. Many additional BsAbs are in preclinical development, however, targeting many different tumor types. The variety of potential effector cells and cancer Ags, along with potential combination therapies, make BsAbs an active area of drug development. In this review, we discuss cancer recognition by the immune system and structural and mechanistic aspects of BsAbs. We summarize key steps in preclinical development and subsequent translation to medical practice. Future directions for BsAbs include combinations with a wide variety of both immunologic and nonimmunologic therapies. Defining their optimum clinical use is at early stages. ***The Journal of Immunology*****, 2019, 203: 585–592.**

In the past 25 years, a variety of new approaches to cancer therapy have joined the traditional arsenal of surgical resection, chemotherapy, and irradiation. Options now include a diverse selection of small-molecule–targeted inhibitors, mAbs against tumor Ags with and without attached toxic cargoes, and several novel immunotherapies (1, 2). The latter category has gained particular traction in recent years. Successful development efforts include immune checkpoint inhibitors to counteract tumors' immune-inhibitory signals (3, 4), reprogramming of T cells to attack tumors with chimeric Ag receptors (CARs) (5, 6), and, finally, bispecific Abs (BsAbs) that promote immune synapse formation between immune effectors and malignant cells (7). Eshhar and colleagues (8, 9) pioneered the concept of T cell reactivation against tumors in the 1980s when they introduced the first CAR designs. These combine extracellular variable regions derived from mAbs to promote specific tumor antigen recognition, with intracellular constant regions (γ or ζ) of the TCR to promote immune activation. These early designs, despite shortcomings from lack of costimulatory signals, paved the way for redirecting T cells against tumor Ags. Modern CARs, with multiple options available now to promote costimulation, have won regulatory approvals to treat CD19-expressing B cell leukemias and lymphomas and are under investigation against a variety of additional targets (5, 6). These approaches share the goal of reengaging T effector lymphocytes with tumor cells. As mentioned, another successful approach has been the development of BsAbs, fusion proteins with specificity for two Ags functioning as activating magnets between effector and tumor cells (10, 11). Only one BsAb, blinatumomab, currently has regulatory approval for clinical use, but the established proof of principle is fueling extensive efforts to expand the approach to additional tumor and effector cell types. In this article, we review the long road from concept to clinical use of BsAbs and novel pharmaceuticals built on this concept at various stages of development.

Bispecific T cell engagers: structure and mechanism of action

T cell–activating BsAbs (TABs) consist of two single-chain variable fragments (scFvs) with binding specificities for different unique Ags (Fig. 1A). Each scFv is a H and L chain variable fragment, derived from a mAb against the desired target, joined with a Ser-Gly peptide linker, typically three or more SGGGG repeats (12). A similar SGGGG linker also holds the two scFvs together. These linkers provide flexibility to permit binding to target cells. The overall molecular mass is ∼55 kDa (13). These fused specificity fragments exclude additional Ab elements like Fc regions and, therefore, rely

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Received for publication May 6, 2019. Accepted for publication June 3, 2019.

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Abbreviations used in this article: AE, adverse event; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; B-ALL, B cell ALL; BCMA, B cell maturation Ag; BET, blinatumomab-expanded T cell; BsAb, bispecific Ab; CAR, chimeric Ag receptor; CI, confidence interval; CRS, cytokine release syndrome; DLBCL, diffuse large B cell lymphoma; EpCAM, epithelial cell adhesion molecule; MRD, minimal residual disease; PD-L1, programmed death ligand 1; r/r, relapsed/refractory; scFv, single-chain variable fragment; TAB, T cell–activating BsAb; Treg, regulatory T cell; TriKE, trispecific killer engager.

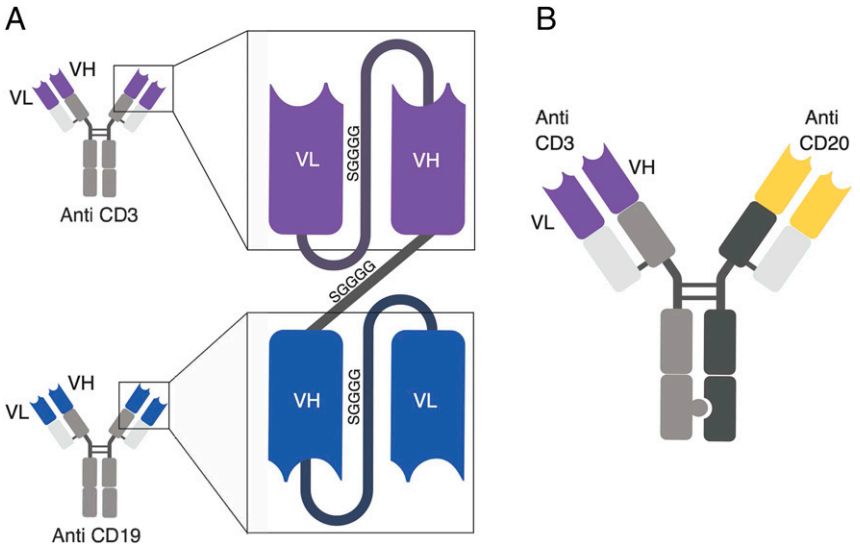

FIGURE 1. Blinatumomab (**A**), the only BsAb approved for clinical use currently, fuses scFvs against CD19 to target malignant B cells, and CD3, to engage T effector cells. Mosunetuzumab (**B**), currently in early-phase clinical evaluation, by contrast is a full-length Ab retaining its Fc tail domain. VH, variable H chain; VL, variable L chain.

exclusively on effector-tumor synapse formation and to some extent T cell expansion, rather than additional recruitments or induction of immune memory. Full-length, Fc-retaining BsAbs, such as the anti-CD20/CD3 molecule mosunetuzumab, currently in early-phase clinical evaluation, are also under development (Fig. 1B; discussed in more detail below).

The most common T effector–engaging scFv targets the CD3 subunit of the TCR, whereas the other scFv targets an Ag expressed on the cell surface of tumors, such as the B cell Ag CD19, the breast cancer receptor tyrosine kinase HER2, or the carcinoembryonic Ag (CEA) found on many gastrointestinal tumors (Fig. 2A) (14). The goal is to promote immune synapse formation between T effectors and tumors (Fig. 2B). Work by Brischwein et al. (15) established that the dual-binding capabilities of TABs are required for this to occur. Specifically, treatment with an epithelial cell adhesion molecule (EpCAM)–specific TAB promoted destruction of target tumor cells via T cell activation. When the same EpCAM-TAB was used on ligand-incompetent tumor cells, however, no T cell activation or other immune response occurred. Immune synapse formation promotes release of perforins and granzymes, which enter tumor cells and trigger apoptosis (16). Elegant work by Offner et al. (17) showed that in the presence of an EpCAM-TAB, synapses formed between ERBB2-specific, CD8-positive T cells and $EpCAM^+$ tumor cells. The requirement for dual-binding of the TAB allows it to maintain specificity for the desired Ag and avoid random T cell activation (18). TAB cytotoxicity effects are enriched by the ability of the molecule to induce proliferation and expansion of unbound T cells to the tumor, leading to a sustained antitumor effects (19, 20). In addition to TABs that target T cells, there is ongoing work attempting to apply the same principles to other immune effectors, most notably NK cells (21–23). Overall, TABs are promising therapeutic tools designed to exploit the intrinsic machinery of the immune system for therapeutic benefit.

Preclinical development

BsAbs have been under investigation in the preclinical literature for 30 years. Work by German scientists beginning in the mid-90s provided major breakthroughs on the road to blinatumomab, creating the initial fusion between CD3 and CD19 scFvs, establishing production with adequate yield as a single polypeptide, and demonstrating activation of unstimulated T cells against various human B cell lymphoma cell lines (12, 24). The effect was later replicated also against patient-derived B cell lymphomas (25). Dreier et al. (26) showed using a B cell lymphoma and human PBMC xenograft mouse model that administration of a CD3/CD19 TAB leads to suppressed tumor growth and increased survival rates. Hoffmann et al. (19) found that CD3/CD19 TAB administration recruited T cells for multiple rounds of target B cell lymphoma tumor elimination.

Multiple additional TAB targets and approaches also have undergone preclinical evaluation. A TAB combining anti-CD3 and an anti–prostate cancer stem cell scFv delayed the growth of xenografted tumors in vivo (27). Increased survival and decreased tumor burden also was observed in vivo in murine melanoma and ovarian cancer xenograft models treated with a TAB conjugated with CD3 and B7H6, a tumor cell surface ligand that specifically binds to NKp30, an NK cell–activating receptor (23).

Adding additional biologic components to the baseline BsAb concept opens a variety of new therapeutic possibilities. For example, Vallera at al. built on the TAB model with a unique trispecific killer engager (TriKE) molecule that directs NK cells to attack acute myeloid leukemia (AML) cells. The TriKE comprised scFvs for NK and AML cells plus IL-5 to enhance proliferation and priming of the NK cells (28). We explore some additional ideas that have emerged built on this concept below, but for a more complete discussion, we recommend the recent review by Suurs et al. (29).

BsAbs in the pipeline

Multiple BsAbs have undergone evaluation in clinical trials. They range from small conjugated specificity fragments to large IgG molecules that retain their Fc domains. These latter may lead to active immunization against targeted tumor Ags, promoting long-lasting antitumor responses, as in the case of catumaxomab, a rat/mouse hybrid BsAb that binds EpCAM and CD3 that was used for the treatment of malignant ascites (30). On the other end of the spectrum, single-chain BsAbs, such as blinatumomab, are much smaller and more pliable, promoting a more efficient cellular linking. In preclinical work, for example, blinatumomab was shown to be effective at

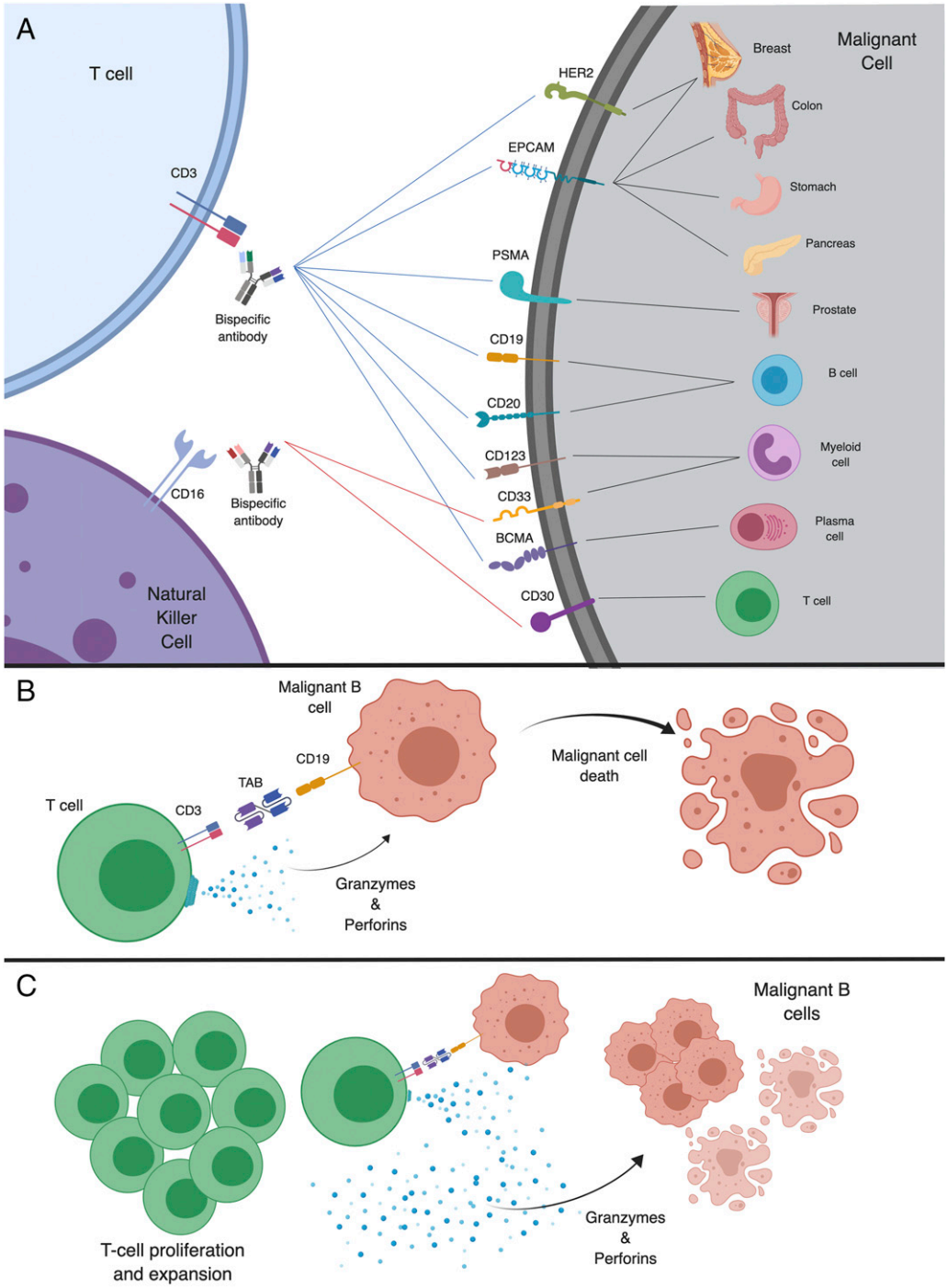

FIGURE 2. (**A**) Tumor Ags (right) under evaluation to promote engagement with either T effector cells via CD3 or NK cells via CD16 (left). CD19/CD3 BsAb (**B**) engages CD3 on T cells and CD19 on malignant B cells leading to synapse formation and release of cytokines, perforins, and granzymes, which causes malignant cell death. There is evidence that BsAbs also promote effector T cell proliferation (**C**). CD, cluster of differentiation; HER2, human epidermal growth factor receptor 2; PSMA, prostate-specific membrane Ag.

much lower effector:target ratios than prior studies with full-length BsAbs (31). Unlike Fc-containing BsAbs, however, single-chain BsAbs are rapidly cleared from circulation, requiring cumbersome continuous infusion (20). Building on the success of blinatumomab, efforts to design more efficacious, and practical compounds with longer half-lives are ongoing. REGN1979 is a hinge-stabilized, full-length Ab with CD20/CD3 bispecificity modified to reduce Fc binding. In a phase I dosage-escalation study, the overall response rate in B cell non–Hodgkin lymphoma patients across dosing levels was 20%, but higher for patients with follicular lymphoma. Grade 3 or higher toxicities occurred in 32% of patients, with infusion-related reactions being most common (32). A phase II clinical trial in relapsed/refractory (r/r) follicular lymphoma is planned.

Another B cell targeting BsAbs is mosunetuzumab, a humanized, full-length, Fc-containing BsAb for CD20/CD3 (Genentech, Roche) (Fig. 1B). It is a full-length IgG1 BsAb with near-native architecture using "knobs-into-holes" technology (14, 33). Multiple phase I/II trials are ongoing

testing BsAbs as monotherapies and in combination with other treatments (Tables I, II). In multiple myeloma, the B cell maturation Ag (BCMA) is among the most targeted Ags because of its high expression on tumor compared with nonmalignant cells (34–36). CAR-T cell therapy against BCMA, for example, has shown some promising early results (37). Other targets for multiple myeloma include CD38 and CD19 (38).

Whereas lymphoid tumors have been successfully targeted, myeloid malignancies have been more challenging. A number of studies are attempting to target CD123 (39, 40) in relapsed AML or myelodysplastic syndrome with a CD3/CD123 BsAb. CD123, the receptor for IL-3, is an attractive target in AML because of frequent expression on AML blasts. Aptevo Therapeutics developed APVO436, referred to as "ADAPTIR," a CD3/CD123 BsAb (41). With Ab-like half-life of 12.5 d due to its modified Fc portion, dosing schedule would be more convenient than scFv fusions like blinatumomab that require continuous infusion. Tagraxofusp is a Food and Drug Administration–approved, CD123-directed cytotoxin consisting of human IL-3 fused to truncated diphtheria toxin and is approved for treating blastic plasmacytoid dendritic cell neoplasm (42, 43). BsAbs with this clinically established target therefore might be a lower-risk approach. APVO436 and other CD123 targeting BsAbs have entered clinical evaluation.

Solid tumors have been even more challenging targets for BsAbs. Hurdles mainly involve safety, stemming from narrow therapeutic windows that have been attributed to relatively low specificity for target Ags (44). In addition, whereas the small size of single-chain BsAbs allows them higher tissue penetration, their short half-life renders them less effective in solid tumors. Efforts to conjugate those constructs and extend half-life are being pursued (45–47). In breast cancer, phase I evaluation of a HER2/CD3 BsAb showed antitumor activity in 5 of 15 patients (48). However, a phase II trial of the drug was terminated by the company to focus on catumaxomab, which targets EpCAM and CD3. This drug won approval by the European Medicines Agency to treat EpCAM0-positive malignant ascites (49), making it the only BsAb other than blinatumomab to gain regulatory approval, but it was withdrawn from the market for commercial reasons.

The BsAb MT110 has the same specificity targets (EpCAM and CD3) and is under evaluation in various epithelial malignancies, including colon, breast, lung, pancreatic, and ovarian. In phase I, however, dosage escalation, unfortunately, was not possible because of significant adverse events (AEs); 1 out of 65 patients had an unconfirmed partial response (44). This study highlights the importance of target selection. Although diarrhea was expected because of EpCAM expression throughout the gastrointestinal tract, its severity and limited response to interventions was unforeseen. This also highlights limitations of preclinical studies in NOD/SCID mice in accurately predicting human response and AEs (50, 51). MT111, a carcinoembryonic Ag (CEA)/CD3 BsAb, produced no objective responses among 39 patients enrolled in a phase I trial (52). In prostate cancer, prostate-specific membrane Ag (PSMA) is being tested in multiple phase I trials with different BsAbs, some with pending results (NCT01723475). Other solid tumor targets are also being tested in early-phase studies (Table I).

BsAbs aimed at NK cell activation also are in, or approaching, clinical evaluation. These typically employ the NK Ag CD16.

Table I. BsAbs undergoing single-agent clinical evaluation

Specificities (Target × Effector Cell)	Drug	Stage	Comments	Trial Identifier
CD19 × CD3	Blinatumomab[a]	Market and multiple phase II/III ongoing studies	Treatment of refractory/relapsed ALL and phase II for r/r NHL	NCT02811679
CD19 × CD3	AFM11	Phase I	NHL and ALL	NCT02106091; NCT02848911
CD20 × CD3	RG6026[a] and REGN1979[a]	Phase I	NHL and CLL	NCT02290951; NCT02290951
CD20 × CD3	Mosunetuzumab[a]	Phase I	NHL, CLL, and DLBCL	NCT02500407; NCT03677154
CLEC12A × CD3	MCLA-117[a]	Phase I	AML	NCT03038230
CD33 × CD3	AMsG 330, GEM333, and AMV564	Phase I	AML and MDS	NCT02520427; NCT03516760; NCT03516591
CD123 × CD3	MGD006, JNJ-63709178[a], and APVO436[a]	Phase I	AML	NCT02152956; NCT02715011; NCT03647800
BCMA × CD3	BI 836909, JNJ64007957[a], PF-06863135[a], and REGN5458	Phase I	Multiple myeloma	NCT02514239; NCT03145181; NCT03269136; NCT03761108
CD38 × CD3	GBR 1342[a]	Phase I	Multiple myeloma	NCT03309111
CD30 × CD16	AFM13	Phase II	Hodgkin lymphoma and cutaneous T cell lymphoma	NCT02321592; NCT03192202
HER2 × CD3	GBR 1302[a]	Phase I	HER2-positive solid tumors	NCT02829372
HER2 × CD137	PRS-343[a]	Phase I	HER2-positive solid tumors	NCT03330561
PSMA × CD3	AMG 160 and ES414[a]	Phase I	Prostate cancer	NCT03792841; NCT02262910
DLL3 × CD3	AMG 757	Phase I	Small-cell lung cancer	NCT03319940
NYESO1/LAGE-1A × CD3	IMCnyeso	Phase I	NYESO1 or LAGE-1A solid tumors	NCT03515551
SSTR2 × CD3	XmAb18087[a]	Phase I	Neuroendocrine tumors and gastrointestinal stromal tumors	NCT03411915
GPC3 × CD3	ERY974[a]	Phase I	GPC3-positive solid tumors	NCT02748837
Gp100 × CD3	IMCgp100	Phase I/II	Uveal melanoma	NCT02570308
GD2 × CD3	Hu3F8-BsAb[a] (GD2/CD3)	Phase I/II	Neuroblastoma, osteosarcoma, and other GD2-expressing solid tumors	NCT03860207

[a]Signifies BsAbs that contain Fc portion. Multiple additional trials with this agent are ongoing (see http://www.clinicaltrials.gov).

Table II. Selected clinical trials of BsAbs in combination with other therapies

Specificities (Target × Effector Cell)	Combination	Stage	Comments	Trial Identifier
BsAbs plus immune checkpoint inhibitors				
CD19 × CD3	Blinatumomab plus pembrolizumab	Phase I/II	r/r B-ALL and r/r DLBCL	NCT03160079; NCT03605589; NCT03340766 (KEYNOTE-348)
CD19 × CD3	Blinatumomab plus nivolumab$^{+/-}$ ipilimumab	Phase I	Poor-risk r/r B-ALL	NCT02879695
CD30 × CD16	AFM13 plus pembrolizmab	Phase I	Hodgkin lymphoma	NCT02665650
HER2 × CD137	PRS-343[a] plus atezolizumab	Phase I	HER2-positive solid tumors	NCT03650348
gpA33 × CD3	MGD007 plus MGA012[a] (anti–PD-1)	Phase I/II	r/r Colorectal cancer	NCT03531632
BsAbs plus stem cell transplant				
CD19 × CD3	Blinatumomab^{+} ASCT	Phase I	Multiple myeloma	NCT03173430
CD19 × CD3	Blinatumomab maintenance post-ASCT	Phase II	ALL, NHL, and DLBCL	NCT02807883; NCT03114865; NCT03072771
CD19 × CD3	BET	Phase I	Indolent NHL/CLL	NCT03823365
BsAbs plus other agents				
CD19 × CD3	Sequential dasatinib plus blinatumomab	Phase II	PH^{+} B-ALL	NCT02744768
CD19 × CD3	Ibrutinib plus blinatumomab	Phase II	r/r B-ALL	NCT02997761
CD19 × CD3	Inotuzumab ozogamicin plus blinatumomab	Phase II	New or r/r CD22^{+} B-ALL	NCT03739814
CD19 × CD3	Lenalidomide plus blinatumomab	Phase I	Relapsed NHL	NCT02568553
CD19 × CD3	Sequiential hyper-CVAD plus blinatumomab	Phase II	Frontline B-ALL	NCT02877303
CD19 × CD3	Ponatinib plus binatumomab	Phase II	r/r B-ALL	NCT03263572
CD20 × CD3	RO7082859[a] plus obinutuzumab	Phase I	r/r NHL	NCT03075696
CD20 × CD3	Polatuzumab plus mosunetuzumab	Phase I/II	DLBCL	NCT03677154
CD19 × CD3	Chemotherapy plus blinatumomab	Phase II	Newly diagnosed, high-risk DLBCL with first-line induction and consolidation in ALL	NCT03023878; NCT03541083

[a]BsAbs with Fc domains.

Rothe et al. (53) reported promising results with AFM13, a CD30/CD16A BsAb, in Hodgkin lymphoma, with an overall disease control rate of 61% in 26 evaluable patients. Phase II evaluation is underway.

BsAbs in clinical practice

In July 2014, the Food and Drug Administration granted breakthrough therapy designation to blinatumomab in adults with r/r Philadelphia chromosome-negative acute lymphoblastic leukemia (ALL) (54). Soon after, the drug received accelerated approval because of significant rates of objective responses in a disease with widely unmet medical need. Indeed, this was quickly expanded to a full approval for adults and children to treat r/r B cell precursor ALL Philadelphia chromosome negative or positive in July 2017 (7, 55, 56). B cell ALL (B-ALL) patients with minimal residual disease (MRD), defined as detectable leukemia cells by flow cytometry or PCR in the presence of hematologic complete remission, received blinatumomab by continuous IV infusion for a maximum of four 28-d cycles. After one cycle, 78% of patients achieved complete MRD response (95% confidence interval [CI], 69–85%). This was expanded upon in a large phase III trial, the TOWER study (7). Compared to standard-of-care chemotherapy, blinatumomab prolonged median survival of patients with r/r ALL from 4.0 to 7.7 mo with a hazard ratio of 0.71 (95% CI, 0.55–0.93; $p = 0.01$). Blinatumomab, therefore, is a new option in this heavily pretreated population.

Goebeler et al. evaluated blinatumomab for r/r non–Hodgkin lymphoma in a phase I dosage-escalation study. Among those patients reaching maximum-tolerated dosage, overall response rate was 69% across NHL subtypes and 55% for patients with diffuse large B cell lymphoma (DLBCL) (57). Median duration of response was reported at 404 d. Longer-term follow-up showed median overall and progression-free survival at 60 and 16 mo, respectively (58). At the time of this review, 40 studies are registered on http://www.clinicaltrials.gov and actively recruiting patients for evaluation of blinatumomab in first-line combination, consolidation, maintenance, and relapsed settings in ALL in addition to many other combinations in various B cell malignancies.

AEs of BsAb therapy

Because Ags targeted by BsAbs are also found on nonmalignant cells, one of the main challenges of this therapeutic approach is to avoid so-called on-target, off-tumor effects. In B cell malignancies, CD19 and CD20 are typically targeted. Not surprisingly, this leads to a drop in WBC counts and, more specifically, B cell aplasia. In this case, however, the target is dispensable, as patients can survive the temporary decline of B cells. Additional hematologic toxicities are less prominent, with leukopenia, neutropenia, and lymphopenia occurring in only 3, 18, and 1%, respectively, in the TOWER study (7). In an exploratory safety analysis performed by Stein et al. (59) with the TOWER study population, exposure-adjusted event rates of blinatumomab were reported to be lower than or equal to standard-of-care chemotherapy in all types of AEs except for cytokine release syndrome (CRS).

Indeed, CRS is characteristic of immunotherapy in general and requires prompt and experienced management. Manifestations of CRS can range from mild fatigue, fever, headache, and arthralgias to more severe and life-threatening problems, such as hypotension, vascular leakage, and circulatory collapse. As the name implies, massive amounts of cytokines are released because of the engagement of effector and target cells and overall activation of the tumor microenvironment. IFN-γ

from T cells and IL-6, IL-10, and TNF-α from macrophages seem to cooperate in promoting this reaction. IL-6 especially has been shown to play an integral role in both humans and mice (60, 61). For this potentially life-threatening syndrome, corticosteroids are given prior to blinatumomab, and the IL-6-targeting mAb tocilizumab is used if CRS occurs, often with great success. Although pretreatment with steroids might seem counterproductive when inducing T cell–mediated cytotoxicity, Aldoss et al. (62) found no correlation between steroid use and response in a retrospective study of 65 patients with r/r ALL.

Whereas the AEs of CRS seems to follow what we know about the biology of CD3 targeting and T cell activation, other side effects were unexpected and not predicted by results in preclinical models. Most prominent is neurotoxicity, which caught clinical investigators by surprise. Symptoms range from subtle personality changes, tremor, dizziness, confusion, and focal neurologic symptoms to more serious episodes of encephalopathy, ataxia, convulsions, and delirium. Grade 3 or higher neurotoxicity AEs occurs in roughly 10–20% of patients treated with blinatumomab (7, 57). The mechanism of this AE remains to be determined. It does not seem to be related to CNS involvement by disease, as those patients typically were excluded from clinical trials (54). One study suggests that variable CD19 expression in human brains might play a role (63), but this toxicity also was seen with mosunetuzumab targeting CD20 (64). Although the very short half-life of blinatumomab is a disadvantage requiring continuous infusion, it actually helps in mitigating side effects, as stopping the infusion typically reverses neurotoxicity within hours. In addition, grade 3 or higher neurologic events can be successfully managed with dexamethasone. Overall, ∼15% of patients experience temporary interruptions and 5% permanently discontinue the drug because of neurologic AEs (54).

Predictors of response and resistance

As with any cancer therapy, BsAbs lead to clinical responses in only a subset of patients. Overall ∼45% of patients respond to blinatumomab (54), and given the potentially severe toxicities, efforts have been made to identify predictive biomarkers. Wei et al. analyzed patients in the TOWER trial retrospectively and reported that higher frequency of $CD45^+CD3^+CD8^+$ cells was associated with higher rates of complete response (odds ratio, 1.39; 95% CI, 1.18–1.65). The opposite was true, and there was increased risk of death with higher counts of $CD45^+CD3^-CD19^+$ cells (hazard ratio, 1.19; 95% CI, 1.09–1.29) (65). Zugmaier et al. (66) studied the kinetics of T cells after blinatumomab infusion. Long-term survivors (MRD responders with overall survival $\geq$ 30 mo) had higher degree of total $CD3^+$ T cell and $CD3^+$ effector memory T cell expansion. Further studies are needed to understand if prior chemotherapy affects this expansion and subsequent response. In other studies, high tumor burden (>50% bone marrow blasts at baseline) and prior history of extramedullary ALL were associated with lower response to blinatumomab (54, 62). Aldoss et al., meanwhile, found no statistical difference in the prevalence of CRS between responders and nonresponders to blinatumomab, but, interestingly, CRS grade > I severity was associated with higher likelihood of response compared with CRS grade I or no CRS (33% versus 9%, respectively; $p = 0.03$). This study found no association between disease burden and response (62).

Relapse precluding consolidation with allogeneic hematopoietic stem cell transplant is unfortunately common among patients treated with blinatumomab. Mechanisms of resistance remain poorly understood for most patients other than 10–20% who present with CD19-negative relapses. Mechanisms of lost CD19 that have been described include disrupted membrane trafficking and export (67) and acquired mutations and alternative splicing (68). Another potential escape mechanism is programmed death ligand 1 (PD-L1) overexpression. Five nonresponder ALL patients had higher PD-L1 expression compared with responders in one study (69). Feucht et al. (70) reported an antileukemic effect of combining blinatumomab with the clinical anti–PD-L1 Ab pembrolizumab in a pediatric B-ALL patient who was refractory to blinatumomab monotherapy. Such findings have sparked a movement for clinical trials combining BsAbs with checkpoint inhibitors, some of which are summarized in Table III. Tumor microenvironment factors also are likely important in many cases. Regulatory T cells (Tregs) can predict resistance with higher levels correlating with poor response (62, 71). This is noteworthy because conditioning regimens can be given to deplete Tregs prior to administration of blinatumomab. One other noteworthy aspect of resistance is the immunogenicity of blinatumomab. Less than 2% of patients treated with blinatumomab tested positive for binding anti-blinatumomab Abs. Of those, seven out of nine had in vitro neutralizing activity. Clinical experience is lacking in other constructs with limited in human experience (72).

Combination therapies and future approaches with BsAbs

Although proof of principle is established for BsAbs' ability to promote T effector engagement with tumors leading to clinical benefit, their clinical utility remains limited by a number of factors. These include on-target, off-tumor effects, low effector to target cell ratio in heavily pretreated patients, and overall pharmacologic limitations. To increase efficacy of BsAbs, multiple phase I and II trials are underway assessing combinations with chemotherapy, immune checkpoint inhibitors, and other treatments (Table II).

To circumvent low effector/target ratios in heavily pretreated patients, Golay et al. created a novel method using blinatumomab-expanded T cells (BET) for adoptive therapy. Starting from only 10 ml peripheral blood, a mean of 515×10^6 $CD3^+$ T cells were expanded in 3 wk. The expanded T cells were mostly effector and central memory cells, whereas Th17 and Tregs were <1%. In DLBCL xenografts, BET plus blinatumomab showed significant antitumor therapeutic effect (73). This unique approach

Table III. Positive and negative predictors of response to blinatumomab and candidate mechanisms of resistance/relapse

Positive Predictors	Negative Predictors
$CD45^+CD3^+CD8^+$	History of EMD
Low Tregs at baseline	EMD at time of treatment
	>50% bone marrow blasts at baseline
	High Tregs in peripheral blood
Candidate mechanisms of resistance/relapse	
CD19 loss 10–20%	
Extramedullary relapse 41% (56)	
PD-L1 overexpression (60)	

aims at reinvigorating the immune system of patients who have received multiple line of bone marrow depleting chemotherapy agents. This is being translated to a phase I clinical trial in patients with indolent NHL and chronic lymphocytic leukemia (NCT03823365). Another study by Golay studied cord blood–derived, cytokine-induced cells combined with blinatumomab for $CD19^+$ tumors and also showed high efficacy in vitro and in vivo. These methods can theoretically be translated with other BsAbs and possibly become a fully off-the-shelf cell therapy after further tested in patients. Additional efforts to enhance the effector cell cytotoxic effects include 41BB BsAbs, which provide a costimulation signal (74) as well as trispecific approaches, which combine a bispecific molecule with a stimulatory cytokine. For example, CD16/IL-15/CD133 TriKEs for high-risk hematologic malignancies (75) are entering clinical evaluation (NCT03214666).

Conclusions

Harnessing the immune system's power to attack malignant cells is an established and evolving paradigm in the treatment of cancer. The success of blinatumomab and ongoing clinical and preclinical studies show the potential of BsAbs to accomplish this goal. Compared with CAR-T therapy, BsAbs for now appear inferior, but multiple approaches to enhance activities and limit toxicities are under exploration. These include efforts to improve tumor Ag selection, enhance delivery in vivo, and novel combinations with potentially synergistic therapies.

Acknowledgments

Figures were created with BioRender (http://biorender.com).

Disclosures

The authors have no financial conflicts of interest.

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