

Bispecific Antibodies in the Treatment of Hematologic Malignancies

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Monoclonal antibody therapies are an important approach for the treatment of hematologic malignancies, but typically show low single-agent activity. Bispecific antibodies, however, redirect immune cells to the tumor for subsequent lysis, and preclinical and accruing clinical data support single-agent efficacy of these agents in hematologic malignancies, presaging an exciting era in the development of novel bispecific formats. This review discusses recent developments in this area, highlighting the challenges in delivering effective immunotherapies for patients.

Bispecific antibodies are engineered to bind to two different antigens or two different epitopes on the same antigen,¹ allowing the construction of a wide range of diverse formats. They represent a fast-growing area of immunotherapy, with over 100 different bispecific antibody formats,² and new avenues for construction are constantly emerging. Bispecific antibodies may be used to link target cells with effector cells or bind two epitopes on the same cell to block more than one signaling pathway. Of the above approaches, redirecting the cytotoxic potential of immune effector cells in the destruction of tumor cells has been an important driver in the development of bispecific antibodies.

Bispecific antibody constructs that retarget immune cells to tumors link an antibody or antibody fragment specific for antigens on a tumor cell and an activating receptor on an effector cell, for example CD3 on T cells or CD16 on natural killer (NK) cells.³ It has been shown that bispecific antibodies can effectively redirect T cells to tumors in a nonmajor histocompatibility complex (MHC)-restricted manner, thus obviating the need for antigen recognition by the T-cell receptor, increasing the number of T cells available to recognize tumor cells of interest.⁴

At the time of this writing, blinatumomab (Blinicyto, Amgen Inc, Newbury Park, CA), which is directed against CD19 and CD3 molecules, is the only bispecific antibody approved globally.⁵ It initially gained accelerated approval in 2014 for Philadelphia chromosome (Ph)-negative relapsed or refractory (r/r) B-cell precursor acute lymphoblastic leukemia (ALL) in adults. Approval was supported by data from a clinical study of 185 adults with Ph-negative r/r B-cell precursor ALL, wherein 32% percent of participants showed complete remission (CR) with a median duration of response of 6.7 months.⁵ In 2015, blinatumomab received accelerated approval for treatment in pediatric patients with ALL and full approval for both adults and children was granted in 2017.⁵ The full approval was supported by data from the TOWER study, wherein blinatumomab nearly doubled median overall survival (OS) vs. standard of care (7.7 months vs. 4 months) with 34% of blinatumomab-treated

patients achieving CR vs. 16% with standard of care.⁵ Data from the ALCANTARA study, which assessed the treatment of patients with Ph-positive r/r B-cell ALL also contributed to the body of evidence, wherein 31% of patients achieved CR and a median duration of response of 6.7 months.⁵ In 2018, the US Food and Drug Administration (FDA) expanded approval for blinatumomab (under accelerated approval) for the treatment of minimal residual disease (MRD)-positive B-cell precursor ALL, and it became the first FDA-approved treatment for these patients.⁶ The approval was supported by data from a single-arm clinical trial of 86 patients in first or second CR (defined as < 5% blasts in bone marrow, platelets >100 × 10⁹/L, absolute neutrophil count >1 × 10⁹/L) with baseline detectable MRD ≥ 0.1%, wherein undetectable MRD with blinatumomab treatment was reported in 70 patients (81.4%), with a median hematologic relapse-free survival of 22.3 months.⁵ Blinatumomab carries a boxed warning reflecting that patients experienced cytokine release syndrome (CRS) and neurologic toxicities⁵ and was approved by the FDA with a Risk Evaluation and Mitigation Strategy in place to monitor these toxicities.

The success of blinatumomab contributed to the explosion of research on bispecific antibodies for other hematologic malignancies targeting various tumor antigens and has led to attempts to modify the standard bispecific configuration in order to increase efficacy and to improve tolerability/feasibility. Herein we review various antibody-based immune effector-cell retargeting approaches in the treatment of hematologic malignancies, and compare these with other established and emerging antibody-based therapies. We also discuss the challenges that remain in translating preclinical studies and the clinical observations on blinatumomab to other bispecific antibodies and indications.

CD3 BISPECIFIC ANTIBODY FORMATS

Early approaches to manufacture bispecific antibodies included the chemical conjugation of two different antibodies or purification from hybridoma fusions.⁷ More recently, advances in genetic

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engineering have resulted in a diverse array of recombinant bispecific antibody formats, allowing for an opportunity to create bespoke bispecific antibodies for specific required mechanisms of action and clinical application.¹

Generally, bispecific antibodies may be categorized in two main classes: those that contain a fragment crystallizable (Fc) region and those that do not. Bispecific antibodies lacking an Fc region are single-chain-based such that individual antibodies contain only the variable regions of heavy and light chains that are joined to each other by a linker. They are normally smaller than the immunoglobulin (Ig)G and IgG-like bispecific molecules comprising an Fc region, and this small size of single-chain-based molecules may confer the advantage of enhanced tissue penetration.⁸ However, their short *in vivo* half-life necessitates more frequent dosing or continuous infusion. If desired, the half-life can be extended using technologies that have otherwise been developed for other protein pharmaceuticals. For example, the direct fusion of bispecific antibody fragments to albumin is possible, resulting in a longer half-life. However, although bispecific antibodies with an extended half-life can ease the logistics of administration, prolonged exposure may be undesirable in the event of observed toxicity.

Many other bispecific antibody approaches (not discussed in further detail here) are also in preclinical or clinical development. Currently, many approaches for hematologic malignancies target CD3 on T cells, but there is a scarcity of tumor antigens targeted, posing a challenge for future approaches, especially for solid tumors where penetration of antibodies into the tumor microenvironment also restricts their antitumor efficacy. Specifically, the dearth of truly tumor-specific cell-surface molecules targetable with antibody-based platforms likely implies that bispecific antibodies can only be used in cases where either (i) the target antigen is highly overexpressed in malignant compared with normal cells (thereby providing a potential therapeutic window), or (ii) lysis of normal target-bearing cells is clinically tolerable (for example, B cells). Therefore, decreasing the toxicity associated with systemic administration of bispecific antibodies recognizing targets with some expression on healthy tissues is an important area of future development. Examples of these approaches are CytomX Therapeutics' probodies, antibodies that only become activated in the tumor microenvironment through the action of tumor-associated protease responsible for cleavage of the antibody prodrug,⁹ and combinatorial targeting of two different tumor antigens to increase specificity, described as a future therapeutic approach for targeting acute myeloid leukemia (AML).¹⁰

Figure 1 lists a summary of the properties of a selection of bispecific antibody approaches discussed in this review.

Bispecific T-cell engagers

Interest in the therapeutic use of bispecific antibodies was invigorated by the success of blinatumomab in patients with ALL.^{11,12} Bispecific T-cell engager (BiTE) antibody constructs are a class of therapeutic antibodies that are made from two single-chain variable fragments (scFv) combined into a single protein chain, and simultaneously target CD3 in the T-cell receptor complex and a tumor antigen on cancer cells. Blinatumomab, for example, consists of an

anti-CD19 scFv in the light chain variable domain (VL)- heavy chain variable domain (VH) orientation linked through a G₄S linker to an anti-CD3 scFv in the VH-VL orientation.¹³

The binding of the BiTE antibody to both the T-cell receptor and tumor antigen leads to the creation of a cytolytic immunologic synapse only with monovalent engagement of the T-cell receptor complex, which prevents the systemic activation of effector cells in the absence of target cells.¹⁴ Typically, to ensure T cells are not triggered in the absence of target, the affinity of the monovalent antibody arm targeting CD3 is designed to be low (in the nM range), whereas the affinity of the antibody targeting the tumor antigen is typically higher and varies depending on the tumor target. The cytolytic synapses formed by BiTE antibodies are essentially identical in structure and composition to typical synapses created by matching T-cell receptor, peptide antigen, and MHC class I molecules.¹⁵ Following synapse formation, polyclonal T-cell activation and expansion results in target cell destruction through the action of lytic granules and cytokines released in the synapse, without need for antigen recognition by the T-cell receptor.¹⁶ Due to its small size (approximately 54 kDa), the half-life of blinatumomab is about 1.25 hours¹⁷ and, as a result, it is administered daily by continuous intravenous infusion at a constant flow rate (after an initial dose escalation) in repeated four-week cycles.¹² Blinatumomab-mediated B-cell lysis is illustrated in **Figure 2**.

Dual-affinity retargeting

The dual-affinity retargeting (DART) bispecific antibody platform format has some similarity to the BiTE format in that it is also a single-chain-based format. The heavy chain of one arm is linked to the light chain of a second arm, which reduces the constraint of intervening linker sequences to achieve an association that is more like that of an IgG molecule. The two arms maintain the covalent linkage between each other, ensuring stability of the molecule. Compared with a single-chain (BiTE) bispecific antibody with identical CD3 and CD19 antibody Fv sequences, DART molecules have been shown to be more potent in the lysis of B cells.⁴ In freshly isolated, resting human PBMCs (peripheral blood mononuclear cells), the cytotoxicity of the DART was found to be greater than that of the BiTE, and the concentration needed to cause 50% of maximal activity (EC₅₀) was up to 60-times lower.⁴ The enhanced killing activity was not associated with an increase in nonspecific activation of T cells or lysis of CD19-negative (CD19⁻) cells.⁴ The architecture of DART molecules allows the maintenance of contact between cells, which could help explain and contribute to the high level of target cell death.⁴

Tandem diabodies

The development of tandem diabodies (TandAbs) has provided a format with two binding sites for each antigen and a molecular weight that avoids first-pass renal clearance, contributing to a half-life that is longer than that seen with smaller bispecific antibodies such as BiTEs,¹⁸ while maintaining the ability to penetrate tumors.^{19,20} TandAbs do not possess Fc domains and are smaller than whole IgG or IgG-derived bispecific antibodies but are larger than BiTEs such as blinatumomab (e.g., AFM11 is 105 kDa,


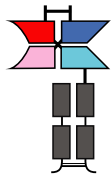
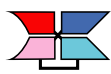
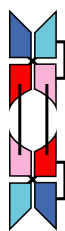
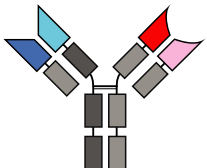

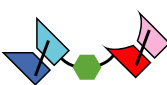
Format	Properties
BiTE 	<ul style="list-style-type: none"> • Two scFVs (heavy and light-chain variable regions) joined together by a linker • Format allows for short distance between tumor cells and T cells • Short serum half-life due to small size • Potent and can induce specific anti tumoral cytotoxicity at low picomolar concentrations in cell culture^{74,75} • Examples: blinatumomab, AMG420 (BCMA BiTE)
DART With Fc:  and without Fc: 	<ul style="list-style-type: none"> • Criss-cross format (heavy chain of one arm joined to light chain of second arm) • Short serum half-life that can be lengthened if Fc region present • Examples may target T cells or CD16-bearing effector cells • Potency has been shown to be superior to BiTEs⁴ • Examples: MGD006 S80880, MDG011
TandAb 	<ul style="list-style-type: none"> • Molecular weight exceeds the renal clearance threshold, offering a longer half-life than BiTEs or DARTs • Examples may target T cells or CD16-bearing effector cells • Potent with cytotoxicity at low picomolar concentrations <i>in vitro</i> • Example: AFM11
Full-length IgG With Fc effector function (Triomabs) Without Fc effector function: 	<ul style="list-style-type: none"> • Full-length IgG confers long half-life • Recruit T cells and Triomabs also activate monocytes, macrophages, dendritic, and NK cells by binding to the Fc region • Formats have also been developed with Fc region that does not bind Fc receptor (effector null) • Examples: catumaxomab (Triomab), PF 06863135 (full-length IgG [effector null])
BiKEs 	<ul style="list-style-type: none"> • BiKEs are similar in design to BiTEs, but target CD16 on NK cells, rather than T cells
TriKEs 	<ul style="list-style-type: none"> • TriKEs incorporate IL-15 sandwiched into the design to drive NK cell expansion <i>in vivo</i> • Both formats direct NK cells to tumors to trigger antibody-dependent cell-mediated cytotoxicity • Examples: 1633 BiKE; 161533 TriKE

Figure 1 Comparison of bispecific antibody formats for redirection of cytotoxic effector cells.⁷¹ BCMA, B-cell maturation antigen; BiKEs, bispecific killer cell engagers; BiTE, bispecific T-cell engager; DART, dual affinity retargeting; Fc, fragment, crystallizable; IgG, immunoglobulin G; NK, natural killer; scFVs, single-chain variable fragment; TandAb, tandem diabodies; TriKEs, trispecific killer cell engagers; Triomabs, trifunctional, bispecific antibodies. Figures adapted from Kontermann RE, Brinkmann U. Bispecific antibodies. *Drug Discov Today*. 2015;20(7):838–847. Published by Elsevier Ltd. <https://doi.org/10.1016/j.drudis.2015.02.008>. Licensed under CC BY-NC-ND 4.0. ©2015 The authors.

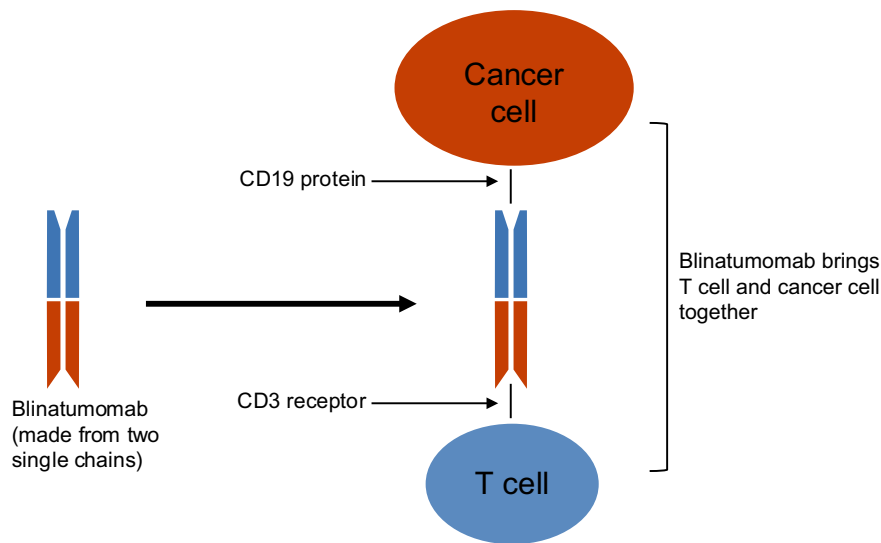


Figure 2 Blinatumomab mode of action. Blinatumomab is a 55-kDa single-chain BiTE antibody. It has Fv fragments from anti-CD3 and anti-CD19 arms joined by a nonimmunogenic linker, bringing together cytotoxic CD3+T cells and CD19+B cells resulting in granzyme-mediated and perforin-mediated B-cell apoptosis. BiTE, bispecific T-cell engager.

whereas blinatumomab is 54 kDa). AFM11 (Affimed, Heidelberg, Germany) is an example of a TandAb in development that recruits immune cells to tumors by simultaneously binding to CD3 on T cells and CD19 on lymphoma cells. In a side-by-side comparison between AFM11 and blinatumomab, AFM11 was found to exhibit more potent antitumor activity *in vitro*, *in vivo*, and *ex vivo*.²¹

Full-length IgG bispecific antibodies, including triomabs

The first bispecific molecules were created in the 1960s, formed by the conjugation of two antibodies of differing specificity. Later, a method was reported for the creation of bispecific antibodies by the somatic fusion of two hybridoma cell lines. This process results in the formation of a quadroma cell that secretes, in a single molecule, whole IgG antibodies with the binding properties of the two parental hybridomas.^{22,23} Triomabs are a family of chimeric IgG-like bispecific antibodies produced using this quadroma approach. They are made from two half-antibodies, each with one light and one heavy chain from parental rat IgG2b and mouse IgG2a isotypes. These IgG-like bispecific antibodies are essentially trifunctional in nature, targeting tumor cells via tumor-associated antigen binding, T cells via CD3, and Fc receptors for IgG (Fc gamma receptor-bearing immune cells [NK cells, macrophages, or dendritic cells]) through Fc gamma receptor binding.¹⁹ Although every triomab has an anti-CD3 rat IgG2b half-antibody for T-cell engagement, the antigen binding site of the mouse IgG2a isotype is interchangeable.²⁴

The triomab bispecific antibody catumaxomab (an anti-EpCAM/anti-CD3; Removab, Fresenius Biotech, Bad Homburg, Germany and Trion Pharma, GmbH, Munich, Germany) was the first bispecific and trifunctional drug, approved for the treatment of malignant ascites by the European Medicines Agency.²⁵ Catumaxomab binds to epithelial cell adhesion molecule and CD3 and, via its Fc fragment, it also adheres to dendritic cells, macrophages, and NK cells.²⁶ Despite its approval based on encouraging clinical trial

data,²⁵ Catumaxomab was voluntarily withdrawn from the US market in 2013 and the EU market in 2017 for commercial reasons.

Full-length bispecific IgG antibodies, similar to triomabs but effector-function null (“Fc dead”), therefore not trifunctional but truly bifunctional, are also in development. Genentech is currently developing RG7828, a humanized full-length T-cell–dependent bispecific antibody targeting CD20 on B cells and CD3. PF-06863135, in development by Pfizer, is a humanized IgG CD3 bispecific mAb that utilizes anti–B-cell maturation antigen (BCMA) and anti-CD3 targeting arms that are paired through hinge-mutation technology within an IgG2a backbone.

HARNESSING NK CELLS—BIKES AND TRIKES

Much focus has also been directed into engineering additional components into bispecific antibody approaches, for example directing immune cells other than T cells to the tumor environment. The potent cytotoxic effector NK cell holds promise to be effectively utilized for immunotherapy, but a big challenge for NK cells in cancer immunotherapy has been the maintenance of NK-cell numbers and function *in vivo* and the development of methods to improve their specificity for tumors. However, approaches are now emerging to take advantage of NK cells.

Bispecific killer cell engagers (BiKEs) and the trispecific killer cell engagers (TriKEs), were developed to better target NK cells to malignant targets. BiKEs are composed of two antibody fragments, one that recognizes a tumor antigen and another directed against CD16 on NK cells.²⁷ Importantly, in TriKEs, the integration of interleukin (IL)-15 drives expansion of NK cells that engage with the tumor target.²⁷ It is suggested that activation of NK cells with paracrine IL-15 may reduce systemic effects compared with systemic administration of IL-15.²⁸

Recent preclinical data support various advantages of TriKEs over BiKEs. For example, in an assay assessing the killing of CD33+HL-60 leukemia cells by normal donor peripheral blood

mononuclear cell PBMCs, increased NK cell–mediated killing was observed with the 161533 TriKE compared with the 1633 BiKE.²⁸ The TriKE also displayed the ability to restore the activity of NK cells against primary AML targets and induced NK cell proliferation.²⁸ Moreover, in a murine xenograft HL-60-Luc tumor model, greater antitumor activity and *in vivo* persistence of human NK cells was observed with the TriKE compared with the BiKE.²⁸ In myelodysplastic syndrome, increased levels of myeloid-derived suppressor cells bearing a high expression of CD155 suppress NK-cell function through engagement with T-cell immunoreceptor with Ig and tyrosine-based inhibition motif (ITIM) domains (TIGIT), a negative regulatory checkpoint expressed on NK cells in myelodysplastic syndrome.²⁹ Although IL-15 is known to enhance NK-cell survival and stimulate activation and proliferation,³⁰ soluble recombinant IL-15 also induces the expression of the inhibitory checkpoint TIGIT on NK cells *in vitro*.³¹ However, when IL-15 was presented in the form of the 161533 TriKE, an anti-CD16–IL-15–anti-CD33 molecule, TIGIT expression was not induced on NK cells.³¹ The data are encouraging and indicate that this first-of-its-kind single-chain TriKE can enhance NK-cell killing without provoking the expression of inhibitory checkpoints. This approach may lead the way for additional modifications that provide important costimulatory or agonistic stimulation to desired effector cells. The TriKE platform also has the advantage of being easily adapted to target different tumors of choice by switching the scFv portion to a specific tumor antigen.

See **Table 1** for a summary of bispecific antibodies in clinical trials for hematologic malignancies.

OTHER ANTIBODY-BASED TECHNOLOGIES

Chimeric antigen receptor T cells

Chimeric antigen receptor (CAR) T cells, like bispecific antibodies, represent a major approach for MHC-independent T-cell immune responses, and are among a growing list of anti-CD19 products in development. CAR T cells express synthetic receptors composed of antibody-derived antigen-binding regions and T-cell receptor-derived signaling components that retarget polyclonal T cells to tumor surface antigens and may be designed in such a manner to enhance T-cell persistence and activity.³²

In 2017, the anti-CD19 T-cell therapy tisagenlecleucel (Kymriah; Novartis, Basel, Switzerland) was approved by the FDA for the treatment of pediatric and young adult patients with r/r B-cell ALL.³³ The approach has also shown potential in patients with B-cell lymphomas and, shortly following the approval of tisagenlecleucel, the anti-CD19 T-cell therapy axicabtagene ciloleucel (Yescarta; Kite Pharma, Santa Monica, CA) was approved for the treatment of adult patients with r/r large B-cell lymphoma.³³ However, resistance can be a problem, and the loss of the target antigen on tumor cells has been cited as mechanism of resistance to anti-CD19 CAR T cells.³⁴ CAR T-cell therapy is also associated with CRS and neurotoxicity.^{35,36} In light of this, in 2018, tocilizumab (a recombinant humanized mAb directed against the IL-6 receptor) was approved by the FDA for the treatment of severe or life-threatening CRS following CAR T-cell therapy.³⁷ It is also important to point out that, unlike monoclonal antibodies (mAbs), which are eventually cleared by the body, CAR T cells may persist and continue to be active unless a kill-switch is engineered

(although no currently approved CAR T-cell approaches incorporate such a kill-switch).

Antibody-drug conjugates

Antibody-drug conjugates (ADCs) are another antibody-based cancer-targeting approach, although not primarily immunotherapeutic in nature. These agents combine the targeting capabilities of antibodies with cancer-killing cytotoxic drugs. The linker attaching mAbs to the drug is stable in the circulation but releases the cell-killing drug in target tumor cells. After gaining access to the tumor cell, the cytotoxin is released and regains its full cytotoxic activity; the goal is to spare healthy cells while killing tumors. Through this approach, the systemic exposure of drug is also limited compared with standard chemotherapeutic drugs or biologics. A number of ADCs are currently on the market for hematologic malignancies and include gemtuzumab ozogamicin (Mylotarg, Pfizer Inc., New York, NY), brentuximab vedotin (Advcetris; Seattle Genetics, Bothell, WA), and inotuzumab ozogamicin (Besponsa, Pfizer Inc, New York, NY). In the setting of r/r B-cell precursor ALL, the single-agent activity of the ADC inotuzumab ozogamicin may be greater than that of blinatumomab (although not tested in comparative studies), with a higher CR rate but, in contrast to blinatumomab, no OS benefit vs. standard of care.³⁸ Biparatopic ADCs which bind to two nonoverlapping epitopes on the same target are also being investigated. The targeting of two different epitopes results in receptor clustering, which enhances internalization and lysosomal trafficking, resulting in more toxins being delivered to target cells.³⁹ Bispecific ADCs against different targets can also be developed to potentially address heterogeneity of target expression on malignant cells and antigen escape mechanisms. Recently a bispecific ADC targeting human epidermal growth factor receptor 2 and the prolactin receptor was shown to kill more effectively than an ADC targeting HER2 alone.⁴⁰

See **Table 2** for a comparison of the properties of various antibody-based approaches that may be used in the treatment of hematologic malignancies.

TRANSLATIONAL STRATEGIES FOR CD3-BISPECIFIC ANTIBODIES

A key determinant of the success of any new drug lies in the ability to accurately translate preclinical data to the clinic to inform both the clinical starting dose as well as the predicted human effective dose. For T-cell–engaging CD3 bispecifics, a minimum anticipated biologic effect level (MABEL) has been used to estimate starting dose due to their immune agonistic properties.⁴¹ A widely applied method for calculating the MABEL-based starting dose is based on the *in vitro* potency threshold, for example effective concentration (EC)₂₀ or EC₅₀, estimated from various human assays for assessing bispecific activity, including but not limited to cytokine release, cytotoxicity, and T-cell activation/proliferation. The recommended starting dose is usually calculated by setting the predicted drug exposure in humans (e.g., maximum concentration) below the threshold estimated from *in vitro* assays. Starting at more than 30% activity (EC₃₀) may be acceptable with sufficient scientific justification. However, this may be dependent on structural properties of the molecule, as well as biology and

Table 1 Immune cell redirecting bispecific antibodies in clinical trials for hematologic malignancies

Format	Molecule	MOA	Targets	Condition	Developer	Phase; NCT#
BiTE	AMG420 (BI 836909)	T-cell recruitment	BCMA + CD3	MM	Boehringer Ingelheim, Amgen (Micromet)	1; [NCT02514239]
	AMG330	T-cell recruitment	CD33 + CD3	AML	Amgen (Micromet)	1; [NCT02520427]
TandAb	AFM11	T-cell recruitment	CD19 + CD3	NHL, ALL	Affimed	1; [NCT02848911 NCT02106091]
	AFM13	Immune cell recruitment	CD30 + CD16	HL	Affimed	2; [NCT02321592]
	AMV564	T-cell recruitment	CD33 + CD3	MDS, AML	Amphivena Therapeutics	1; [NCT03516591 NCT03144245]
DART	MGD006 S80880	T-cell recruitment	CD123 + CD3	AML, MDS	Macrogenics, Servier	1; [NCT02152956]
	MDG011 JNJ-64052781	T-cell recruitment	CD19 + CD3	B-cell malignancies	Macrogenics, Johnson & Johnson	1; [NCT02743546]
TriKE	161533	NK-cell recruitment and MDSC inhibition	CD16 + CD33 with IL-15 crosslinker	MDS, AML, ASM	Oxis Biotech	1,2; [NCT03214666]
cLC-hetero-H-chain IgG	MCLA117	T-cell recruitment	CLEC12A + CD3	AML	Merus N.V.	1; [NCT03038230]
	REGN1979	T-cell recruitment	CD20 + CD3	NHL, HL, CLL	Regeneron	1; [NCT02651662 NCT02290951]
bsmAb ^a	RG7828, BTCT 4465A	T-cell recruitment	CD20 + CD3	NHL, CLL	Genentech	1; [NCT02500407 NCT03671018 NCT03677141 NCT03677154]
	JNJ 63709178 Duobody	T-cell recruitment	CD123 + CD3	AML	Janssen, Genmab	1; [NCT02715011]
	JNJ-64007957 Duobody	T-cell recruitment	BCMA + CD3	MM	Janssen, Genmab	1; [NCT03145181]
	PF-06863135	T-cell recruitment	BCMA + CD3	MM	Pfizer	1; [NCT03269136]
scFv-Fc-(Fab)-fusions	Xmab14045	T-cell recruitment	CD123 + CD3	AML, B-cell ALL, BPDCN, CML	Xencor, Novartis	1; [NCT02730312]
GEMoaB	GEM333	T-cell recruitment	CD33 + T cells	AML	GEMoaB Monoclonals	1; [NCT03516760]
BEAT	GBR1342	T-cell recruitment	CD38 + CD3	MM	Glenmark Pharmaceuticals	1; [NCT03309111]

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ASM, advanced systemic mastocytosis; BCMA, B-cell maturation antigen; BEAT, bispecific engagement by antibodies on the T-cell receptor; BiTE, bispecific T-cell engager; BPDCN, blastic plasmacytoid dendritic cell neoplasm; bsmAb, bispecific monoclonal antibody; CL, cutaneous lymphoma; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; DART, dual affinity retargeting; HL, Hodgkin's lymphoma; IgG, immunoglobulin G; MDS, myelodysplastic syndromes; MM, multiple myeloma; MOA, mechanism of action; NHL, non-Hodgkin's lymphoma; TandAb, tandem diabodies; TriKE, trispecific killer engager.

^aIgG assembled from half-antibodies.

disease indication, and acceptable limits should be addressed on a case-by-case basis. In the case of AMG211, a carcinoembryonic antigen and CD3-engaging BiTE, tumor lysis was determined to be the most sensitive measure of activity and the associated EC₂₀ value was used to define a starting dose of 52 µg/day.⁴¹ In addition, another orthogonal method using mechanistic pharmacokinetic (PK)/pharmacodynamic models determines the MABEL dose through the combination of *in vitro* and *in vivo* data.⁴² Specifically, multiple *in vitro* endpoints, including but not limited to cytokine release and cytotoxicity experiments, have been employed in exposure–response analyses. The MABEL dose for Amgen's CD33 BiTE (AMG330) was selected using the calculated EC₅₀ value from a combination of *in vitro*, *in vivo* (including toxicology), and *ex vivo* data, resulting in a starting dose of 0.5 µg/

day.⁴¹ For Pfizer's extended half-life DART-bispecific antibody directed against CD3 and P-cadherin (PF-06671008), calculation of the concentration of tumor synapse that achieves 20% of the maximal effect (EC₂₀) for each assay in combination with an *in vivo* model that included predicted human PK, distribution of the drug to the tumor as well as formation of the tumor synapse, allowed for projection of a MABEL-based starting dose of 1.5 ng/kg/week.⁴² This approach can be compared with other conventional methods to ensure a conservative and safe starting dose while providing a more comprehensive view of the predicted pharmacologic response in humans.

One important step to consider when determining the MABEL dose is to carefully evaluate the relevance of the chosen *in vitro* cytotoxicity or activation assay. Typically, researchers demonstrate

Table 2 Comparison of properties of antibody-based approaches in the treatment of cancer

Properties	Bispecific antibodies	Monoclonal antibodies	Antibody–drug conjugates	CAR T cells
Cytotoxicity	Mediated by redirecting immune cells to the tumor	Mediated by Fc γ receptor activation on effector cells or by blocking action of growth factors	Mediated by cytotoxic payload attached to antibody	Patients' own T cells are modified to bind to antigen on cancer cells and kill them
Molecular weight	Few kDa to 1,000 kDa	Few kDa to 150 kDa	Few kDa to 1,000 kDa	Not applicable
Serum half-life	Varies from hours to days	Days to weeks	Days ⁷²	Weeks – years
Dosing regimen	Weekly to monthly cycles	Varying dosage and dosing regimens. Ranges from weekly to monthly to six months	Weekly to monthly cycles	Typically single administration. Can be split over multiple injections (e.g., three injections, each one day apart) ⁷³
Common toxicities	Neutropenia, infections, severe cytokine release syndrome and neurological symptoms	Hives or itching, chills, fatigue, fever, muscle ache, nausea, vomiting, diarrhea, skin rash, hypotension	Anemia, neutropenia, peripheral neuropathy, thrombocytopenia, hepatic toxicity, ocular toxicity	Cytokine release syndrome, tumor lysis syndrome, neurotoxicity

CAR, chimeric antigen receptor.

potency of CD3-bispecific molecules in assays that use healthy donor T cells at high effector-to-target (E:T) ratios (i.e., 1:1 to as high as 10:1). Under these conditions, which often do not mimic physiological E:T ratios and patient T-cell quality, EC₅₀ values are reported in low picomolar ranges. This may result in selection of starting doses that are much below the concentrations required to redirect T cells in the patient, and consequently, dose escalation trials can take several years. For example, Amgen's BCMA BiTE (AMG 420) has been in phase I since 2015, while the first positive clinical results were reported three years later in patient cohorts that were dosed several logs higher than the initial dose cohort.⁴³ Therefore, there is a growing need in the field to reevaluate the type of *in vitro* assays that are chosen for determining the MABEL dose for CD3 bispecifics. Assays that use more physiological E:T ratios, patient T cells, and patient tumors, although challenging to set up and develop, should be attempted in an effort to improve our predictions of efficacious doses in patients. Similarly, development of *in vivo* preclinical toxicology models is challenging, mostly because of the need to use immunodeficient mice for engraftment of human cancer cells and immune cells. These mice lack other components of the immune system, and thus do not have the ability to induce CRS. Furthermore, prolonged engraftment of human T cells in immunodeficient mice generally leads to xenogeneic graft-vs.-host disease, which limits the duration of these experiments and does not occur in humans. Nonhuman primates could serve as a good toxicology species to estimate the extent of CRS due to recognition of healthy tissue in cases when target expression is conserved. However, risk of CRS in response to tumor cannot be estimated in nonhuman primates. Furthermore, subtle differences in healthy tissue expression of tumor-associated targets between humans and nonhuman primates could further prevent accurate assessment of toxicity. For example, Genentech's preclinical stage C-type lectin-like molecule (CLL-1) CD3 bispecific targets CLL-1, expressed on AML blasts, and at high levels on human neutrophils but at lower

levels on cynomolgous monkey neutrophils, making it difficult to precisely translate the risk of CRS to humans.⁴⁴ Thorough evaluation of target expression in humans and chosen toxicology species by various methods (RNA and protein, when possible) is required. Finally, tissue cross-reactivity assays that assess potential for off-target binding of developed antibodies are also important for building a better understanding of toxicity.

CYTOKINE RELEASE SYNDROME

Although regarded as a breakthrough in the treatment of certain cancers in some patients, the use of some immunotherapy approaches in the clinic has revealed potentially fatal adverse effects, most notably CRS. This systemic inflammatory response, which correlates with T-cell activation and high levels of cytokines,⁴⁵ has been documented since the early 1990s, following use of several antibody-based therapies. The success of T-cell-redirecting immunotherapies has reignited further interest in CRS, as it is a frequent serious adverse event associated with such therapies; for example, studies have shown it is an important adverse event of blinatumomab⁴⁶ and CD19-targeted CAR T cells.^{35,36,47} With the increasing use of T-cell-engaging immunotherapies, it is paramount that specialists who are dosing patients with these agents should be knowledgeable about the presentation and complications that CRS may cause and its clinical management. However, as these therapies are relatively new, the optimal management of patients is evolving, and requires collaboration between areas of expertise such as radiology, hematology/oncology, critical care, and neurology.⁴⁸ Although increases in cytokines are seen in many patients, the magnitude of elevation may not correspond with the clinical response to immunotherapy, and CRS does not appear to be required for a response to T-cell-redirecting immunotherapies.^{48,49} Advances in the identification of biomarkers for predicting CRS will aid clinical management of CRS and may equip physicians with agents to treat CRS while maintaining the beneficial activity of

the immunotherapy.⁴⁸ In addition, advances in the design on T-cell-engaging immunotherapies should be explored, with a focus on reducing the risk of CRS.

MECHANISMS OF RESISTANCE TO CD3-BISPECIFIC ANTIBODIES

When developing a CD3-bispecific antibody, although it may mediate promising antitumor efficacy *in vitro* and in animal models, there are several challenges to overcome to translate that success to the clinic. For example, presence of tumor microenvironment-intrinsic inhibitory pathways that limit the function of redirected T cells, and the escape of tumor cells by downregulation of tumor antigen. Substantial clinical activity in a number of cancers has been reported with immune checkpoint inhibitors targeting cytotoxic T lymphocyte-associated protein 4 (CTLA-4), the programmed cell death-1 receptor (PD-1), or its ligand PD-L1.⁵⁰ Interestingly, in AML cell lines overexpressing individual T-cell ligands, expression of PD-L1 and PD-L2 reduced the cytolytic activity of the BiTE antibody construct AMG 330, and T-cell redirection upregulated PD-1 on T cells and PD-L1 on AML blasts *ex vivo*.^{51,52}

Recent evidence suggests that the expression of coinhibitory molecules and loss of costimulatory molecules may be an important aspect of tumor immune escape. The effect of cosignaling molecules on the ability of T cells to mount a response against leukemia mediated by blinatumomab was investigated in a recent study.⁵³ Results showed increased PD-L1 levels in relapsed pediatric patients with ALL and pediatric patients with ALL refractory to blinatumomab. In addition, levels of the exhaustion markers PD-1 and T-cell Ig and mucin domain-containing molecule-3 were significantly upregulated on T cells compared with physiologic controls. T-cell proliferation and effector function correlated with the expression of cosignaling molecules and were target-cell dependent. Enhanced *in vitro* T-cell function was observed with blockade of the inhibitory pathways PD-1, PD-L1, and CTLA-4, while blocking costimulatory CD28-CD80/86 interaction significantly reduced T-cell function. Moreover, therapy with blinatumomab and PD-1 blockade resulted in an anti-leukemic *in vivo* response in a 12-year-old with refractory ALL.⁵³ The authors concluded that ALL cells actively regulate T-cell function through expression of cosignaling molecules and modify the efficacy of the T cells in their fight against ALL.⁵³ It is important to note that these findings are from a pediatric group of patients in a small study and there is no systematic evaluation of coinhibitory signals with blinatumomab therapy in adults. However, it is possible that antibodies blocking this pathway could be beneficial in combination with bispecific antibodies to treat hematological malignancies.

Another recent study reported that under BiTE antibody induced inflammatory conditions, PD-L1 was shown to be increased on primary AML cells, which inhibited blast-cell lysis.⁵¹ BiTE-mediated lysis, T-cell proliferation, and interferon- γ secretion were all enhanced by blockade of the PD-1/PD-L1 interaction. This combination of BiTE antibody construct and blockade of the PD-1/PD-L1 interaction was shown to be especially helpful in settings of protracted AML cell lysis.⁵¹ It is suggested that combinatorial approaches of BiTE antibody construct and

PD-1/PD-L1 blockade could be a promising future strategy to improve efficacy.⁵¹ It must be noted, however, that use of bispecific antibodies results in a strong activation of T cells and production of proinflammatory cytokines⁵⁴ and may also create a situation where immunosuppressive strategies evolve in tumor cells.

Antigen escape

A recent study of four patients with ALL treated with blinatumomab reported that in three of the patients with on-treatment emerging resistance, the CD19⁻ escape variant was detected after just two courses of treatment with blinatumomab.⁵⁵ This was followed by cytological relapse no later than after three courses. The fourth patient showed a late relapse, with CD19⁻ clones appearing at 19 months following completion of blinatumomab treatment. Apart from CD19 negativity, all of the four patients showed a cellular phenotype that was identical to the primary diagnosis.⁵⁵ The investigators suggested CD19 negativity was the result of an isolated molecular event, as, in a comprehensive molecular investigation of one of the patients, they found evidence of disrupted CD19 membrane export in the post endoplasmic reticulum compartment. The data show that, in some cases, imminent relapses may be detected early on with standard flow cytometry analysis.⁵⁵

Although CD19⁻ relapses have been observed in up to 20% of patients with ALL treated with blinatumomab,⁵⁵ a recent study assessing patients with r/r ALL after failure of blinatumomab therapy found that at the time of drug failure among 61 patients evaluated for immunophenotype, 56 (92%) had CD19⁺ blasts, whereas just five (8%) had CD19⁻ disease.⁵⁶ These data suggest that although the patient outcome in r/r ALL is poor following blinatumomab failure, its use does not exclude some patients from further CD19-directed treatments such as CAR T-cell therapy.

In a study of r/r ALL in children and young adults treated with CD19 CAR T cells, the overall intent-to-treat MRD-negative (MRD⁻) CR rate was 89% ($n = 40$ of 45).⁵⁷ However, 18 of the 40 patients with MRD⁻ CR experienced relapse, seven of whom were associated with the inability to detect cell surface CD19.⁵⁷

Myeloid lineage switch

Myeloid lineage switch (MLL) constitutes a variant of CD19⁻ relapse. Rearrangements of the *MLL* gene occur frequently in infants with ALL or AML, and while actual conversions of leukemia cell lineage are rare, this most commonly occurs in the setting of *MLL* rearrangement.⁵⁸ In a recent report, a 3-month-old infant with B-cell precursor ALL and *MLL* rearrangement was treated with blinatumomab but relapsed on day 15 with a leukemic lineage switch to CD19⁻ monoblastic AML (with identical karyotype as pre-blinatumomab).⁵⁸ It has been speculated that CD19-specific selective pressure may result in a different blast differentiation program, or to the selection of minor myeloid CD19⁻ leukemic subpopulations leading to myeloid relapses.⁵⁹ Recently, two *BCR-ABL1* fusion-positive B-cell precursor ALL patients with CD19⁻ myeloid lineage relapse after blinatumomab therapy were described.⁶⁰ Although blinatumomab eliminated the aggressive B-cell clone, it had no effect on an ancestral CD19⁻ *BCR-ABL1*-positive precursor responsible for the CD19⁻ relapse. The authors

suggested that novel treatment approaches should target CD19⁺ malignant precursor cells as well as the B-cell leukemic bulk.⁶⁰

Regulatory T cells

Regulatory T cells (Tregs) may play a role in tumor development and immunosuppression by inhibiting effector cells. In murine tumor models, treatment with an anti-CTLA-4 antibody led to a reduction in the number of intratumoral Tregs and better tumor control.⁶¹ More recently, administration of an anti-CD27 antibody in mouse tumor models and in patients with advanced solid tumors resulted in antitumor activity associated with T-cell stimulation and the depletion of Tregs.^{62,63} Hence, alterations in the amount of Tregs may impact the effectiveness of immunotherapy. It has been shown that high percentages of Tregs in samples of patients with ALL not responding to blinatumomab treatment was predictive in determining the nonresponse, and the depletion of Tregs in nonresponding patient samples restored blinatumomab-triggered T-cell proliferation.⁶⁴ It is considered that in the nonresponders, blinatumomab-activated Tregs are able to suppress effector T-cell proliferation and lysis of ALL cells. These data suggest therapeutic depletion of Tregs may convert blinatumomab nonresponders to responders.

Tumor burden

Results from a large multicenter phase II trial of blinatumomab indicate that the tumor burden at the time of treatment may be a critical factor for the clinical activity of bispecific antibody constructs; a higher tumor burden was seen more frequently in patients with r/r ALL not responding to blinatumomab treatment.¹² Moreover, a recent analysis of the outcome of blinatumomab in the treatment of ALL found greater numbers of pretreatment blasts in nonresponders compared with responders.⁶⁴

Antidrug antibodies

Treatment with any antibody drug therapy carries the potential for patients to develop antidrug antibodies (ADAs). Although not a mechanism of resistance, ADAs may reduce efficacy by affecting the PK of the immunotherapy through interference with clearance mechanisms and by targeting domains critical for efficacy.⁶⁵ ADAs can also have toxic effects, including hypersensitivity reactions.⁶⁶ A recent ADA-focused review of extracted data from 81 oncology clinical trials of biologic agents reported most biological anticancer immunotherapy drugs are immunogenic and induce ADAs.⁶⁷ The report also found that even among agents on the market, gaps in the data on ADA formation were apparent.⁶⁷ Routine investigation of the relationship between ADAs and efficacy, toxicity, and PK may shed light on the clinical relevance of ADAs and help explain the variability seen in drug responses and safety. Standardized reporting of ADAs is critical to understanding the relevance of ADA formation, and the development of trials investigating clinical prevention strategies is needed.

LONG-TERM SURVIVAL

Long-term survival after BiTE treatment may be associated with a higher degree of T-cell expansion.⁶⁸ A long-term follow-up analysis in a phase II study evaluated OS and relapse-free survival

in 36 adults with r/r B-cell precursor ALL treated with blinatumomab.⁶⁸ Results showed long-term survivors (MRD responders with OS \geq 30 months) during treatment cycles 1 and 2 had a greater level of T-cell and effector memory T-cell expansion.⁶⁸ This compared with only minor or even absent T-cell expansion in patients with OS < 30 months, and none of the patients without MRD response was considered a long-term survivor. The data suggest that the expansion of T cells could be an important factor for patients with r/r ALL.⁶⁸

Survival following treatment with BiTEs may be enhanced with subsequent allogeneic hematopoietic stem cell transplantation (HSCT). In an analysis of the long-term outcomes of a phase II study in MRD, survival after treatment with blinatumomab and HSCT in continuous CR was evaluated.⁶⁹ After follow-up \geq 3 years, in patients \leq 35 years of age, 16 of 26 (62%) who underwent HSCT were alive, compared with two of nine (22%) of those who had not received HSCT. In patients aged >35 years, 19 of 48 (40%) and 13 of 27 (48%) were alive with and without HSCT, respectively. These results suggest that in transplant-eligible patients in continuous CR, HSCT should be considered as a consolidation option after blinatumomab-induced remission, particularly in patients aged 35 years or younger.⁶⁹

FUTURE OF BISPECIFIC ANTIBODIES IN THE CLINIC

As bispecific antibody formats continue to be developed, design modifications to permit improved PK characteristics without the need for continuous infusion will need to take account of the potential for such alterations to compromise the therapeutic window of this platform. Addition of costimulatory molecules or activating cytokines (in a similar manner as for TriKEs) for all formats, including anti-CD3 BiTEs, will create molecules with the ability to recruit additional effector functions. Further progress could involve approaches designed to neutralize or abrogate counter-regulatory mechanisms such as Tregs or inhibitory immunoreceptors (e.g., PD-1), either by combined therapy (e.g., coinfusion of anti-PD-1) or by specific recruitment of the cell types of interest. It may be possible to extend this strategy to solid tumors, but this would require identification of tumor-specific surface antigens. Recruitment of other effector cells, for example, macrophages using anti-CD64, is also an important area for further investigation.

CONCLUSIONS

Recruiting the immune system in the fight against cancer holds great potential, with success already observed in the treatment of hematologic malignancies, and approaches such as bispecific antibodies and CAR T cells can overcome some of the limitations of conventional mAb approaches. Many bispecific antibody formats with differing mechanisms of action are currently being investigated, each with their own advantages and limitations. In addition to approaches that harness T cells, newer formats such as BiKEs and TriKEs take advantage of NK cells, broadening the scope in immune-cell retargeting approaches. The future should bring a better understanding of PK, improved delivery methods, and ways to manage toxicities and ultimately improve patient inconvenience and avoidance of hospitalizations. Although blinatumomab is the

first approved bispecific, future agents will likely not require continuous infusion. As subcutaneous dosing is explored, there may be an opportunity to avoid hospitalization for infusion at the first dose.

Resistance remains a challenge, and future advances in reducing the incidence of resistance will likely be informed by careful correlative studies of patients receiving bispecific antibodies. For example, combination approaches using bispecific antibodies with other agents such as checkpoint inhibitors have been designed based on relevant preclinical observations, and combined use of bispecific antibodies with conventional treatments or other immunotherapies has been reported in more than 1,000 open clinical trials.⁷⁰ More research on combination approaches and the further development of antibody formats will bring new avenues and therapies for the immunotherapeutic treatment of hematologic malignancies.

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CONFLICT OF INTEREST

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