

Bispecific antibodies in cancer immunotherapy

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Abstract: Following the clinical success of immune checkpoint antibodies targeting CTLA-4, PD-1 or PD-L1 in cancer treatment, bispecific antibodies are now emerging as a growing class of immunotherapies with potential to further improve clinical efficacy and safety. We describe three classes of immunotherapeutic bispecific antibodies: (a) cytotoxic effector cell redirectors; (b) tumor-targeted immunomodulators; and (c) dual immunomodulators. Cytotoxic effector cell redirectors are dominated by T-cell redirecting compounds, bispecific compounds engaging a tumor-associated antigen and the T-cell receptor/CD3 complex, thereby redirecting T-cell cytotoxicity to malignant cells. This is the most established class of bispecific immunotherapies, with two compounds having reached the market and numerous compounds in clinical development. Tumor-targeted immunomodulators are bispecific compounds binding to a tumor-associated antigen and an immunomodulating receptor, such as CD40 or 4-1BB. Such compounds are usually designed to be inactive until binding the tumor antigen, thereby localizing immune stimulation to the tumor environment, while minimizing immune activation elsewhere. This is expected to induce powerful activation of tumor-specific T cells with reduced risk of immune-related adverse events. Finally, dual immunomodulators are bispecific compounds that bind two distinct immunomodulating targets, often combining targeting of PD-1 or PD-L1 with that of LAG-3 or TIM-3. The rationale is to induce superior tumor immunity compared to monospecific antibodies to the same targets. In this review, we describe each of these classes of bispecific antibodies, and present examples of compounds in development.

Keywords: bispecific antibody, cancer, checkpoint, co-stimulation, immuno-oncology, immunotherapy

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Introduction

Immunotherapeutic antibodies have revolutionized cancer treatment over the past few years, demonstrating superior tolerability and major improvements in long-term survival.^{1–3} The first immuno-oncology antibody to reach the market was the CTLA-4 inhibitor ipilimumab, which was approved for melanoma in 2011. It was followed by the PD-1 antibodies nivolumab and pembrolizumab in 2014, and the PD-L1 antibodies atezolizumab, avelumab and durvalumab in 2016 and 2017. To date, immuno-oncology antibodies are approved for a range of tumor types including melanoma, Hodgkin's lymphoma, Merkel cell carcinoma, non-small cell lung, head

and neck, renal, bladder, colorectal, liver, gastric, and esophageal cancer.

In metastatic melanoma, long-term survival (beyond three years) in response to ipilimumab therapy is approximately 20%,² and for PD-1 inhibitors the corresponding numbers are in the range of 35–50%.^{3,4} While these numbers are impressive, it has to be acknowledged that the majority of patients do not respond. In addition, immune-related adverse events such as colitis, diarrhea, dermatological toxicity, endocrinopathy, hepatotoxicity and pneumonitis limit the clinical use of immunotherapeutic antibodies.^{5,6}

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Combination therapies seem to improve long-term survival beyond what is possible with a monotherapy, but improved efficacy comes at the cost of disabling side effects.³

Bispecific immunotherapies have the potential to improve clinical efficacy as well as safety and can be seen as the next generation of immunotherapies. As a result, interest in bispecific antibodies has grown considerably in recent years, and there are now numerous bispecific immunotherapies in clinical and preclinical development. The majority of these are T-cell redirecting compounds targeting CD3 and tumor-associated antigens.^{7,8} The concept builds on the fact that the compound will bind and activate T cells *via* CD3, and redirect these T cells to the tumor area *via* the tumor antigen binding property. One obvious disadvantage with this approach is that CD3 will recruit T cells indiscriminately. As a result, we now see a second wave of bispecific antibodies emerging where novel approaches are being explored in order to achieve more selective recruitment and activation of tumor-specific T cells, or a more powerful immunomodulation by targeting two distinct immunoregulatory pathways.

Rationale for developing bispecific antibodies in cancer immunotherapy

A bispecific antibody can be seen as two therapeutic drugs merged into one superior entity harboring the effect of both drugs. While this may appear to be the ultimate goal, this is not usually sufficient. In fact, most companies developing bispecific antibodies do so with the objective of generating a drug with superior properties compared to the combination of the two monospecific drugs, or in order to create a drug with properties that cannot be achieved with a mixture of monospecific compounds.

Thus, the demands to justify development of a bispecific antibody are high. This is due to the fact that the development of bispecifics is considerably more challenging than development of conventional monospecific antibodies. The inherent properties of different bispecific formats must be carefully considered in order to obtain optimal clinical efficacy and safety along with acceptable developability properties and a cost-effective manufacturing process. In addition, the dosing regimen of the two targets cannot be independently controlled for a bispecific antibody, as would be the case for a combination therapy with two monospecific compounds. On the other

hand, the development of a bispecific monotherapy may be less complex than the co-development of two monospecific drugs, for example in terms of establishing the therapeutic dose and dosing regimen. This aside, there should generally be a clear biological rationale behind every bispecific drug being developed.

The benefits of bispecific *versus* monospecific antibodies can be divided into improved efficacy and improved safety. Bispecific antibodies present a range of opportunities to improve efficacy. One concept includes cytotoxic effector cell redirectors such as T-cell or natural killer (NK)-cell redirecting compounds, in which the cytotoxic function of the effector cells is directed to malignant cells expressing a particular tumor antigen.^{8,9} A similar concept is that of tumor-targeted immunomodulators.¹⁰⁻¹² Such compounds focus the immune-activating pharmacologic effects to the tumor area, thereby achieving improved efficacy as well as reduced systemic immune-related adverse effects compared to systemic immunomodulation. Another concept includes dual immunomodulators, in which two different immune-activating entities are merged into one molecule.^{13,14} Such compounds may hold the combined activity of both original drugs, but also allow for additional synergies and unexpected novel biological effects that could not be achieved by combination treatment with the corresponding monospecific antibodies. For instance, increasing cell-to-cell interactions and clustering of costimulatory receptors may promote stabilization of immunological synapses, thereby triggering signaling.

Factors influencing the effect of bispecific antibodies

The functional properties, and thus ultimately the clinical success, of a bispecific antibody will depend on three major factors: (1) the biological rationale; (2) the format of the bispecific compound; and (3) the absence or presence and properties of an Fc domain.

Biological rationale

The biological rationale for a compound takes into account the biological targets and their modes of action as well as target-binding properties. For tumor-targeting approaches such as T-cell and NK-cell redirection and tumor-targeted immunomodulation, the choice of tumor antigen is critical. The expression pattern of the tumor antigen

will impact safety as well as efficacy, and the ideal tumor antigen is highly upregulated on a large proportion of tumor cells, and absent in normal tissue. In addition, the preferred immune-activating target may need to be adapted to the choice of tumor antigen and target indications. For instance, it could be hypothesized that T-cell redirection using CD3 activation may be better suited for liquid tumors, as indicated by the clinical effects of blinatumomab and catumaxomab,^{15–18} although current development will reveal whether such compounds may also be effective in the treatment of solid tumors. In contrast, costimulatory receptors such as 4-1BB found to be upregulated on tumor-specific tumor-infiltrating T cells¹⁹ may be more suitable for solid tumors, where tumor-infiltrating T cells could preferentially be activated by a tumor-targeted immunomodulator acting on, for example, 4-1BB. For dual immunomodulators, the ability of the two targets to induce superior synergy when targeted by a bispecific compound compared to combination therapy with the corresponding monospecific entities is an important aspect. This is sometimes difficult to predict and may require extensive screening of bispecific compounds in order to identify the optimal bispecific combination. Finally, binding affinity for each target is an important factor that may impact on effect, safety and dosing, and will depend on the relative expression pattern and levels of the two targets. For instance, for T-cell redirecting compounds, a relatively higher affinity for the tumor antigen compared to CD3 may improve tumor-localized T-cell activation and reduce systemic CD3 targeting and toxicity.²⁰

Bispecific format

In addition to the various biologically functional properties of a bispecific antibody, physicochemical properties such as size, binding valency, isoelectric point (pI), and manufacturability of the bispecific format will impact the behavior and success of the compound. Various bispecific antibody formats have been described elsewhere^{7,8} and will not be further discussed here, but examples of the formats used for various approaches and their potential impact will be discussed in the sections below.

Fc domain

One aspect related to the chosen bispecific format having impact on the functional activity and mode of action of the molecule is whether the format

contains an Fc domain, and if so, its sequence. The Fc domain provides a compound with various attributes, including sustained circulation half-life by neonatal Fc receptor (FcRn) recycling,²¹ as well as effector functions mediated by FcγR binding, such as antibody-dependent cellular cytotoxicity and complement-mediated lysis.^{22–24} Depending on the desired mode of action, various approaches have been taken. The most common approaches include: absence of Fc to obtain a small molecular size, short half-life and lack of Fc function; Fc with a low level of effector function but retained FcRn binding; and Fc with a retained or enhanced FcγR and/or C1q affinity for potent target cell destruction. Examples of compounds employing these approaches are presented below.

Classes of bispecific antibodies in cancer immunotherapy

Based on the types of biological targets and modes of action, bispecific immunotherapies can be divided into three main categories (Table 1):

- (1) Cytotoxic effector cell redirectors, including
 - (a) T-cell redirectors
 - (b) NK-cell redirectors
- (2) Tumor-targeted immunomodulators
- (3) Dual immunomodulators.

Among these concepts, T-cell redirecting therapies are by far the most advanced, with two approved products and several compounds in clinical development.⁷ In contrast, the other classes are mainly in early stages of research and development, with few programs in the clinical phase.

Cytotoxic effector cell redirectors

T-cell redirection. T-cell redirecting therapies are bispecific compounds engaging a tumor-associated antigen and the T-cell receptor (TCR)–CD3 complex, thereby redirecting T-cell cytotoxicity to malignant cells. In this approach, T-cell activation is independent of antigen specificity and a large proportion of the T-cell pool is activated (Figure 1). The concept of T-cell redirection has been explored for many years, with favorable clinical effects demonstrated in, for example, blinatumomab.^{17,18,25} However, few T-cell redirecting therapies have reached the market or late-stage clinical development, probably due to significant toxicity, manufacturing problems, immunogenicity, and low response rates in solid tumors.^{26–28}

Table 1. Summary of various classes of immunotherapeutic bispecific antibodies, including examples of each class.

Class	Description	Targets	Examples	Stage*
T-cell redirectors	Redirects T cells to malignant cells by targeting a tumor antigen and CD3	CD19 × CD3	Blinatumomab	Market
		EpCAM × CD3	Catumaxomab	Marketed (withdrawn)
		CD20 × CD3	XmAb13676 BTCT4465A RO7082859	I
		CD123 × CD3	MGD006 JNJ-63709178 Xmab14045	I
		BCMA × CD3	JNJ-64007957 BI 836909	I
		B7H3 × CD3	MGD009	I
		CEA × CD3	RO6958688 MT111	I
NK-cell redirectors	Redirects NK cells to malignant cells by targeting a tumor antigen and CD16A	PSMA × CD3	Pasotuximab ES414/MOR209	I
		CD30 × CD16A	AFM13	II
		EGFR × CD16A	AFM24	PC
Tumor-targeted immunomodulators	Directs potent costimulation to the tumor-infiltrating immune cells by targeting a tumor antigen and costimulatory molecules such as CD40 or 4-1BB	BCMA × CD16A	AFM26	PC
		TA × CD40	ABBV-428	I
		HER2 × 4-1BB	PRS343	I
		FAP × 4-1BB	4-1BB agonist	PC
Dual immunomodulators	Simultaneous targeting of two immunomodulating targets, resulting in blockade of inhibitory targets, depletion of suppressive cells or activation of effector cells	5T4 × 4-1BB	ALG.APV-527	PC
		PD-L1 × TGF-β	M7824	I
		PD-1 × LAG-3	MGD013	I
			FS118	PC
		PD-1 × TIM-3	MCLA-134	PC
	PD-1 × CTLA-4	XmAb20717	PC	
	CTLA-4 × OX40	ATOR-1015	PC	

* I, clinical phase I; II, clinical phase II; PC, preclinical development; TA, tumor antigen.

Unique features of T-cell redirecting therapies include lack of dependency on tumor specificity through TCR engagement, as well as high sensitivity to CD3-mediated activation. In addition,

many T-cell redirecting therapies are independent of costimulation through CD28 or IL-2.^{20,29–31} It is hypothesized that molecules such as the so-called *Bispecific T-cell Engagers* (BiTEs) may

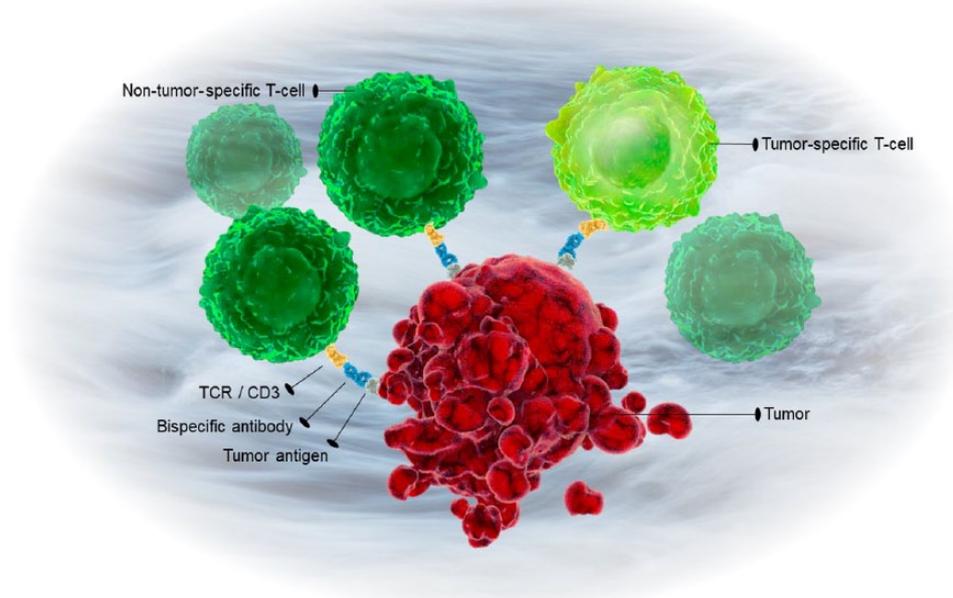


Figure 1. T-cell redirection. T-cell redirecting therapies are bispecific compounds targeting a tumor antigen expressed by the malignant cell, and CD3 expressed by the T-cell. Upon binding to both targets, the compound redirects T-cell cytotoxicity toward the malignant cells. This effect is independent of T-cell specificity, and is mainly mediated by non-tumor-specific T cells.

cluster TCR–CD3 complexes within the induced immunological synapse, triggering signaling in the absence of costimulation.^{20,32,33} Another hypothesis is that memory T cells are the predominant effector cells in T-cell redirection-mediated cytotoxicity as they seem to require less stimulation to be fully activated.^{20,34} T-cell activation mediated by T-cell redirectors can be achieved at very low antibody doses or toward tumor antigens with low expression levels, in the range of hundreds to thousands of tumor antigens per cell.^{20,26,35,36}

However, T-cell redirecting therapies may be associated with suboptimal antitumor effects. Such compounds may mainly be effective toward tumor cells expressing the specific target antigen, in contrast to tumor cells not expressing or down-regulating the target antigen. Tumor cells expressing low levels of antigen may be spared, resulting in only partial killing of the tumors and development of treatment resistance.³⁷ In addition, antigen-independent activation of T cells leads to rapid and powerful activation of a large T-cell pool, which may explain the toxicity seen by such compounds.^{38,39} The toxicities observed with T-cell redirecting compounds prompt further mechanistic studies of T-cell activation and

dosing regimens to support the development of treatments with an improved safety profile. Despite the challenges, the field has grown considerably in recent years. Two T-cell redirecting compounds have reached the market, blinatumomab (Blinicyto[®]) and catumaxomab (Removab), although the latter was withdrawn from the market in June 2017. In addition, there are currently more than 25 T-cell redirecting compounds in clinical phase I studies, developed for hematological as well as solid tumors.⁸

Blinatumomab is a CD19–CD3 BiTE compound approved for B-cell acute lymphatic leukemia (B-ALL) and currently undergoing phase I and II clinical studies in other hematological malignancies such as diffuse large B-cell lymphoma [ClinicalTrials.gov identifier: NCT01741792], non-Hodgkin's lymphoma [ClinicalTrials.gov identifier: NCT02811679] and multiple myeloma [ClinicalTrials.gov identifier: NCT03173430]. The molecular format is a bispecific tandem scFv, thus lacking an Fc domain and having a serum half-life of 2 h.^{34,40,41} The short half-life may be a benefit of the compound, enabling a short wash-out period upon toxic side effects. However, it is also associated with an inconvenient dosing regimen involving continuous infusions and long periods of

hospitalization, often 1–2 weeks after the first dose. In a recent phase III trial of single-agent immunotherapy with relapsed or refractory ALL, blinatumomab demonstrated a significant survival benefit compared to chemotherapy, and significantly higher rates of hematologic remission and longer survival than conventional chemotherapy.¹⁷

Catumaxumab is an EpCAM–CD3 bispecific antibody that was marketed for the treatment of malignant ascites. The molecular format of this compound is very different to that of blinatumomab. It is an asymmetric Fc-containing full-length antibody comprising one heavy and light chain pair binding to each target.^{42–44} The two antibodies used to generate the bispecific compound are of mouse and rat origin, respectively, thus resulting in a hybrid compound. In addition to the T-cell activation induced by CD3 engagement, the clinical effect of the compound is also suggested to be mediated by FcγR engagement of innate immune cells, as well as the immunogenicity induced by the mouse/rat origin of the compound.^{42,44}

Additional examples of T-cell redirecting compounds are listed in Table 1.

NK-cell redirection. An alternative to utilizing the cytotoxic potential of T cells for tumor eradication is to redirect and activate the cytotoxic function of NK cells to malignant cells. Several receptors capable of activating the cytotoxic function of NK cells have been described, including CD16 (FcγRIII), NKp30, NKp46, NKG2D, and DNAM-1.^{45–47} To date, few NK-cell redirecting compounds have been described. One example is AFM13, a CD30–CD16A bispecific compound in the tetravalent TandAb format.⁴⁸ CD16A, or FcγRIIIA, is an FcγR expressed by NK cells and macrophages.^{23,24} Upon binding to the Fc domain of an antibody attached to its cognate antigen, CD16A induces antibody-dependent cellular cytotoxicity (ADCC) of the antigen-expressing cell.⁴⁹ In AFM13, this function is utilized to direct NK-cell cytotoxicity to lymphoma cells expressing CD30. A slow off-rate of the CD16A binding, resulting in a longer duration of binding compared to other CD16A binding compounds, has been shown to be important for the efficacy in target cell killing.⁵⁰ In a phase I clinical study, AFM13 showed signs of clinical activity, activation of NK cells and a decrease in soluble CD30. In addition, the compound was well tolerated, and data indicate that NK-cell redirecting

therapies may be better tolerated compared to T-cell redirecting therapies.⁴⁸ AFM13 is currently in phase II clinical development and two additional CD16A bispecific compounds, AFM24 and AFM26, targeting EGFR or BCMA, respectively, are in preclinical development.^{51,52}

In addition to bispecific NK-cell redirecting compounds, several companies and academic research groups are exploring tri- and tetraspecific approaches to improve tumor selectivity as well as NK-activating capacity. One example of such a concept is the TriFlex format, in which tumor selectivity of antibody-induced cellular cytotoxicity *via* CD16 is increased by dual targeting of the tumor antigens BCMA and CD200.⁵³ Other concepts include TriKE and TetraKE, in which NK activation *via* CD16 is further potentiated by including interleukin-15 in the construct.^{54–56}

Tumor-targeted immunomodulators

Tumor-targeted immunomodulators belong to an emerging class of bispecific immunotherapies. Most compounds are still in preclinical phases of research and development and clinical data are lacking. However, explorative preclinical studies suggest great potential.^{57–59}

The concept builds on the stimulation of tumor-infiltrating, preactivated immune cells such as tumor-specific effector T cells (Figure 2), and the capacity to promote T-cell cytotoxicity, survival and long-term immunological memory. The ability to activate many different clones of tumor-specific T cells may enable recognition of tumor cells with tumor antigen heterogeneity. In that sense, tumor-targeted immunotherapies differ from T- or NK-cell redirecting therapies, in which the activity is dependent on expression of the tumor antigen targeted by the compound.^{20,50,60} In brief, T- or NK-cell redirecting therapies direct a large pool of nonspecific T cells to tumor cells expressing a defined antigen, whereas tumor-targeted immunomodulators more selectively activate a smaller pool of tumor-specific T cells. The former results in a massive acute immune attack with less likelihood of inducing immunological memory, while the latter induces a slow-onset immune attack strongly associated with immunological memory and thus a potential for sustained antitumor effects.

One of the few tumor-targeted immunotherapies in clinical development is ABBV-428,¹⁰ a tumor-targeting CD40 bispecific compound. CD40 is a

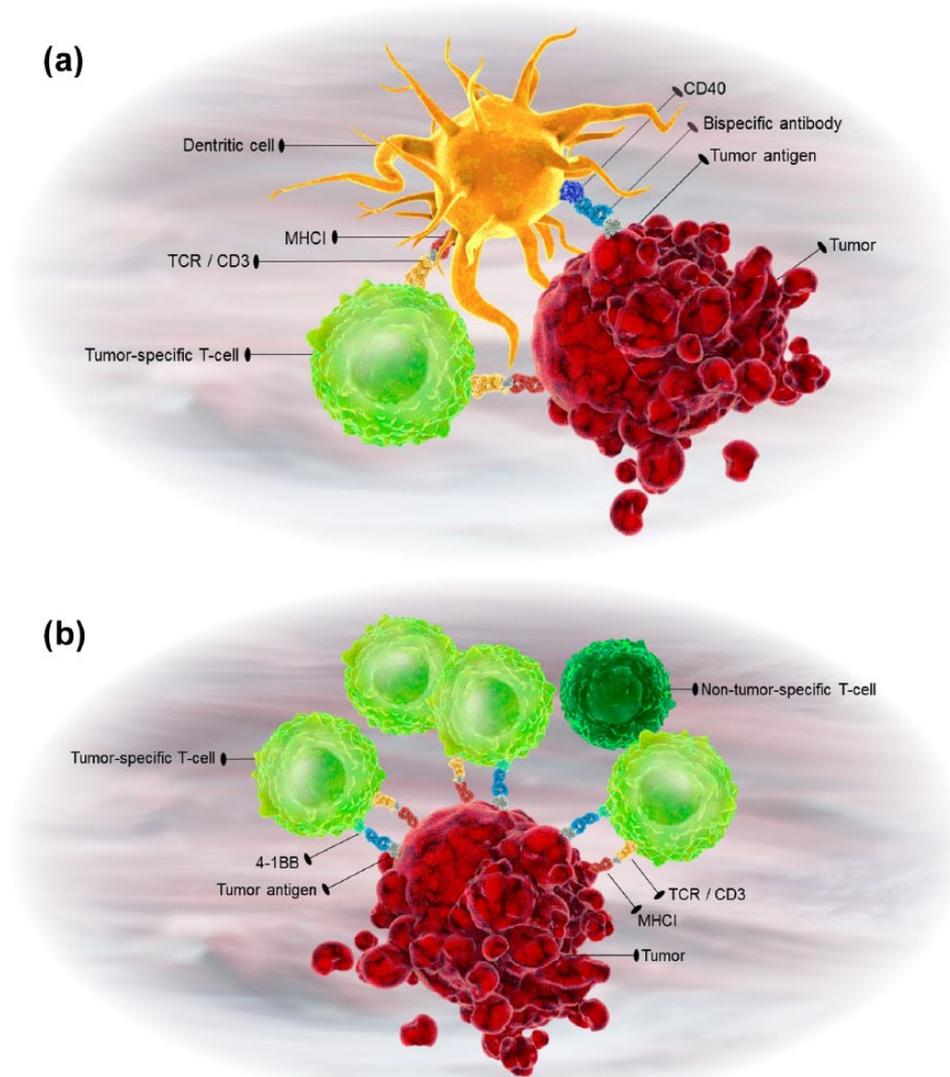


Figure 2. Tumor-targeted immunomodulators. Tumor-targeted immunomodulators are bispecific compounds targeting a tumor antigen expressed by the malignant cell, and an immunomodulatory receptor expressed by tumor-infiltrating immune cells. By activation of the immunomodulatory receptor, the preexisting, tumor-specific immune response induced in the tumor environment by the interplay between antigen-presenting cells, T cells and tumor cells is stimulated. Tumor-targeted immunomodulators may either activate antigen-presenting cells, for example *via* the CD40 pathway, stimulating their ability to induce a potent antitumor immune response (a); or they may act directly on tumor-specific T cells, for example by activation of 4-1BB (b).

potent stimulator of antigen-presenting and myeloid cells and has been shown to promote tumor immunity in a vast number of model systems.^{61–68} However, due to the potent immunostimulating role of CD40, systemic toxicity may in some cases be a challenge for clinical development.^{69–72} Stimulation of CD40, as well as other members of the tumor necrosis factor receptor superfamily (TNFRSF), is dependent on receptor oligomerization.⁷³ Therefore, most agonistic CD40 antibodies need to be crosslinked – for instance by the

interaction between the Fc domain of the antibody and Fc γ R expressed on immune cells. By generating a bispecific molecule with a silent Fc domain and a tumor-binding entity, crosslinking and CD40 activation is induced upon tumor antigen binding, and ABBV-428 has been shown to activate CD40 only in the presence of the tumor antigen.¹⁰ Interestingly, the liver toxicity and systemic inflammatory response observed with a monospecific CD40 antibody was not seen with the tumor-targeted bispecific CD40 agonist.

Another interesting concept is tumor-targeted activation of 4-1BB, which is upregulated on effector T cells, and the stimulation of which promotes cytotoxic function as well as induction of immunological memory.^{74–76} Also, 4-1BB is upregulated on tumor-infiltrating T cells, and has been proposed as a marker for tumor-specific T cells.¹⁹ In addition to its function on T cells, it has been shown to promote the cytotoxic function of NK cells,^{77,78} and to activate tumor vasculature.⁷⁹ Since 4-1BB is a potent costimulatory receptor, systemic activation may result in severe toxicity. Indeed, the initial clinical development of the agonistic 4-1BB monospecific antibody urelumab was terminated due to fatal hepatotoxicity, and was later restarted at a significantly lower dose level.⁸⁰ In contrast, the agonistic 4-1BB antibody utomilumab has been well tolerated even at high doses, possibly attributed to the fact that this antibody requires crosslinking in order to be active.⁸¹ Utomilumab has an Fc with low activity (Fc γ 2), reducing crosslinking and likely biological activity. Thus, in order to obtain optimal safety and efficacy of a 4-1BB agonist, tumor-targeted activation is a promising approach.

A number of companies are developing bispecific tumor-targeted 4-1BB compounds, composed of a tumor antigen binding moiety and a 4-1BB agonist. An important common feature of these compounds is the lack of significant 4-1BB activation in the absence of tumor antigen binding, thereby ensuring tumor-localized immune activation. One example of such a bispecific compound is PRS343.¹² The compound is based on a HER2 antibody to which a 4-1BB agonistic anticalin is fused to the C-terminus of the heavy chain. It contains a silenced Fc γ 4 domain to avoid systemic Fc γ R-mediated crosslinking and activation of 4-1BB. This program is in phase I clinical development.⁸² Another tumor-targeting 4-1BB agonist is a fusion protein composed of a trimeric 4-1BB ligand, a Fab moiety targeting fibroblast activation protein (FAP), and a silenced Fc domain lacking affinity for Fc γ Rs and C1q.¹¹ Importantly, it was shown that hepatic toxicity of conventional 4-1BB monospecific antibodies is dependent on Fc γ R interaction, whereas no such toxicity was observed for this compound. Clinical development will soon be initiated. A third example of a similar approach is ALG.APV-527. This is a tetravalent, bispecific antibody based on the ADAPTIR platform, comprising a 4-1BB agonistic antibody and an antibody targeting the tumor antigen 5T4.^{83,84} Thus,

in contrast to the tumor-targeted 4-1BB agonists described above, this compound is fully antibody-based. Additional tumor-targeted 4-1BB agonists that have been described include bispecific molecules based on the DART[®] and DARPin formats.^{85,86}

One aspect to consider for tumor-targeted immunomodulators is the potential effect of the selected bispecific format on receptor signaling. As mentioned above, members of the TNFRSF require oligomerization for signaling to occur. This may be facilitated by formats allowing bivalent or trivalent interaction with the receptor. In fact, this approach is used for most compounds of this class.^{10–12,85,86} In addition, tumor-targeted immunomodulators appear to require higher tumor antigen density compared to T-cell redirecting therapies,^{85,87,88} making such therapies more suitable for tumor antigens with high expression levels. Indeed, antigens chosen for tumor-targeted 4-1BB agonists include HER2, FAP and 5T4, which are expressed at high levels on tumor and stromal cells.

Dual immunomodulation

Dual immunomodulators are bispecific compounds that bind two distinct immunomodulating targets (Figure 3). Most compounds in this class combine the targeting of two T-cell checkpoint pathways such as PD-1, PD-L1, LAG-3 and TIM-3, with the majority of compounds combining targeting of PD-1 or PD-L1 with that of LAG-3 or TIM-3. The general rationale for these types of compounds is based on the fact that each of the targets inhibits cytotoxic effector functions and blockade has been shown to promote tumor immunity. In addition, additive or synergistic effects have been obtained by combination therapies in the clinic and in animal models.^{3,89–93} As mentioned above, a true benefit of dual immunomodulators would be achieved if superior efficacy is obtained by the bispecific compound compared to combination therapy with the corresponding monospecific entities. Whether this is the case remains to be demonstrated. However, it may be speculated that some properties of dual immunomodulators may indeed result in synergistic effects not achieved by monospecific combinations. For instance, the avidity effects obtained by targeting two checkpoint molecules on the same cell, in particular by multivalent compounds, may lead to prolonged inhibition of

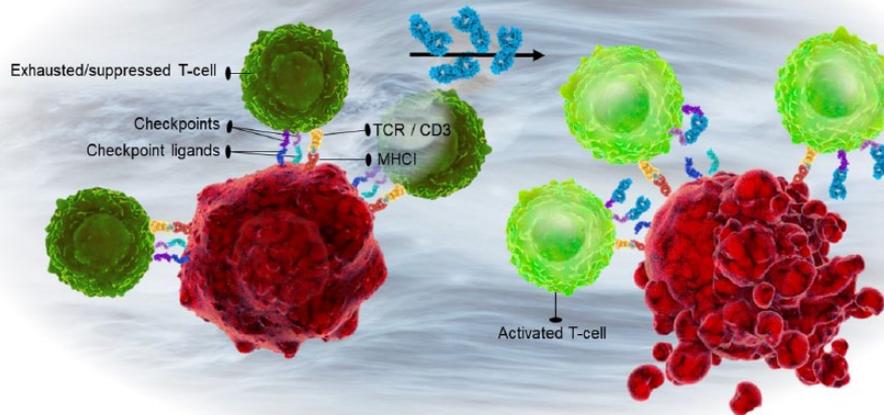


Figure 3. Dual immunomodulators. Dual immunomodulators are bispecific compounds binding two distinct immunomodulating targets. Most such compounds block two inhibitory checkpoint pathways, thereby reducing the immunosuppressive mechanisms inhibiting tumor immunity.

immunosuppressive pathways or more effective depletion of regulatory cells. In addition, cell clusters formed upon bispecific compounds binding to targets expressed on different cells may facilitate paracrine activation induced by stimulating cytokines produced by the cells being activated by the compound. Finally, the clusters induced by the dual immunomodulators may promote stabilization of immunological synapses and thereby induce stronger signaling.

In contrast to the tumor-targeted immunomodulators described above, both targets of dual immunomodulators have immunomodulating functions. However, several immunomodulating receptors and ligands are upregulated in the tumor environment. The dual binding of two such targets may therefore result in enrichment of the compound in the tumor, thereby resulting in a certain degree of tumor localization of the pharmacological effect.

M7824 is a bispecific immunotherapy designed to simultaneously block two immuno-inhibitory molecules, namely PD-L1 and TGF β .¹³ Both targets are used by tumors to evade immune-mediated destruction, suppressing tumor immunity by inhibiting effector cells such as cytotoxic T cells and NK cells, and inducing immunosuppressive cells such as regulatory T cells or M2 macrophages.^{94,95} This compound differs from

the majority of immune checkpoint bispecifics, which mainly act on targets expressed by T cells. Two phase I clinical studies are being conducted with this compound [ClinicalTrials.gov identifiers: NCT02699515, NCT02517398].

MGD013 is a dual immunomodulator targeting PD-1 and LAG-3. Both targets are expressed on exhausted T cells and tumor-infiltrating lymphocytes, and inhibition of the two targets has been shown to exert a synergistic effect on tumor immunity in mice.⁹¹ MGD013 is based on the DART[®] format, and has been shown to inhibit binding of the ligands for PD-1 (PD-L1 and PD-L2) and LAG-3 (MHCII) and to stimulate T-cell activation.¹⁴ This compound is currently in phase I [ClinicalTrials.gov identifier: NCT03219268]. Examples of other preclinical-stage dual-checkpoint compounds include: FS118, targeting PD-1 and LAG-3;⁹⁶ MCLA-134, targeting PD-1 and TIM-3;⁹⁷ and XmAb-20717, targeting CTLA-4 and PD-1.⁹⁸

ATOR-1015 is a dual immunomodulator targeting CTLA-4 and OX40.⁹⁹ The compound consists of a CTLA-4 inhibitory protein fused to an OX40 agonistic human IgG1 antibody. The CTLA-4 inhibitor is an optimized version of CD86, one of the natural ligands of CTLA-4, and has been affinity-matured to bind CTLA-4 with high affinity while having low affinity for its other counter-receptor, the costimulatory

receptor CD28. ATOR-1015 depletes T cells expressing high levels of CTLA-4 and OX40, such as regulatory T cells in the tumor environment. The activity is several-fold stronger when both targets are engaged simultaneously, which directs the immune activation to the tumor area and reduces systemic toxicity. ATOR-1015 is in late preclinical development.¹⁰⁰

Concluding remarks and future directions

Since the approval of ipilimumab in 2011 and subsequent approvals of PD-1 and PD-L1 inhibitors, cancer immunotherapy has become well-established as a highly effective therapeutic option and is now one of the cornerstones of cancer therapy. Building on the success of these pioneering immunotherapies, bispecific immunotherapies are emerging as a second wave of immunotherapeutic compounds. In order for these compounds to be successful, they should address the limitations of current immunotherapies, as well as offer advantages in terms of efficacy or safety.

The number of bispecific immunotherapeutic antibodies in development has grown considerably over the past years. Most compounds are in early clinical or preclinical phases, and clinical effect and safety remain to be demonstrated. T-cell redirecting therapy is the largest class, with more than 25 compounds in clinical development. Two such compounds, blinatumomab and catumaxomab, have reached the market, while none of the others have been developed beyond phase I. Safety issues seem to be the key challenge, together with low efficacy in solid-tumor indications. The related NK-cell redirecting antibodies seem to be better tolerated, but are still in early phases of clinical development, which makes clinical efficacy difficult to predict.

Tumor-targeted immunotherapy is a class of bispecific compounds with great potential to address safety as well as efficacy issues seen with other immunotherapies. In particular, since such compounds are usually designed to lack activity in the absence of tumor antigen, the safety profile may be superior to most other immunotherapeutic compounds. This may be a significant advantage for combination therapy with most other immunotherapies, reducing the risk of severe adverse events. In addition, since immune activation is localized to the tumor environment, more efficacious compounds can be developed with fewer safety concerns. This also opens up possibilities for improved clinical efficacy.

Finally, dual immunomodulators are an interesting class of compounds developed to induce synergistic effects by simultaneously modulating the activity of two immunoregulating targets. A potential disadvantage of such compounds may be the risk of toxicity related to the strong immune activation. The field is dominated by checkpoint inhibitors, combining inhibition of PD-1 or PD-L1 with other immunosuppressive targets such as TGF- β , LAG-3 and TIM-3. Based on the clinical success of the PD-1/PD-L1 inhibitors and a general interest in checkpoint inhibition in the biotech/pharma industry, the number of such compounds in development is expected to grow. In contrast, very few efforts have been made to develop dual immunostimulators targeting, for example, costimulatory molecules of the TNFR and immunoglobulin superfamilies. However, as the clinical development of agonistic antibodies to, for example, CD40, 4-1BB, OX40, CD27 and ICOS progresses, interest in the development of such dual costimulators can be expected to grow.

To summarize, the field of bispecific immunotherapies is growing rapidly. Over the coming years, clinical data will emerge to improve our understanding of the potential of such compounds.

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Conflict of interest statement

The authors are employees of Alligator Bioscience, a company developing mono- and bispecific antibodies in cancer immunotherapy.

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