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Bispecific antibodies in cancer immunotherapy

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Abstract

Among antibody-based cancer therapies, bispecific antibodies (biAbs) have gained momentum in preclinical and clinical investigations following the regulatory approvals of the trailblazing T-cell engaging biAb (T-biAb) blinatumomab. Discussed herein are recent strategies that aim at boosting the potency and mitigating the toxicity of T-biAbs, broadening their therapeutic utility from hematologic to solid malignancies, and generating T-biAbs *in situ*. In cancer immunotherapy, T-biAbs are facing fierce competition with chimeric antigen receptor T cells (CAR-Ts), a battle for clinical and commercial viability that will be closely watched. However, innovative combinations of T-biAbs and CAR-Ts have also transpired. NK-cell engaging biAbs (NK-biAbs) are reemerging as an alternative that addresses liabilities of T-biAbs. Beyond NK-biAbs, other biAbs designed to recruit cellular and molecular components of the innate immune system will be covered in this reflection on new tools, technologies, and targets.

Introduction

Since October 1, 2017, >500 publications listed in PubMed have reported or reviewed a large variety of bispecific antibodies (biAbs) at various stages of basic, preclinical, and clinical investigations toward cancer immunotherapy. To provide an overview and opinion of current trends, this review focuses on novel insights and concepts that emerged over the past two years. For a comprehensive portrayal of the history of bispecific antibodies in cancer immunotherapy, for a deeper analysis of their opportunities, challenges, and various formats, and for a look at their rich clinical pipeline, the reader is referred to two recent reviews ••[1] •[2].

Among next-generation antibody-based cancer therapies [3], biAbs feature prominently. Thus far, biAbs in cancer immunotherapy have been dominated by T-cell engaging biAbs (T-biAbs) including blinatumomab (Blinicyto®; Amgen), the currently only T-biAb that received marketing authorization in both the USA (FDA approval in 2014) and the EU (EMA approval 2015). Since then, progress has been made toward more potent and safe interventions by T-biAbs and toward broadening their indications from hematologic to solid malignancies. Here I first summarize recent developments of T-biAbs and then look beyond T-cell engagement to other cellular and molecular components of the immune system in the cancer patient that can be redirected by biAbs. Progress on tools, technologies, and targets of

cancer immunotherapeutic strategies involving canonical biAbs exclusively consisting of immunoglobulin (Ig) domains will be discussed along the way. Noncanonical biAbs that combine Ig domains with other proteins, peptides, aptamers, or small molecules will not be covered.

T-biAbs in hematologic malignancies

As one of the most promising classes of cancer immunotherapeutics, T-biAbs mediate potent and selective cytotoxicity by recruiting and activating T cells with one arm and targeting tumor cell surface receptors with the other arm [4,5]. The majority of T-biAbs in preclinical and clinical investigations use an anti-CD3 (specifically CD3 ϵ) antibody fragment as T-cell engaging arm. As component of the T-cell receptor (TCR) complex, CD3 engagement by a T-biAb mediates the formation of cytolytic synapses between T cell and tumor cell that resemble natural cytolytic synapses formed by the interaction of the TCR with a peptide presented by a major histocompatibility complex (MHC) class I or II molecule. Although the concept is simple, reducing it to practice is confounded by numerous molecular and cellular factors. For example, efficient formation of cytolytic synapses is influenced by the affinity and avidity of both T-biAb arms and by the epitope, density, and fluidity of their respective targets on T cells and tumor cells [4].

The 2014 FDA and 2015 EMA approvals of CD3 $\times$ CD19 T-biAb blinatumomab for the treatment of adults and children with refractory or relapsed (R/R) pre-B cell acute lymphoblastic leukemia (pre-B-ALL) [6,7] followed by its 2018 FDA and 2019 EMA approvals for the treatment of adults and children with pre-B ALL in first or second complete remission with minimal residual disease [8–10], are milestones marking the therapeutic utility of T-biAbs. Although blinatumomab has remained the only FDA-approved biAb for cancer therapy thus far, at least 25 other T-biAbs are currently investigated in phase I and II clinical trials for the treatment of hematologic and solid malignancies ••[1]. As trailblazing T-biAb, blinatumomab, which is also in clinical trials for *de novo* pre-B ALL and R/R B-cell non-Hodgkin lymphoma (B-NHL) therapy [11], has continued to provide key insights to the short-term and long-term potency and safety of T-biAbs. Adverse events that can be fatal include cytokine release syndrome (CRS) and neurologic toxicity. Thus, hospitalization during treatment with blinatumomab is recommended. Gaining control over these adverse events by advanced T-biAb engineering and T-biAb target discovery has become a major effort. In a phase III clinical trial with 405 randomly assigned R/R pre-ALL adult patients, blinatumomab resulted in significantly longer overall survival compared to standard-of-care chemotherapy (median 7.7 versus 4.0 months) [6]. Although the median response duration was shorter than one year, the study revealed an improvement in post-treatment health-related quality of life [12].

With the recent FDA and EMA approvals of CD19-targeting chimeric antigen receptor T cell (CAR-T) therapy tisagenlecleucel (Kymriah®; Novartis) for pediatric (25 years and younger) R/R pre-B-ALL and CD22-targeting antibody-drug conjugate (ADC) inotuzumab ozogamicin (Besponsa®; Pfizer) for adult R/R pre-B ALL, two powerful competitors of blinatumomab have emerged. The unfolding battle of these three mechanistically different antibody-based cancer therapies in overlapping indications will provide an assessment of the

clinical and commercial viability of T-biAbs. Especially side-by-side comparisons between blinatumomab and tisagenlecleucel will be closely watched [13]. Conceptually similar to T-biAbs •[14], CAR-Ts constitute another promising class of cancer immunotherapeutics [15]. Two CD19-targeting CAR-Ts, tisagenlecleucel and axicabtagene ciloleucel (Yescarta®; Gilead), have received FDA and EMA approval thus far for pediatric R/R pre-B-ALL (tisagenlecleucel) and adult R/R B-NHL (tisagenlecleucel and axicabtagene ciloleucel) [16]. Unlike any other drugs before, CAR-Ts are living drugs. They are built by transducing autologous T cells from cancer patients with CARs that fuse an extracellular antibody fragment, typically a scFv, to a transmembrane segment followed by the cytoplasmic signaling domain of a T cell costimulatory receptor (typically CD28 or 4-1BB) and the cytoplasmic signaling domain of CD3 ζ of the T-cell receptor complex. As such, a CAR links antibody-mediated binding to T-cell activation. Compared to T-biAbs, CAR-Ts face two key challenges. First, the Good Manufacturing Practice (GMP) production of CAR-Ts is logistically challenging [17,18]. It involves the collection, activation, transduction, expansion, cryopreservation, and infusion of autologous T cells. Second, as living drugs, CAR-Ts can persist forever in the cancer patient. The FDA/EMA-approved CAR-Ts do not have on/off switches that permit control over their on-target-on-tissue, on-target-off-tissue, and off-target-off-tissue activities and, like T-biAbs, carry the risk of CRS and neurological toxicities. Another important criterion when comparing T-biAbs and CAR-Ts is resistance. CD19 modulation causing resistance is seen in up to 25% of patients on CD19-targeting CAR-T therapies [19]. CD19 epitope loss was shown to be caused by truncating mutations [20]. Perhaps due to weaker selection pressure, CD19 epitope loss following blinatumomab treatment appears to be less frequent [21]. However, as T-biAbs become more potent, cancer immunoevasion by antigen modulation will have to be addressed by targeting multiple antigens simultaneously. Thus, it can be anticipated that T-biAbs and CAR-Ts, in simulation of the natural immune system, move from monoclonal to polyclonal treatments. While both T-biAbs and CAR-Ts provide a promising platform for further improvements in terms of potency, safety, and scope, side-by-side comparisons of these conceptually similar but methodologically different interventions are needed [13,22].

Blinatumomab also faces competition from next-generation CD3 $\times$ CD19 T-biAbs for pre-B ALL, B-NHL, and chronic lymphocytic leukemia (CLL) therapy and CD3 $\times$ CD20 T-biAbs for B-NHL and CLL therapy. Several of these are in phase I and II clinical trials and use different biAb formats ••[1] [4]. In fact, T-biAbs use numerous different formats and assemblies to combine two different antibody fragments for binding to T cells and tumor cells •[23]. For example, blinatumomab is composed of an anti-CD3 single-chain Fv (scFv) adjoined to an anti-CD19 scFv (Fig. 1a). The antibody fragments, typically scFv or Fab, can be connected through an Fc module (Fig. 1b) for prolonged circulatory half-life mediated by the neonatal Fc receptor (FcRn) on endothelial cells •[23]. Whereas blinatumomab has a circulatory half-life of approximately two hours and is given as continuous intravenous infusion, Fc-containing biAbs stay in the blood for weeks similar to conventional mAbs and thus allow intravenous bolus injection. However, most T-biAbs use mutated Fc domains that bind to FcRn but not to Fc γ receptors (Fc γ R) in order to avoid off-target T-cell activation by T-biAbs bound to Fc γ R-expressing immune cells in circulation and in tissues. As such, T-biAbs with silenced Fc domains also infiltrate tumor tissues more efficiently [24]. Notable

preclinical advances that have emerged for CD3 × CD19 and CD3 × CD20 T-biAbs in the past two years include combination treatments with the FDA/EMA-approved Bruton tyrosine kinase inhibitor ibrutinib in CLL for increased potency •[25] and pretreatment with FDA/EMA-approved anti-CD20 mAb obinutuzumab for increased safety in R/R B-NHL •[26], respectively.

In addition to CD19 and CD20, clinically investigated T-biAb targets in hematologic malignancies that are each pursued by more than one company include BCMA for multiple myeloma (MM); CD123 for acute myeloid leukemia (AML), chronic myeloid leukemia (CML), myelodysplastic syndrome (MDS), and both B-ALL and T-ALL; CD33 for AML; and CD38 for MM •[1]. None have reached phase III clinical trials yet. Among these, BCMA is particularly interesting in the sense that both a CAR-T [27] and an ADC [28] are in phase II clinical trials, providing another testing ground for comparing the potency, safety, and economy of powerful next-generation antibody-based cancer therapies [29]. At least six different CD3 × BCMA T-biAbs, all based on different formats, are currently in clinical trials for MM •[1], with preclinical data published for several of them [30,31] including a head-to-head comparison of an ADC and a T-biAb in which the latter prevailed [32]. Interestingly, a recent study based on a BCMA-targeting CAR-T showed that γ -secretase inhibitors mitigate the shedding of cell surface BCMA and the concomitant release of soluble BCMA, collectively augmenting the potency of the CAR-T [33]. It is conceivable that combinations of CD3 × BCMA T-biAbs with γ -secretase inhibitors will also boost their potency. Another important indication for T-biAbs is AML, a highly heterogeneous hematologic malignancy with poor prognosis. Only one fourth of newly diagnosed AML patients survive 5 years. Screening of the B-cell repertoire of an AML patient who had been cured by allogeneic hematopoietic stem cell transplantation, yielded a human mAb to a unique sialylated epitope on CD43 overexpressed on AML cells. Conversion of this mAb to a T-biAb using a chemoenzymatic assembly strategy [34] permitted selective depletion of AML cells in the presence of healthy human hematopoietic cells in mouse models •[35].

T-biAbs in solid malignancies

Although collectively three T-biAbs and CAR-Ts have been approved by the FDA and the EMA for the treatment of leukemia and lymphoma, their therapeutic utility for solid malignancies, which make up 90% of all cancers, remains to be established clinically [13]. A CD3 × EpCAM T-biAb, catumaxomab (Removab®; Fresenius), received 2009 EMA approval for intraperitoneal treatment of malignant ascites but was withdrawn from the market in 2017. A major impediment for T-biAbs in solid malignancies is the hypoxia-induced immunosuppressive tumor environment that impedes T-cell infiltration, activation, and proliferation [36,37]. Another challenge is the identification of cell surface receptors that are selectively expressed on cancer cells and can serve as targets for T-biAbs and CAR-Ts that do not harm healthy cells and tissues. Nonetheless, more than a dozen T-biAbs that target solid malignancies are currently investigated in clinical trials •[1]. Targets that are each pursued by more than one company include HER2 in breast and gastric cancer and PSMA in prostate cancer. Other notable targets with recently published preclinical data are glypican 3 (GPC3) in liver, lung, and other cancers [38]; ROR1, which is expressed in both hematologic and solid malignancies [39]; p95HER2 in breast cancer, a carboxyl-terminal

fragment of HER2 not found on healthy cells and tissues [40]; and Mucin 16 (MUC16) in ovarian cancer •[41]. Interestingly, the ability to generate so called TCR-like mAbs that bind peptides presented by MHC class I or II molecules with high specificity and affinity [42], has allowed to begin to expand the target range of T-biAbs from extracellular to intracellular proteins [43,44]. Of course, one of the advantages T-biAbs have over TCRs, namely bypassing MHC restriction, does not apply to T-biAbs that use TCR-like antibody fragments as tumor cell binding arm. To avoid on-target-off-tumor toxicity of T-biAbs that target cell surface receptors with broader expression profiles, probody [45] approaches are being pursued that are based on proteolytic liberation of either tumor targeting or T-cell engagement in the tumor microenvironment [46].

Another interesting target for T-biAbs is EGFRvIII in glioblastoma multiforme (GBM) [47]. Systemically administered T-biAbs have to be able to cross the blood-brain barrier (BBB) to be effective in GBM. Like blinatumomab, the CD3 × EGFRvIII T-biAb in this study uses a tandem scFv format (Fig. 1a) for enhanced diffusion and retainment in the brain due to its lower molecular weight and absence of an Fc domain, respectively. Mediated by FcRn, which is highly expressed on brain endothelial cells, conventional and engineered antibodies with Fc domain that have crossed the BBB undergo efflux from the brain to the blood by transcytosis [48]. Although T-biAbs in tandem scFv or other low molecular weight assemblies without Fc domain may be preferred formats for the treatment of cancers inside the brain, enhanced BBB crossing may pose a liability for T-biAbs targeting cancers outside of the brain. In case of blinatumomab, neurologic toxicity is a common adverse event that may be triggered by BBB crossing events [49]. On the other hand, enhanced diffusion of lower molecular weight T-biAbs may benefit deep tumor tissue penetration in solid malignancies.

However, enhanced diffusion of T-biAbs alone is not sufficient for overcoming the hypoxia-induced immunosuppressive tumor environment in solid malignancies. To augment T-cell infiltration, activation, and proliferation, combination therapies of T-biAbs with checkpoint blockade such as anti-PD1 mAbs are worth considering [50] and have shown promise in preclinical studies [51]. Interestingly, an effort to identify and characterize T-cell populations that respond to blinatumomab unveiled PD1 and LILRB1 as suppressing receptors on distinct CD45RA+ CCR7– CD8+ T cell subsets. Anti-PD1 and anti-LILRB1 mAbs synergistically enhanced the potency of blinatumomab *in vitro* [52]. An alternative to inhibiting suppressing receptors on T cells with antagonizing mAbs is activating stimulating receptors on T cells with agonizing mAbs. In order to confine T-cell activation to the tumor environment, T-biAb compositions that target a tumor or tumor stroma antigen with one arm and agonize T cells with the other arm, are attractive on their own and in combination with T-biAbs as shown recently for a combination of 4–1BB × FAP and CD3 × CEA T-biAbs in xenogeneic and syngeneic mouse models of gastrointestinal cancer •[53]. The same study showed that analogous combinations, namely 4–1BB × CD19 and CD3 × CD20 T-biAbs, can also be applied to cancer immunotherapy of hematologic malignancies. Using an innovative 150-kDa assembly that combines three anti-4–1BB scFv with three anti-EGFR VHH [54] or three anti-CEA VHH [55] nanobodies around the collagen XVIII homotrimerization domain, conceptually similar T-biAbs without Fc domain have been reported.

A panel of biAbs with combined checkpoint blockade, such as PD1 $\times$ CTLA4 biAbs, are currently in phase I and II clinical trials •[1]. Mechanistically related T-biAbs that combine targeting PD-L1, the ligand of PD1, on tumor cells with targeting a different suppressing receptor on T cells, such as CTLA4, LAG3, and TIM3 are also clinically investigated. Targeting ligands of suppressing receptors on T cells can be combined with CD3 engagement as is the case for a recently described CD3 $\times$ B7-H4 T-biAb [56]. Collectively, if adverse events are deemed manageable, future translation of T-biAbs to clinical trials for solid malignancies will likely entail combinations of T-biAbs and checkpoint blockade whether mediated by a mixture of drugs with single functionality or by a single drug with multiple functionalities.

***In situ* expression of T-biAbs**

In general, an inflamed tumor microenvironment is more susceptible for both checkpoint blockade and T-biAbs. Fueled by the 2015 FDA and EMA approvals of the first oncolytic virus (OV), talimogene laherparepvec (Imlygic®; Amgen), which is a live, attenuated herpes simplex virus 1 (HSV-1) with a human GM-CSF transgene and administered to R/R melanoma patients by intratumoral injection [57], OVs have emerged as an exciting tool for triggering inflammation in conjunction with checkpoint blockade [58]. In addition to designing and engineering OVs to selectively and directly lyse tumor cells and stimulate systemic immune responses without harming healthy cells and tissues, they can be equipped with an expression cassette encoding a therapeutic transgene, such as a functional T-biAb [59]. As such, the T-biAb gets delivered for *in situ* expression by tumor cells. This Trojan Horse concept is very attractive as it also obviates a need for manufacturing and administering purified T-biAbs. OVs that have been engineered for T-biAb expression include adenovirus [60] (Fig. 1c) and measles virus [61]. Two independent preclinical studies used an adenovirus to deliver a CD3 $\times$ FAP T-biAb to cancer-associated fibroblasts [62,63]. T-biAb-delivering OVs have also been used in combination with CAR-Ts [64].

In addition to OVs, several different methods for *in situ* expression of T-biAbs have emerged [65]. Virus-free delivery of T-biAbs as transgene for *in situ* expression in mouse models has been achieved by intravenously administering mRNA encapsulated in liver-targeting nanoparticles [66] or by electroporating plasmid DNA into the tibialis anterior muscle •[67]. Sustained *in situ* expression of the tandem scFv format substantially increased its circulatory half-life. Already well established is the concept of *ex vivo* transduction of autologous T cells with T-biAb transgenes as an alternative to CAR transgenes. Concomitant transduction of CD80 and 4-1BBL, the ligands of stimulating receptors CD28 and 4-1BB, respectively, was found to potentiate the antitumor activity of T-biAb-secreting T cells [68]. Introducing both T-biAb and CAR transgenes into T cells, as recently done for an EGFRvIII-targeting CAR-T expressing a CD3 $\times$ EGFR T-biAb in mouse models of GBM, is an attractive approach for addressing tumor heterogeneity and for confining T-biAb expression to the tumor tissue •[69] (Fig. 1d).

CAR-T switches

An interesting new concept that factually bridges T-biAbs and CAR-Ts are so called switchable CAR-Ts that are recruited and activated by a bispecific adapter protein [70,71].

In one example, the antibody component of a universal CAR binds with high affinity and specificity to a nonhuman linear peptide. A mAb in Fab format that recognizes a cancer cell surface antigen and also displays the linear peptide on one of its N- or C-termini then serves as a bispecific adaptor protein that can switch on the otherwise dormant CAR-T. Thus, activity and specificity of the universal CAR-T is controlled by the bispecific adaptor protein, mitigating CRS and other adverse events [72]. Canonical biAbs have also been used as bispecific adapter proteins [73].

Beyond T-biAbs

Substantial progress has been made on employing biAbs for recruiting and activating cellular and molecular components of the innate immune system, including NK cells, macrophages, and complement system [74]. The recent success of T-biAbs has outshined the beginnings of biAbs in cancer immunotherapy that were initially used for redirecting Fc γ R-expressing macrophages, NK cells, and other innate immune cells via CD64 (Fc γ RI) and CD16 (Fc γ RIII) [2]. Although conventional mAbs also bind Fc γ Rs via their Fc domain, such NK cell- and macrophage-engaging biAbs (NK-biAbs and M ϕ -biAbs) enable higher specificity and affinity, do not compete with endogenous immunoglobulins, and do not depend on Fc γ R polymorphisms. Compared to T-biAbs, adverse events of NK-biAbs are generally milder [75]. CD16 $\times$ CD30 NK-biAbs for the therapy of Hodgkin lymphoma and cutaneous T-cell lymphoma and CD16 $\times$ CD33 NK-biAbs for the therapy of AML and myelodysplastic syndrome (MDS) are in phase I and II clinical trials ••[1]. Including IL-15 as third functionality into a CD16 $\times$ CD33 NK-biAb enhanced its ability to recruit and activate NK cells in mouse and *ex vivo* models of AML and MDS [76,77]. In addition to CD16, NK-biAbs engaging NK cells via other activating receptors, such as NKG2D [78] and Nkp46 [79], have been investigated preclinically.

The discovery of CD47 as “don’t eat me signal” that protects malignant and normal cells from phagocytosis by engaging inhibitory receptor SIRP α on macrophages has resulted in a flurry of novel cancer immunotherapy strategies directed at interrupting the CD47/SIRP α axis [80]. This includes preclinical studies with a SIRP α $\times$ CD70 M ϕ -biAb in B-NHL [81]. Although biAbs can be categorized into trans- and cis-acting depending on whether they bridge two targets on the same or on different cells, respectively, the vast majority of biAbs in cancer immunotherapy are trans-acting. Among the few exceptions are biAbs that target CD47 in addition to a second tumor cell surface receptor for higher specificity. For example, CD47 $\times$ CD20 and CD47 $\times$ CD19 biAbs were developed to confine CD47/SIRP α axis blockade to malignant and normal B cells [82,83].

The Fc domain of conventional mAbs mediates antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) by engaging Fc γ Rs on NK cells, macrophages, and other innate immune cells. In addition, it mediates complement-dependent cytotoxicity (CDC), a mechanism that is increasingly harnessed for cancer immunotherapy [84]. To do so requires the neutralization of CD46, CD55, or CD59 which are often overexpressed on cancer cells to evade complement-mediated lysis. In B-NHL, cis-acting CD55 $\times$ CD20 and CD59 $\times$ CD20 biAbs were shown to enhance CDC when compared to conventional anti-CD20 mAbs [85]. Another interesting strategy for using a

cis-acting biAb to enhance CDC is based on inducing cell surface hexamerization of mAbs in IgG1 format by a single amino acid substitution in the Fc domain. Such hexameric IgG1 are much more efficient in recruiting the also hexameric C1q complement component which is the initiating step that triggers CDC [86]. Combining two different hexameric IgG1s, one targeting CD20 and the other CD37, resulted in the formation of mixed hexameric complexes with further enhanced CDC [87,88]. These mixed hexamers can be considered noncovalent cis-acting biAbs. Finally, biAbs that recruit C1q with one arm and target tumor cells with the other arm afford yet another strategy for engaging the complement system in cancer immunotherapy [89].

Conclusions

To prove their worth in cancer immunotherapy, biAbs will have to achieve additional regulatory approvals. Looking at their rich clinical and preclinical pipeline in terms of targets, formats, indications, mechanisms of action, and stakeholders, it appears likely that we will see biAbs capturing market shares in the treatment of both hematologic and solid malignancies. More importantly, biAbs have retained their potential to emerge as one of the most effective interventions toward controlling, if not curing, cancer.

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Highlights

- Opportunities and challenges of T-biAbs in hematologic and solid malignancies
- Conventional versus *in situ* delivery of T-biAbs
- T-biAbs versus CAR-Ts in cancer immunotherapy
- Employing biAbs to engage the innate immune system

Figure 1. Conventional and *in situ* delivery of T-biAbs.

T-biAbs mediate potent and selective cytotoxicity by recruiting and activating T cells (blue) with one arm (also blue) and targeting tumor cells (orange) with the other arm (also orange). Note that the blue arm typically engages CD3 ϵ of the TCR complex whereas the orange arm targets one of many tumor cell surface receptors that can be restricted to the tumor cell (tumor-specific antigen, TSA) or shared with expandable healthy cells (tumor-associated antigen, TAA). **(a)** Conventional T-biAbs in tandem scFv format as shown or other low molecular weight assemblies without Fc domain are administered as continuous intravenous infusion due to their short circulatory half-lives. Alternatively, they can be administered as mRNA via viral or nonviral transduction of liver and muscle cells. **(b)** T-biAbs with Fc domain, as exemplified here by a scFv-Fc format, have long circulatory half-lives and are administered as bolus injection similar to conventional mAbs. **(c)** As part of their *ex vivo* production, CAR-T cells can be transduced with a T-biAb expression cassette for *in situ* delivery, facilitating a one-two punch with the CAR-T targeting a TSA and the T-biAb targeting a TAA. **(d)** OVs, as exemplified here by an adenovirus, can deliver a T-biAb expression cassette to tumor cells. In addition to programming tumor cells for T-biAb production, inflammation triggered by the oncolytic activity of the OV can turn cold into hot tumors, i.e. mitigate immunosuppression in the tumor environment to augment the potency of the T-biAb.