OVERCOMING THE CHALLENGES ASSOCIATED WITH CD3+ T-CELL REDIRECTION IN CANCER

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The development of bispecific antibodies that redirect the cytotoxic activity of CD3+ T cells to tumours is a promising immunotherapeutic strategy for the treatment of haematological malignancies and solid cancers. Since the landmark FDA approval at the end of 2014 of the anti-CD3 × anti-CD19 bispecific antibody blinatumomab (Blincyto®) for the treatment of relapsed/refractory B-cell acute lymphoblastic leukaemia, ~100 clinical trials investigating the safety and efficacy of CD3+ bispecific T-cell redirectors for cancer have been initiated. However, despite early success, numerous challenges pertaining to CD3+ T-cell redirection in the context of cancer exist, including the recruitment of counterproductive CD3+ T-cell subsets, the release of systemic cytokines, the expansion of immune checkpoint molecules, the presence of an immunosuppressive tumour microenvironment, tumour antigen loss/escape, on-target off-tumour toxicity and suboptimal potency. The aim of the present review is to discuss novel approaches to overcome the key challenges associated with CD3+ bispecific T-cell redirection in order to achieve an optimal balance of anti-tumour activity and safety.

BACKGROUND

The development of antibody therapeutics has transformed the field of cancer immunotherapy. The first successful monoclonal antibody (mAb) therapy in oncology was rituximab (Rituxan®), a mouse–human chimeric immunoglobulin G1 (IgG1) mAb targeting the CD20 antigen on B cells for the treatment of non-Hodgkin’s lymphoma. Signalling-induced cell death and Fc-mediated effector functions, including antibody-dependent cellular cytotoxicity (ADCC) and complement fixation, have been identified as key processes that contribute to the multifactorial mechanism of the action of rituximab.

The concept of engineering mAbs directed against cancer antigens expressed on haematological malignancies was subsequently extended to solid cancers and was followed by the design of novel bispecific antibody formats capable of engaging multiple targets. CD3+ bispecific T-cell redirection antibody therapeutics, for example, mechanistically function by bridging T cells with cancer cells. In so doing, the cytolytic activity of CD3+ T cells can be redirected towards tumour cells to facilitate their elimination, independent of the usual requirement for the T cell to be bound to a major histocompatibility complex (MHC) molecule (MHC restriction) (Fig. 1). A major advantage associated with bispecific antibodies is that functional activity can be achieved, which would otherwise not be achievable in the context of monovalent antibody combinations. Nearly 25 years after its initial conceptualisation, the first bispecific CD3+ T-cell redirector, catumaxomab (Removab®), was approved by the European Union (EU) for the treatment of malignant ascites in 2009. However, this anti-CD3 × anti-epithelial cell adhesion molecule (EpCAM) antibody was shown to induce off-target hepatotoxicity in patients due to its binding to hepatic macrophages and was later withdrawn from the market due to commercial reasons.

The Food Drug Administration (FDA) subsequently approved the anti-CD3 × anti-CD19 bispecific T-cell engager blinatumomab (Blincyto®) for the treatment of patients with Philadelphia chromosome-negative B-cell acute lymphoblastic leukaemia (ALL). FDA approval of this drug was based on the results of the multicentre, open-label, single-arm Phase 2 BLAST clinical trial (NCT01207388), in which 78% of patients with B-cell ALL achieved a complete minimal residual disease (MRD) response after just one cycle of treatment. Compared with MRD non-responders, patients who achieved complete MRD responses also demonstrated longer overall survival (12.5 versus 38.9 months; \( P = 0.002 \)) and relapse-free survival (5.7 versus 23.6 months; \( P = 0.002 \)). The unprecedented clinical success of blinatumomab has since laid the foundation for the development of CD3+ T-cell-engaging bispecific antibodies. Although blinatumomab is structurally formatted as a bispecific T-cell engager (BiTE®), additional bispecific antibody designs have been described for CD3+ T-cell redirection (Fig. 2). Next-generation CD3+ bispecific T-cell redirectors are under preclinical and clinical investigation. To date, ~100 clinical trials have been initiated for CD3+ bispecific T-cell redirection in cancer.

Blinatumomab is now approved for the treatment of patients with Philadelphia-negative and -positive relapsed/refractory ALL, as well as those patients with ALL who are in remission but show signs of MRD; however, its use is associated with elevated levels of cytokine release and neurotoxicity, although these toxicities can be clinically managed. Indeed, CD3+ T-cell redirection has been reported to be associated with a number of challenges that might...
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Fig. 1  Mechanism of action of CD3+ bispecific T-cell redirection in cancer. The schematic depicts an IgG-like bispecific antibody simultaneously binding a tumour-associated antigen (TAA) on a cancer cell and CD3 epsilon on a T cell to redirect the cytotoxic activity of T cells to these tumour cells. Limiting its use or reduce its anti-tumour efficacy, including the recruitment of counterproductive CD3+ T-cell subsets, dose-limiting cytokine storm, the presence of an immunosuppressive tumour microenvironment (TME), T-cell dysfunction and exhaustion due to expression of immune checkpoint molecules, tumour antigen escape, on-target off-tumour toxicity and suboptimal potency, all of which will be outlined in this article (Table 1). Continued progress in the clinic and momentum in the field of cancer immunotherapy will therefore rely on addressing these major obstacles, and therefore novel strategies to overcome the challenges associated with the use of T-cell redirection are also described herein.

RECRUITMENT OF PAN T-CELL POPULATIONS
CD3+ bispecific T-cell engagers function by redirecting all T-cell populations towards cancer cells to facilitate efficient tumour cell killing. However, several disadvantages are associated with polyclonal T-cell activation and redirection, including the recruitment of ‘counterproductive’ CD3+ T-cell subsets, such as naïve/exhausted T cells, regulatory T (TREG) cells and other CD4+ T-cell populations. In fact, resistance to blinatumomab has been associated with a good clinical outcome in cancer.20 Menares et al.21 also demonstrated, in 2019, a crosstalk between TRM T cells and dendritic cells that leads to an amplified CD8+ CTL-mediated anti-tumour immune response. In view of these results, research efforts are underway to improve the selectivity of T-cell-engaging bispecific antibodies in favour of more potent tumour cell killing accompanied by reduced toxicity.

CYTOKINE RELEASE SYNDROME
Cytokine release syndrome is a severe immune response that is characterised by the rapid, systemic release of pro-inflammatory cytokines, including interleukin (IL)-6, IL-10, tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ). Elevated levels of these core cytokines induce immune cells to produce excessive amounts of additional cytokines, creating an amplified phenomenon referred to as a ‘cytokine storm’.16,23 This immune-related adverse event is frequently observed in response to chimeric antigen receptor (CAR)-T cell therapy or T-cell redirection therapy and presents a major challenge in the context of CD3+ T-cell redirection because the infused bispecific antibodies indiscriminately bind to all available T cells, leading to a massive release of cytokines. Indeed, blinatumomab and two FDA-approved CAR-T cell therapies, tisagenlecleucel (Kymriah) and axicabtagene ciloleucel (Yescarta), have been shown to induce cytokine release syndrome in patients with leukaemia and lymphoma.24 Clinical manifestations of cytokine release syndrome include fever, fatigue, myalgia, respiratory distress, capillary leakage, vasodilatory shock and eventual organ system failure.25 CD19-CAR-T cells armed with anti-CD3 × anti-HER2 or anti-CD3 × anti-epidermal growth factor receptor (EGFR) bispecific antibodies have been developed to address the cytokine release syndrome.25 This approach is self-limiting and holds potential for reducing the risk of the occurrence of a cytokine storm as the amount of bispecific antibody on the bispecific antibody-armed CAR-T cell decreases after multiple cell divisions.

Managing the cytokine release syndrome using pharmacological approaches
A number of pharmacological approaches have been investigated to overcome the cytokine release syndrome associated with T-cell redirection therapies. Monocyte activation mediated by TNF-α generated by T-cell triggering has been demonstrated to be a primary source of systemic cytokine release after treatment with anti-CD3 × anti-HER2 bispecific antibodies and, interestingly, TNF-α blockade alone was sufficient to impair cytokine release without affecting the cytotoxic activity of the T-cell-directing antibodies.26 Furthermore, administration of the immunosuppressant dexamethasone prior to treatment with an anti-CD3 × anti-glypican 3 (GPC3) bispecific antibody led to significant inhibition of cytokine release without compromising the anti-tumour activity in a humanised mouse model.27 In another study, pre-treatment with the anti-IL-6 receptor antibody tocilizumab prior to administration of an anti-CD3 × anti-prostate-specific membrane antigen (PSMA) bispecific antibody attenuated the cytokine release syndrome while maintaining therapeutic activity in patients with perforin/granzymes and can initiate killing immediately upon crosslinking with tumour cells.18,19 However, a bispecific antibody targeting the CD8 co-receptor and prostate stem cell antigen (PSCA) constructed to test this approach was able to efficiently engage only pre-activated CD8+ T cells and redirect them for tumour cell lysis—freshly isolated CD8+ T cells could not be activated/redirected—thus signifying the importance of both pre-activation and co-ligation in inducing selective tumour killing.17 The tissue-resident memory (TRM) CD8+ CD69+ CD103+ T-cell phenotype has also emerged as an important T-cell population for selective retargeting to tumour cells, as this memory CD8+ T-cell subset expresses high levels of cytotoxic molecules and is associated with a good clinical outcome in cancer.26

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castration-resistant metastatic prostate cancer. However, in this same study, dexamethasone treatment prior to bispecific antibody therapy was shown to negatively impact anti-tumour efficacy, as evidenced by suppression of T-cell proliferation and inhibition of tumour lysis. Similarly, the data from a Phase 1 clinical trial showed that dexamethasone treatment did not increase the maximum tolerated dose of an anti-CD3 × anti-carcinoma embryonic antigen (CEA) bispecific antibody (NCT01284231). Taken together, the results of these studies suggest that co-administration of CD3+ T-cell-directing therapies with steroids,
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Table 1. Mitigation strategies to overcome the challenges associated with CD3+ bispecific T-cell redirection in cancer.

| Challenges associated with CD3+ T-cell redirection in cancer | Resulting consequences | Mitigation strategies |
|-------------------------------------------------------------|------------------------|----------------------|
| Recruitment of pan T-cell populations                      | Activation/redirection of counterproductive CD3+ T-cell subsets (e.g., Tregs, naive/exhausted T cells, CD4+ T cells that secrete excessive amounts of cytokines) | Selective redirection of CD8+ cytotoxic T lymphocytes or CD8+ tissue-resident memory T cells |
| Cytokine release syndrome                                   | Cytokine storm (life-threatening immune-related adverse event); dose-limiting; efficacy-limiting; narrow therapeutic window | Pharmacological intervention with IL-6R blockers, or corticosteroids; quantitative cytokine modelling to improve clinical dosing strategy; sequence-based antibody discovery to decouple cytokine release from anti-tumour activity |
| Expression of immune checkpoint molecules                  | T-cell dysfunction and exhaustion lead to suppressed anti-tumour immunity | Combination therapy with co-inhibitory receptor antagonists (e.g., anti-PD-1, anti-CTLA-4) or co-stimulatory receptor agonists (e.g., anti-4-1BB, anti-CD28) |
| Immunosuppressive tumour microenvironment                  | Hostile TME components (e.g., stromal cells, immune cells, cytokines, soluble factors) contribute to suppression of anti-tumour immune response | Combination therapy with IL-10 inhibitors, adenosine receptor antagonists, or VLA4 inhibitors; oncolytic adenoviruses armed with CD3 redirectors that target immunosuppressive stromal cells |
| Tumour antigen escape                                       | Resistance to CD3+ T-cell redirection | Development of CD3+ T-cell redirectors with dual tumour antigen targeting |
| Lack of tumour antigen selectivity                          | On-target off-tumour toxicity | Protease-activated T-cell engagers; hemibodies; development of a multivalent avidity-based antigen-binding strategy to generate bispecific antibodies that selectively redirect T cells towards tumour cells while avoiding healthy tissue |
| Suboptimal potency                                          | Diminished anti-tumour immune response | Consideration of target antigen copy number and size; modulation of binding domain positioning (e.g., epitope distance) and valency |

anti-TNF-α blockers or IL-6 receptor targeting antibodies might, in certain circumstances, help to mitigate the cytokine storm associated with T-cell redirection.29 The outcomes of such combinatorial approaches are context-dependent and could be influenced by the specific mechanism of a given T-cell redirector. Thus, a deeper mechanistic understanding of the relationship between T-cell activation and the cytokine storm is warranted. The identification of clinical biomarkers that are predictive of a cytokine release syndrome will aid in the future development of novel therapeutic agents and approaches that are capable of mitigating the cytokine storm in the context of T-cell redirection. Of note, regression modelling has been used to identify a cytokine signature in patients that is predictive of the development of the cytokine release syndrome after CAR-T cell treatment.29 Novel strategies to mitigate the cytokine release syndrome Although the cytokine release syndrome has the potential to be life-threatening inflammatory response was not predicted by pre-clinical toxicology models.32,33 Preventing future occurrences of this magnitude in other T-cell-based immunotherapies quickly became a priority. Since this devastating Phase 1 clinical trial, different clinical dosing regimens for T-cell-engaging therapeutic antibodies have been considered. A priming dose strategy has been tested in the clinic to mitigate the toxicities induced by the cytokine release syndrome—this approach involves administration of a lower initial dose followed by a higher maintenance dose (NCT02152956).32,33 As the methodology of selecting an optimal priming dosing regimen is empirical by nature, quantitative cytokine modelling was later introduced to improve the design of clinical dosing strategies to achieve efficacy while minimising cytokine release syndrome-related toxicities. Indeed, a novel pharmacokinetic–pharmacodynamic (PK-PD) model developed in 2019 simulates the cytokine release profiles in response to treatment with T-cell-directing therapies.33 A quantitative systems pharmacology model was subsequently established to enable the accurate prediction of safety- and efficacy-related biomarkers to reduce the risk of cytokine storm with the anti-CD3 × anti-CD20 bispecific antibody mosunetuzumab in non-Hodgkin lymphoma (NCT02500407).34 Although quantitative modelling has been used to determine the optimal dosing to mitigate the cytokine release syndrome associated with current T-cell-engaging antibodies, next-
generation CD3+ T-cell-recruiting bispecific antibodies have been engineered to directly address the challenge of limiting the dose to prevent cytokine release syndrome. Initial anti-CD3+ antibody generation efforts were often biased towards high-affinity CD3 binders, which elicited not only potent tumour cell killing, but also high levels of cytokine release. The identification of naturally occurring anti-CD3 antibodies with varying binding affinities and T-cell activation profiles soon became a priority. A next-generation sequence (NGS)-based antibody discovery platform established in 2019 to identify anti-CD3 antibodies from humanised rats that bind to multiple CD3 epitopes with a diverse range of binding strengths was used to identify lead anti-CD3-binding domains, which were then formatted as bispecific antibodies. In particular, the anti-CD3+anti-BCMA bispecific T-cell redirector that emerged from this antibody screening was shown to elicit robust tumour antigen-specific killing in a mouse xenograft model with almost undetectable cytokine release. Importantly, this work provides proof of concept for uncoupling of tumour cell cytotoxicity from cytokine release, thus relieving the dose constraints associated with T-cell engagers and broadening the therapeutic index. Collectively, these results show that PK-PD modelling and sequence-based antibody discovery strategies can be used to mitigate cytokine release syndrome-related toxicities from T-cell-redirecting antibody therapeutics.

Additional next-generation T-cell-recruiting bispecific antibodies have been described, including an elegant body of work that demonstrated the use of bispecific antibodies to retarget ex vivo expanded T cells to solid and haematological malignancies such as non-Hodgkin’s lymphoma, multiple myeloma, pancreatic cancer, breast cancer and prostate cancer. These armed T-cell infusions not only show preclinical and clinical anti-tumour efficacy, with or without stem cell transplantation, but, importantly, avoid cytokine release syndrome. The lack of apparent dose-limiting toxicity provides a rationale for the use of bispecific antibody-armed activated T-cell infusions as a strategy to overcome the challenge of cytokine release syndrome.

**EXPRESSION OF IMMUNE CHECKPOINT MOLECULES**

Inhibitory checkpoint molecules and stimulatory checkpoint molecules function alongside each other to ensure that the immune system can mount a response against foreign bodies but that this response is controlled, in terms of type, magnitude and duration, in order to maintain self-tolerance and prevent autoimmunity. It is well established that inhibitory molecules expressed on the surface of T cells or tumour cells can promote T-cell dysfunction and exhaustion, leading to a suppressed anti-tumour immune response. Examples of such co-inhibitory immune checkpoint molecules include programmed death-1 (PD-1), programmed death-ligand 1 (PD-L1), cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), T-cell immunoglobulin mucin domain-containing protein 3 (TIM3), lymphocyte-activation gene 3 (LAG3) and T-cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT). Enrichment of immune checkpoint receptor expression was observed in CD8+CD69+CD103+ human T cells with a TRM phenotype, and anti-PD-1 treatment led to a significant expansion of this T-cell population in melanoma patients. Moreover, anti-PD-1 therapy was shown to enhance the infiltration of tissue-resident CD8+ memory T cells in a mouse model of melanoma.

CD3+ bispecific T-cell redirection combined with immune checkpoint co-inhibitors

Combinatorial immunotherapy approaches have been designed to counteract the mechanisms of T-cell dysfunction/exhaustion using inhibitory checkpoint-blocking antibodies to improve the clinical outcomes of individuals receiving CD3+ bispecific T-cell redirection therapies. Interestingly, resistance to blinatumomab treatment has been attributed to an increase in PD-L1 expression on CD19+ leukaemic cells. Since this discovery, a number of clinical trials evaluating the safety and efficacy of blinatumomab in combination with pembrolizumab (anti-PD-1 therapy) for the treatment of lymphoma or leukaemia have been initiated (NCT03160079, NCT03605589, NCT03340766, NCT03512405). Furthermore, the addition of the dual immune-checkpoint blockade to blinatumomab therapy is under investigation in the clinic (e.g., a Phase 1 trial of blinatumomab and nivolumab (an anti-PD-1 antibody) with or without ipilimumab (an anti-CTLA-4 antibody) for the treatment of leukaemia is underway (NCT02879695)). A bifunctional checkpoint inhibitory T-cell engager (CiTE) that redirects T cells to CD33 (expressed on acute myeloid leukaemia (AML) cells), combined with the extracellular domain of PD-1 to bind weakly to, but inhibit engagement of PD-L1, demonstrated early preclinical success in mitigating adaptive immune escape in patients with AML. Notably, anti-CD3+anti-PD-1+anti-HIV and anti-CD3+anti-TIGIT+anti-HIV trifunctional T-cell engagers have demonstrated efficacy in enhancing CD8+T-cell effector functions in HIV-infected rhesus macaques; this model can probably be applied in the context of cancer. Immune checkpoint inhibition coupled with CD3+ bispecific T-cell redirection has also been examined in solid tumours. The safety and efficacy of MGD007 (anti-CD3+anti-gpA33 bispecific antibody) in combination with MGA012 (an anti-PD-1 antibody) is currently under clinical investigation in a Phase 1/2 trial for metastatic colorectal cancer (NCT03531632). A quantitative systems pharmacology model and biomarker strategy revealed that combination therapy comprising an anti-CD3+anti-CEA bispecific antibody with anti-PD-L1 treatment led to an enhancement of anti-tumour activity in patients with colorectal cancer.

CD3+ bispecific T-cell redirection combined with immune checkpoint co-stimulators

Although antagonising co-inhibitory receptors on T cells has proven to be a promising approach for enhancing the anti-tumour activity of CD3+ T-cell redirectedors, there is also evidence that agonistic antibodies that are capable of activating co-stimulatory receptors on T cells, including members of the tumour necrosis factor receptor (TNFR) superfamily (e.g., 4-1BB, OX40, GITR, CD27) or immunoglobulin superfamily (e.g., CD28, ICOS), can be used to boost immune responses to CD3+-T-cell engaging therapies. Notably, an agonistic anti-4-1BB antibody was shown to restore the functionality of exhausted CD8+ tumour-infiltrating lymphocytes in a mouse model of melanoma. Likewise, stimulation of 4-1BB signalling with an agonistic anti-4-1BB antibody led to the prolonged persistence of cytotoxic T lymphocytes and their enhanced function in a B16F10-OVA melanoma mouse model. Anti-4-1BB co-stimulation was also demonstrated to enhance the anti-tumour activity of an anti-CD3+anti-PSMA bispecific antibody both in vitro and in a syngeneic mouse model of prostate cancer. Furthermore, combination therapy of fibroblast activation protein (FAP)-4-1BBL with a CEA-targeted T-cell bispecific antibody plus CD19+4-1BBL with a CD20-targeted T-cell bispecific antibody led to significant tumour regression in mouse models and intratumoural accumulation of activated CD8+ cytoxic T cells. Mechanistically, in the presence of T-cell receptor signalling, tumour antigen-targeted 4-1BB agonists induce 4-1BB stimulation in response to crosslinking with tumour antigen-expressing cells without relying on FcγR-mediated crosslinking. As well as 4-1BB, other co-stimulatory molecules such as CD28 have been shown to improve anti-tumour immune responses to CD3+-T-cell-redirecting antibodies. Treatment of cancer cells with anti-CD3+anti-tumour antigen and anti-CD28+anti-tumour antigen cross-interacting bispecific antibodies led to enhanced tumour cell-dependent T-
Physiologically relevant models to assess combinatorial approaches

Given the complexity of the TME, designing optimal preclinical models that recapitulate human physiology and the disease phenotype remains a priority—the development of preclinical models that translate to human immunity was described by Hegde et al.,53 as being a top key challenge in cancer immunotherapy. Three-dimensional multicellular tumour spheroids and ‘tumour on a chip’ microfluidics devices are examples of next-generation platforms that have been developed to increase preclinical-to-clinical translation of drug candidates.54,55 Such complex in vitro models are engineered to incorporate extra-cellular matrix components, soluble factors, tissue barriers, hypoxia gradients, interstitial pressure and vascular perfusion to recreate organ-specific architecture. Stromal cells, immune cells and endothelial cells can also be integrated into these micro-physiological tumour models to enable a deeper understanding of the interactions between cancer cells and their surrounding TME. Indeed, lysis mediated by T-cell-receptor (TCR)-engineered T cells of hepatocellular carcinoma cells embedded in a 3D collagen microenvironment was demonstrated using a microfluidics platform.66 Moreover, proof of concept was established for the assessment of tumour cell cytotoxicity by TCR-engineered T cells under hypoxic and inflammatory conditions in the microfluidics device. Physiologically relevant complex in vitro models can therefore represent powerful preclinical tools that can be used to evaluate such combinatorial immunotherapies to better predict translational success.

TUMOUR ANTIGEN ESCAPE AND LACK OF TUMOUR ANTIGEN SPECIFICITY

The loss of expression of tumour antigens is another factor that can compromise the efficacy of CD3+ bispecific T-cell redirection therapies. For example, 10–20% of patients with B-cell ALL have been shown to experience resistance to blinatumomab treatment due to CD19 antigen loss.65 The emergence of CD19+ leukemic blasts during blinatumomab therapy has been mechanistically linked to disruptions in the trafficking of CD19 to the membrane.

Overcoming tumour antigen escape

The development of novel CD3+ T-cell redirectors with the ability to target two tumour antigens is a potential strategy that can be used to overcome tumour antigen escape and restore anti-tumour immunity. In fact, the strategy of simultaneously targeting two tumour antigens using bispecific tandem CARs has already been demonstrated to mitigate tumour antigen escape.68,69 A single-nucleotide polymorphism (SNP) present in the V domain of CD33 is known to limit the anti-tumour activity of anti-CD33 antibodies due to antigen loss, but the development of a novel bispecific antibody that targets CD3 and the C2 domain of CD33 resulted in T-cell-mediated tumour lysis independent of the SNP genotype.70 These findings indicate a therapeutic benefit in designing CD3+ T-cell redirectors that target the C2 domain and not the V domain of the CD33 epitope for the treatment of AML.

Improving tumour antigen specificity

Identifying antigenic targets that are specific to tumour cells and not expressed on healthy tissue is another challenge associated with developing CD3+ bispecific T-cell redirectors, especially for the treatment of solid malignancies. Even low-level antigen expression on normal cells can lead to detrimental on-target off-tumour toxicities in which the cytolytic activity of CD3+ T cells is directed towards healthy tissue. Preclinical studies suggest that tuning the affinity of CD3+ bispecific T-cell engagers towards target antigens is a rational approach to achieve an optimal balance of anti-tumour activity and safety. To reduce the occurrence of T-cell autoreactivity towards antigen-expressing
vital organs, a multivalent avidity-based antigen-binding design strategy has been developed to generate an anti-CD3 × anti-HER2 bispecific antibody capable of selectively killing HER2-expressing tumour cells. Selective redirection of CD3+ T cells towards HER2+ cancer cells was achieved by engineering the anti-CD3 × anti-HER2 bispecific antibody with two low-affinity anti-HER2 Fab arms that engage HER2-overexpressing tumour cells with a high antigen density relative to healthy tissue. This seminal work provides proof of concept for redirecting the cytotoxicity of T cells to tumour cells while sparing normal tissue and could be applicable to other solid tumour targets that are challenged by on-target off-tumour toxicity.

Another means to address the lack of tumour antigen specificity associated with CD3+ T-cell redirection involves the use of a conditionally active COBRA (Conditional Bispecific Redirected Activation) T-cell engager platform. COBRAs are prodrugs that are selectively activated upon cleavage by matrix metalloproteases (MMPs) in the protease-rich TME. This selective activation enables them to remain as inactive prodrugs in normal tissue where the proteolytic activity is tightly regulated but become active T-cell engagers in the context of tumours, where proteases are ubiquitously expressed. Indeed, COBRA constructs with an MMP-9 cleavable linker targeting anti-CD3, anti-EGFR and antihuman serum albumin were shown to induce tumour regression in a xenograft mouse model of colorectal cancer. Additional protease-activated T-cell engagers have also been described. Tumour specificity was demonstrated using an anti-folate receptor 1 (FOLR1) T-cell bispecific antibody engineered with an anti-idiotypic anti-CD3 ‘mask’ joined to an anti-CD3 Fab region via a protease-cleavable linker. ‘Unmasking’ of the anti-CD3 targeting moiety by cleavage by active proteases in the TME led to selective killing of FOLR1-expressing tumour cells in vitro and in vivo, while sparing normal cells with low FOLR1 expression. Next-generation designs of masked antibodies that are capable of being selectively unmasked at tumour sites have been optimised. Incorporation of a heterodimeric coiled-coil domain into an antibody construct was shown to be an effective and generalisable approach for masking the complementary-determining regions (CDRs) of an antibody, with selective cleavage of coiled-coil peptides by tumour-associated proteases restoring antigen binding while overcoming on-target off-tumour toxicity. Moreover, preclinical proof-of-concept studies demonstrated that such probody therapeutics improved the safety profile of EGFR-targeting antibodies and broadened the therapeutic index. A first-in-human Phase 1/2 clinical trial evaluating the safety and tolerability of a PD-L1 probody therapeutic (CX-072) as monotherapy or in combination with ipilimumab or vemurafenib for the treatment of solid tumours or lymphomas (NCT03013491) is currently underway.

In addition to protease-activated T-cell engagers, a novel split T-cell engaging antibody format—referred to as a hemibody (e.g., an antigen-specific scFv fused to the VH or VL region of an anti-CD3 antibody)—has been described. Simultaneous engagement of two complementary hemibodies with their respective antigens on a single target cell activated the CD3-binding site and redirected CD3+ T cells for tumour cell lysis. Using this approach, hemibodies can selectively kill dual-antigen-positive tumour cells while sparing cells that express a single antigen, thereby overcoming the limitation of off-target toxicity commonly associated with CD3+ T-cell redirection.

SUBOPTIMAL POTENCY
Improving the potency of CD3+ bispecific T-cell redirectors is an ongoing challenge. Both the size of the target antigen and the distance of the target epitope to the T-cell membrane have been identified as important factors contributing to the anti-tumour activity of CD3+ T-cell engagers. Interestingly, anti-CD3 × anti-chondroitin sulphate proteoglycan 4 (CSPG4) bispecific antibodies demonstrated greater potency, in terms of T-cell-mediated tumour lysis, when the size of the target antigen was smaller. Moreover, binding of the anti-CD3 × anti-CSPG4 bispecific antibody to the membrane-proximal domain D3 of CSPG4 rather than the distal domain elicited a more potent anti-tumour immune response by the redirected T cells. Additional factors, such as the copy number and valency of tumour antigens, have also been shown to affect the potency of bispecific T-cellredirectors. Collectively, these preclinical data suggest that awareness of target antigen size/copy number and modulation of binding domain positioning and valency should be considered in the design of more potent, best-in-class CD3+ T-cell redirectors.

CONCLUDING REMARKS AND FUTURE DIRECTIONS
Antibody-based therapy—particularly CD3+ bispecific T-cell redirection—has revolutionised the field of cancer immunotherapy. From a preclinical aspect, anti-tumour activity in both haematological malignancies and solid cancers has been achieved with CD3+ T-cell engaging bispecific antibodies by addressing some of the major obstacles inherent to this therapeutic class, such as the expression of inhibitory immune checkpoint molecules, the presence of an immunosuppressive TME, suboptimal potency, escape of tumour antigens, cytokine storm and on-target off-tumour toxicity. Encouragingly, many of these novel CD3+ bispecific T-cell antibodies have progressed to clinical trials and next-generation designs of CD3+ bispecific T-cell redirectors are being characterised. Intriguingly, preclinical evidence has demonstrated that in vitro BiTE-mediated tumour cell cytotoxicity is enhanced by the expression of CD80/CD86 co-stimulatory molecules on tumour cells. The engagement of CD80/CD86-activating ligands on cancer cells with their cognate receptor (CD28) on naive T cells provides co-stimulation to support T-cell activity. These data are further corroborated by robust evidence suggesting that CAR-T cell potency is enhanced upon the incorporation of co-stimulatory domains (e.g., CD28, 4-1BB) into the CAR backbones. However,
Table 2. Clinical trials of CD3+ bispecific T-cell redirectors for haematological cancers.

| Targets | Clinical trial Phase | Clinical trial identifier | Disease indication(s) | Organisation(s) | Antibody |
|---------|----------------------|--------------------------|-----------------------|-----------------|----------|
| CD3 × CD19 | Launched | NCT01207388 | B-cell precursor ALL | Amgen | Blinatumomab; Blincyto; AMG 103 |
| CD3 × CD19 | Phase 1 | NCT03571828 | B-cell NHL; DLBCL; MCL; FL | Amgen | AMG 562 |
| CD3 × CD19 | Phase 1 | NCT04056975 | B-cell lymphoma | Generon | A-319 |
| CD3 × CD19 | Phase 1 | CTR20191955 | B-cell lymphoma | Lvyzhu Biopharmaceutical | K193 |
| CD3 × CD20 | Phase 1 | NCT02651662 | B-cell NHL; CLL | Regeneron | REGN1979 |
| CD3 × CD20 | Phase 1 | NCT04082936 | B-cell NHL; DLBCL; FL; MZL | IGM | IGM-2323 |
| CD3 × CD20 | Phase 1 | NCT02924402 | B-cell NHL; CLL; small lymphocytic lymphoma; MZL | Xencor | Plamotamab; XmAb13676 |
| CD3 × CD20 | Phase 1 | NCT02500407 | B-cell NHL; CLL; DLBCL | Genentech; Hoffmann-La Roche | Mosunetuzumab; BTCT4465A; RO7030816 |
| CD3 × CD20 | Phase 1 | NCT03075696 | B-cell NHL; DLBCL; FL | Hoffmann-La Roche | Glofitamab; RO7082859 |
| CD3 × CD33 | Phase 1 | NCT0244946 | B-cell NHL; multiple myeloma; plasma cell neoplasm | Barbara Ann Karmanos Cancer Institute | CD20Bi-ATC |
| CD3 × BCMA | Phase 1 | NCT02514239 | Multiple myeloma | Glenmark Pharmaceuticals | GBR 1342 |
| CD3 × BCMA | Phase 1 | NCT02445563 | Multiple myeloma | Amgen | AMG 424 |
| CD3 × BCMA | Phase 1 | NCT03647800 | Multiple myeloma | AbbVie; TeneoBio | TNP-383B |
| CD3 × BCMA | Phase 1 | NCT03269136 | Multiple myeloma | Pfizer | PF-0663135 |
| CD3 × BCMA | Phase 1 | NCT03145181 | Multiple myeloma | Janssen | JNJ-64007957 |
| CD3 × BCMA | Phase 1 | NCT03486067 | Multiple myeloma | Celgene | CC-93269 |
| CD3 × BCMA | Phase 1 | NCT03671018 | Multiple myeloma | Boehringer Ingelheim; Amgen | BI 836909; AMG 420 |
| CD3 × BCMA | Phase 1 | NCT03933735 | Multiple myeloma | AbbVie; TeneoBio | TNP-383B |
| CD3 × BCMA | Phase 1 | NCT03269136 | Multiple myeloma | Pfizer | PF-0663135 |
| CD3 × BCMA | Phase 1 | NCT03145181 | Multiple myeloma | Janssen | JNJ-64007957 |
| CD3 × BCMA | Phase 1 | NCT03486067 | Multiple myeloma | Celgene | CC-93269 |
| CD3 × BCMA | Phase 1 | NCT03671018 | Multiple myeloma | Boehringer Ingelheim; Amgen | BI 836909; AMG 420 |
| CD3 × BCMA | Phase 1 | NCT03933735 | Multiple myeloma | AbbVie; TeneoBio | TNP-383B |
| CD3 × BCMA | Phase 1 | NCT03269136 | Multiple myeloma | Pfizer | PF-0663135 |
| CD3 × BCMA | Phase 1 | NCT03145181 | Multiple myeloma | Janssen | JNJ-64007957 |
| CD3 × BCMA | Phase 1 | NCT03486067 | Multiple myeloma | Celgene | CC-93269 |
| CD3 × BCMA | Phase 1 | NCT03671018 | Multiple myeloma | Boehringer Ingelheim; Amgen | BI 836909; AMG 420 |
| CD3 × BCMA | Phase 1 | NCT03933735 | Multiple myeloma | AbbVie; TeneoBio | TNP-383B |
| CD3 × BCMA | Phase 1 | NCT03269136 | Multiple myeloma | Pfizer | PF-0663135 |
| CD3 × BCMA | Phase 1 | NCT03145181 | Multiple myeloma | Janssen | JNJ-64007957 |
| CD3 × BCMA | Phase 1 | NCT03486067 | Multiple myeloma | Celgene | CC-93269 |
| CD3 × BCMA | Phase 1 | NCT03671018 | Multiple myeloma | Boehringer Ingelheim; Amgen | BI 836909; AMG 420 |
| CD3 × BCMA | Phase 1 | NCT03933735 | Multiple myeloma | AbbVie; TeneoBio | TNP-383B |
| CD3 × BCMA | Phase 1 | NCT03269136 | Multiple myeloma | Pfizer | PF-0663135 |
| CD3 × BCMA | Phase 1 | NCT03145181 | Multiple myeloma | Janssen | JNJ-64007957 |
| CD3 × BCMA | Phase 1 | NCT03486067 | Multiple myeloma | Celgene | CC-93269 |
| CD3 × BCMA | Phase 1 | NCT03671018 | Multiple myeloma | Boehringer Ingelheim; Amgen | BI 836909; AMG 420 |
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| CD3 × BCMA | Phase 1 | NCT03145181 | Multiple myeloma | Janssen | JNJ-64007957 |
| CD3 × BCMA | Phase 1 | NCT03486067 | Multiple myeloma | Celgene | CC-93269 |
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| CD3 × BCMA | Phase 1 | NCT03269136 | Multiple myeloma | Pfizer | PF-0663135 |
| CD3 × BCMA | Phase 1 | NCT03145181 | Multiple myeloma | Janssen | JNJ-64007957 |
| CD3 × BCMA | Phase 1 | NCT03486067 | Multiple myeloma | Celgene | CC-93269 |
Table 3. Clinical trials of CD3+ bispecific T-cell redirectors for solid cancers.

| Targets              | Clinical trial Phase | Clinical trial identifier | Disease indication(s)                                                                 | Organisation(s)                                                                 | Antibody         |
|----------------------|----------------------|---------------------------|--------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|------------------|
| CD3 × B7-H3          | Phase 1              | NCT03406949               | Advanced solid tumours                                                               | MacroGenics                                                                    | Orfotamab; MGD009|
| CD3 × CEACAM         | Phase 1              | NCT02291614               | Gastrointestinal adenocarcinoma                                                      | Agenen; MedImmune                                                               | AMG 211; MEDI-565|
|                      |                      | NCT01284231               |                                                                                      |                                                                                 |                  |
| CD3 × CEACAM         | Phase 1/2            | NCT03866239               | Colorectal cancer; NSCLC                                                             | Hoffmann-La Roche                                                              | Cibisatamab; RO6958688|
|                      |                      | NCT03337998               |                                                                                      |                                                                                 |                  |
|                      |                      | NCT02665073               |                                                                                      |                                                                                 |                  |
|                      |                      | NCT02324257               |                                                                                      |                                                                                 |                  |
| CD3 × CLDN18.2       | Phase 1              | NCT04260191               | Gastric and gastro-oesophageal junction adenocarcinoma                               | Agenen                                                                        | AMG 910          |
|                      |                      | NCT033319940              |                                                                                      |                                                                                 |                  |
| CD3 × DLL3           | Phase 1              | NCT03296969               | Glioblastoma                                                                         | Agenen                                                                        | AMG 757          |
|                      |                      | NCT0248805                |                                                                                      |                                                                                 |                  |
| CD3 × EGFRvIII       | Phase 1/2            | NCT01420874               | Pancreatic cancer; colorectal cancer                                                   | Barbara Ann Karmanos Cancer Institute; University of Virginia                 | EGFRvIII-ATC; EGFR-BATs|
|                      |                      | NCT02620865               | glioblastoma                                                                         |                                                                                 |                  |
|                      |                      | NCT03269526               |                                                                                      |                                                                                 |                  |
|                      |                      | NCT03344260               |                                                                                      |                                                                                 |                  |
| CD3 × EpCAM          | Phase 1              | CTR20181212               | Malignant ascites                                                                    | Wuhan YZY Biopharma                                                           | M701             |
| CD3 × EpCAM          | Withdrawn from market| Withdrawn from market     | Malignant ascites                                                                    | Trion Pharma                                                                   | Catumaxomab; Removab|
| CD3 × GD2            | Phase 1/2            | NCT02173093               | Neuroblastoma                                                                        | University of Virginia                                                        | GD2Bi-aATC       |
|                      |                      | NCT03531632               | Colorrectal cancer                                                                    | MacroGenics                                                                    | MGD007           |
|                      |                      | NCT02248805               |                                                                                      |                                                                                 |                  |
| CD3 × gp100          | Phase 1/2            | NCT03707392               | Uveal melanoma; malignant melanoma                                                    | Immunocore; University of Pennsylvania                                         | IMCgp100         |
|                      |                      | NCT02535078               |                                                                                      |                                                                                 |                  |
|                      |                      | NCT02570308               |                                                                                      |                                                                                 |                  |
|                      |                      | NCT01211262               |                                                                                      |                                                                                 |                  |
|                      |                      | NCT01209676               |                                                                                      |                                                                                 |                  |
| CD3 × GPC3           | Phase 1              | NCT02748837               | Solid tumours                                                                        | Chugai Pharmaceutical                                                          | ERY974           |
| CD3 × GUCY2c         | Phase 1              | NCT04171141               | Gastrointestinal tumours; colorectal cancer; oesophageal adenocarcinomas            | Pfizer                                                                        | PF-07062119      |
|                      |                      | NCT03661424               |                                                                                      |                                                                                 |                  |
| CD3 × HER2           | Phase 1/2            | NCT03406585               | Prostate cancer; breast cancer; leptomeningeal metatases                              | Barbara Ann Karmanos Cancer Institute; University of Virginia                 | HER2Bi-ATC; HER2-BATs|
|                      |                      | NCT03272334               |                                                                                      |                                                                                 |                  |
|                      |                      | NCT00027807               |                                                                                      |                                                                                 |                  |
| CD3 × HER2           | Phase 1              | NCT03448042               | Solid tumours                                                                        | Genentech                                                                      | BTRC4017A        |
| CD3 × HER2           | Phase 1/2            | NCT03983395               | Breast cancer                                                                        | Glenmark Pharmaceuticals                                                        | GBR 1302; ISB 1302|
|                      |                      | NCT02171194               | Advanced solid tumours                                                               | Wuhan YZY Biopharma                                                           | M802             |
| CD3 × MAGE-A4        | Phase 1/2            | NCT03973333               | Advanced solid tumours                                                               | Immunocore Ltd                                                                 | IMC-C103C         |
|                      |                      | NCT03872206               | Ovarian cancer; fallopion tube cancer; peritoneal cancer; pancreatic cancer; mesothelioma | Harpoon Therapeutics                                                           | HPNS36           |
| CD3 × MSLN × ALB     | Phase 1/2            | NCT03540199               | Kidney cancer; breast cancer; lung cancer; gastric cancer; pancreatic cancer; colorectal cancer | Fuda Cancer Hospital, Guangzhou; Benhealth Biopharmaceutical | Activated CIK and CD3-MUC1 |
|                      |                      | NCT03524261               |                                                                                      |                                                                                 |                  |
|                      |                      | NCT03501056               |                                                                                      |                                                                                 |                  |
|                      |                      | NCT0354395                |                                                                                      |                                                                                 |                  |
|                      |                      | NCT03509298               |                                                                                      |                                                                                 |                  |
|                      |                      | NCT03146637               |                                                                                      |                                                                                 |                  |
| CD3 × MUC1           | Phase 2              | NCT03564340               | Ovarian cancer; fallopion tube cancer; primary peritoneal cancer                      | Regeneron; Sanofi                                                               | REGN4018         |
| CD3 × MUC16          | Phase 1/2            | NCT04117958               | Gastric and gastro-oesophageal junction cancer                                       | Agenen                                                                        | AMG 199          |
| CD3 × MUC17          | Phase 1              | NCT02659631               | Triple-negative breast cancer; colorectal cancer                                       | Pfizer                                                                        | PF-06671008      |
| CD3 × P-cadherin     | Phase 1              | NCT04262466               | Advanced solid tumours                                                               | Immunocore Ltd                                                                 | IMC-F106C         |
| CD3 × PSACA          | Phase 1              | NCT03927573               | Pancreatic cancer; breast cancer; urogenital cancer; NSCLC                           | Celgene                                                                        | GEM3PSCA         |
| CD3 × PSMA           | Phase 1              | NCT03926013               | Prostate cancer; renal cell carcinoma                                                | Janssen                                                                        | JNJ-63898081     |
| CD3 × PSMA           | Phase 1              | NCT03929841               | Prostate cancer                                                                      | Agenen                                                                        | AMG 160          |
| CD3 × PSMA           | Phase 1              | NCT01723475               | Prostate cancer                                                                      | Amgen; Bayer   | Pasotuxizumab; AMG 212 |
| CD3 × PSMA           | Phase 1              | NCT02629210               | Prostate cancer                                                                      | Aptevo Therapeutics                                                           | E5414            |
| CD3 × PSMA × ALB     | Phase 1              | NCT03577028               | Prostate cancer                                                                      | Harpoon Therapeutics                                                           | HPN424           |
| CD3 × SSTR2          | Phase 1              | NCT03141191               | Neuroendocrine tumours; gastrointestinal stromal tumours                              | Xencor                                                                        | Tidutamab; XmAb18087|
| CD3 × STEAP1         | Phase 1              | NCT04221542               | Prostate cancer                                                                      | Amgen                                                                         | AMG 509          |
| CD3 × ST4            | Phase 1              | NCT04424641               | Solid tumours                                                                        | Genmab; AbbVie                                                                | GEN1044          |

CEACAM carcinoembryonic antigen-related adhesion molecule, CLDN18.2 claudin-18 isoform 2, DLL3 delta-like canonical notch ligand 3, EGFRvIII epidermal growth factor receptor vIII, EGFR epidermal growth factor receptor, EpCAM epithelial cell adhesion molecule, gpA33 glycoprotein A33, gp100 glycoprotein 100, GUCY2c guanylate cyclase 2c, HER2 human epidermal growth factor receptor 2, MAGE-A4 melanoma-associated antigen A4, MSLN mesothelin, ALB albumin, MUC1 mucin 1, MUC16 mucin 16, MUC17 mucin 17, PRAME preferentially expressed antigen in melanoma, PSCA prostate stem cell antigen, PSMA prostate-specific membrane antigen, SSTR2 somatostatin receptor type 2, STEAP1 six transmembrane epithelial antigen of the prostate 1, NSCLC non-small cell lung cancer, SCLC small-cell lung cancer.
although co-stimulation via CD28 has provided benefit to BiTEs and CAR-T cells alike by enhancing anti-tumour activity without inducing toxicity, cases have arisen where too much co-stimulation has induced life-threatening immune-related adverse events such as cytokine storm, as observed using the anti-CD28 super-agonist TGN1412.\textsuperscript{24} As such, optimising co-stimulatory signalling is important for achieving anti-tumour activity while minimising toxicity. Accordingly, simultaneous multiple interaction T-cell-engaging (SMITE) bispecific antibodies, comprising a combination of two BiTE antibodies, have been developed.\textsuperscript{85} In fact, T-cell co-stimulation with an anti-CD28 \texttimes anti-ROR1 BiTE was shown to enhance the anti-tumour activity of a CD3 \texttimes ROR1 BiTE in K562 cells transduced with ROR1. In addition, co-activation of T cells with a CD28 \texttimes PD-L1 BiTE and a paired CD3 \texttimes CD19 BiTE was demonstrated to overcome BiTE resistance by reversing immune checkpoint inhibition in PD-L1-expressing cancer cells.\textsuperscript{85} Furthermore, a bispecific antibody retrotagged CAR, comprising an extracellular folate receptor fused to intracellular TCR and CD28 costimulatory domains in tandem, has been developed.\textsuperscript{86} This work demonstrated proof of concept for combining bispecific antibodies with genetically engineered T cells, which led to simultaneous TCR activation/co-stimulation and induced a tumour-antigen-specific T-cell response. CD3\textsuperscript{+}- Redirecting tri-specific antibodies have also been designed to bridge T cells to tumour cells in the presence of co-stimulation. For example, the anti-CD3 \texttimes anti-CD28 \texttimes anti-CEA tri-specific antibody was shown to mediate tumour-specific cytolysis of CEA-expressing cancer cells in vitro.\textsuperscript{87} Along the same lines, an anti-CD3 \texttimes anti-CD28 \texttimes anti-CD38 tri-specific T-cell redirector antibody has been developed (Fig. 3), which, when administered in vivo, led to the proliferation of effector/memory T-cell populations and the suppression of tumour growth in CD38-expressing melanoma.\textsuperscript{88} Next-generation CD3\textsuperscript{+} T-cell engagers that exploit signalling through co-stimulatory molecules appear to represent promising antibody therapeutics for cancer immunotherapy.

A novel T-cell redirection strategy called STAB immunotherapy, which involves the endogenous secretion of T cell-redirecting bsAbs (bispecific antibodies), has also emerged as a promising platform in oncology.\textsuperscript{89} In this approach, T cells are genetically engineered to secrete bispecific T-cell-redirecting antibodies intratumourally (known as ‘on-tumour’ STABs) or at a distant site (‘off-tumour’ STABs). This platform addresses some of the challenges associated with systemic antibody administration, such as inadequate tumour penetration, on-target off-tumour toxicity, and short serum half-life. Proof of concept for STAB cancer immunotherapy has been demonstrated in preclinical in vitro and in vivo studies.\textsuperscript{90–92}

Overall, substantial progress has been made towards the development of CD3\textsuperscript{+} bispecific T-cell redirectors for cancer immunotherapy. A diverse panel of haematological and solid tumour targets have been characterised for CD3\textsuperscript{+} T-cell redirection, and the safety and efficacy of \textasciitilde100 antibodies in this therapeutic class are currently under investigation in the clinic for the treatment of haematological cancers and solid tumours (Tables 2 and 3). Nonetheless, a number of challenges associated with CD3\textsuperscript{+} T-cell redirection exist that can limit anti-tumour efficacy. As many of these challenges are common to other cancer immunotherapy approaches such as CAR-T cell therapy,\textsuperscript{93} lessons learned from these studies can potentially be applied to CD3\textsuperscript{+} T-cell redirection. Future directions in the field of cancer immunotherapy are likely to involve identifying optimal combination therapies with CD3\textsuperscript{+} bispecific T-cell redirectors that enhance anti-tumour immunity while minimising toxicity.

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REFERENCES

1. Salles, G., Barrett, M., Foa, R., Maurer, J., O’Brien, S., Valente, N. et al. Rituximab in B-Cell hematologic malignancies: a review of 20 years of clinical experience. Adv. Ther. 34, 2232–2273 (2017).
2. Weiner, G. J. Rituximab: mechanism of action. Semin Hematol. 47, 115–123 (2010).
3. Goebeler, M. & Bargou, R. C. T-cell-engaging therapies—BITEs and beyond. Nat. Rev. Clin. Oncol. 17, 418–434 (2020).
4. Deshaies, R. J. Multispecific drugs herald a new era of biopharmaceutical innovation. Nature 580, 329–338 (2020).
5. Labrijn, A. F., Janmaat, M. L., Reichert, J. M. & Parren, P. W. H. I. Bispecific antibodies: a mechanistic review of the pipeline. Nat. Rev. Drug Discov. 18, 585–608 (2019).
6. Borflak, J., Langer, F., Spanel, R., Schondorfer, G. & Dittrich, C. Immune-mediated liver injury of the cancer therapeutic antibody catumaxomab targeting EpCAM, CD3 and Fcy receptors. Oncotarget 7, 28059–28074 (2016).
7. Nagorsen, D., Kufer, P., Baeuerle, P. A. & Bargou, R. Blinatumomab: a historical perspective. Pharm. Ther. 136, 334–342 (2012).
8. Golkugetur, N., Dombret, H., Bonifacio, M., Reichle, A., Graux, C., Faur, C. et al. Blinatumomab for minimal residual disease in adults with B-cell precursor acute lymphoblastic leukemia. Blood 131, 1522–1531 (2018).
9. Suurs, F. V., Lub-de Hooge, M. N., de Vries, E. G. E. & de Groot, D. J. A. Review of bispecific antibodies and antibody constructs in oncology and clinical challenges. Pharm. Ther. 201, 103–119 (2019).
10. Nie, S., Wang, Z., Moscoso-Castro, M., D’Souza, P., Lei, C., Xu, J. et al. Biology drives the discovery of bispecific antibodies as innovative therapeutics. Antib. Therapeutics 3, 18–62 (2020).
11. Yu, L. & Wang, J. T-cell redirecting bispecific antibodies in cancer immunotherapy: recent advances. J. Cancer Res. Clin. Oncol. 145, 941–956 (2019).
12. Pennock, N. D., White, J. T., Cross, E. W., Cheney, E. E., Tamburini, B. A. & Kedi, R. M. T cell responses: naïve to memory and everything in between. Adv. Physiol. Educ. 37, 273–283 (2013).
13. Golubovskaya, V. & Wu, L. Different subsets of T cells, memory, effector functions, and CAR-T immunotherapy. Cancers 8, 36 (2016).
14. Chraa, D., Naim, A., Olive, D. & Badou, A. T lymphocyte subsets in cancer immunity: friends or foes. J. Leukoc. Biol. 105, 243–255 (2019).
15. Duell, J., Dittrich, M., Bedke, T., Mueller, T., Eisele, F., Rosenwald, A. et al. Frequency and CAR-T immunotherapy. Cancers 2181 (2017).
16. Michalk, I., Feldmann, A., Koristka, S., Amdt, C., Cartellieri, M., Ehnring, A. et al. Characterization of a novel single-chain bispecific antibody for retargeting of T cells to tumor cells via the TCR co-receptor CD8. PLoS ONE 9, 95517 (2014).
18. Zhang, N. & Bevan, M. J. CD8+ T cells: foot soldiers of the immune system. *Immunity* 35, 161–168 (2011).
19. Feldman, A., Arndt, C., Topfer, K., Starnova, S., Krone, F., Cartellieri, M. et al. Novel humanized and highly efficient bispecific antibodies mediate killing of prostate stem cell antigen-expressing tumor cells by CD8+ and CD4+ T cells. *J. Immunol.* 189, 3249–3259 (2012).
20. Thakur, A., Hahn, S., Hans, S., T. Granier, C., Saldman, A., Anson, M. et al. Targeting resident memory T cells for cancer immunotherapy. *Front. Immunol.* 9, 1722 (2018).
21. Menares, E., Galvez-Cancino, F., Caceres-Morgado, P., Ghorani, E., Lopez, E., Diaz, X. et al. Tissue-resident memory CD8+ T cells amplify anti-tumor immunity by triggering antigen spreading through dendritic cells. *Nat. Commun.* 10, 4401 (2019).
22. Kohnke, T., Krupka, C., Tischer, J., Knosel, T. & Subklewe, M. Increase of PD-L1 expression in CD3+CD8+ T cells in non-small cell lung cancer. *Eur. J. Cancer* 100, 51–58 (2019).
23. Ishiguro, T., Sano, Y., Komatsu, S. I., Kamata-Sakurai, M., Kaneko, A., Kinoshita, Y. et al. Tissue-resident memory CD8+ T cells amplify anti-tumor immunity by triggering antigen spreading through dendritic cells. *J. Clin. Oncol.* 36, 56 (2018).
24. Suntharalingam, G., Perry, M. R., Ward, S., Brett, S. J., Castello-Cortes, A., Brunner, M. D. et al. Cytokine storm in phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *Clin. Exp. Immunol.* 151, 108–1028 (2008).
25. Thakur, A., Scholler, J., Schall, D. L., June, C. H. & Lum, L. G. Enhanced cytokotoxicity against solid tumors by bispecific antibody-armed CD19 CAR T-cells: a proof-of-concept study. *J. Cancer Res. Clin. Oncol.* 146, 2007–2016 (2020).
26. Li, J., Piskol, R., Ybarra, R., Chen, Y. J. J., Li, J., Slaga, D. et al. CD3 bispecific antibody-induced cytokine release is dispensable for cytotoxic T cell activity. *Sci. Transl. Med.* 11, eaaw5681 (2019).
27. Ishiguro, T., Sano, Y., Komatsu, S. I., Kamata-Sakurai, M., Kaneko, A., Kinoshita, Y. et al. Anti-CD19 bispecific antibody-mediated cytokine release in mice treated with rituximab or anti-CD3 antibodies. *Front. Oncol.* 9, 4291 (2019).
28. Kauer, J., Horner, S., Osburg, L., Muller, S., Marklin, M., Heitmann, J. S. et al. Tolciluzumab, but not demethylasemone, prevents CRS without affecting antitumor activity of bispecific antibodies. *J. Immunother. Cancer* 8, 000621 (2020).
29. Khadka, R. H., Sakemura, R., Kenderian, S. S. & Johnson, A. J. Management of cytokine release syndrome: an update on emerging antigen-specific T cell engaging immunotherapies. *Immunotherapy* 11, 851–857 (2019).
30. Teachy, D. T., Lacey, S. F., Shaw, P. A., Melenhorst, J. J., Maude, S. L., Frey, N. et al. Identification of predictive biomarkers for cytokine release syndrome after chimeric antigen receptor T cell therapy for acute lymphoblastic leukemia. *Cancer Discov.* 6, 664–679 (2016).
31. Hunning, T. The storm has cleared: lessons from the CD228 superagonist TGN1412 trial. *Nat. Rev. Med.* 12, 317–318 (2012).
32. Jacobs, K., Godwin, J., Foster, M., Vey, N., Uy, G. L., Rizzi, D. A. et al. Lead-in dose optimization to mitigate cytokine release syndrome in AML and MDS patients treated with flotuzumab, a CD123 x CD3 DART molecule for T-cell redirected therapy. *Blood* 130, 3856 (2017).
33. Chen, X., Kampf-Schroer, C., Wong, G. W. & Xuan, D. A modeling framework to understand the characteristics of tumor-infiltrating immune cells and their engagement by bispecific CAR T cells. *J. Theor. Biol.* 250, 523–534 (2008).
34. Lum, L. G., Thakur, A., Liu, Q., Deol, A., Al-Kadhimi, Z., Ayash, L. et al. CD20-targeted T cells after stem cell transplantation for high risk and refractory non-Hodgkin’s lymphoma. *J. Biol. Med. Transplant. Vascular Tissue Eng.* 19, 925–933 (2013).
35. Lum, L. G., Thakur, A., Kondadassua, S. V., Al-Kadhimi, Z., Deol, A., Tomaszewski, E. N. et al. Targeting CD138 x CD20- clonalogenic myeloma precursor cells decreases these cells and induces transferable antitumoral immunity. *J. Biol. Med. Transplant. Vascular Tissue Eng.* 22, 869–878 (2016).
36. Lum, L. G., Thakur, A., Choi, M., Deol, A., Kondadassua, V., Schalk, D. et al. Clinical and immune responses to anti-CD3 x anti-EGFR bispecific antibody armed activated T cells (EGFR-BATs) in pancreatic cancer patients. *Oncoimmunology* 9, 1773201 (2020).
37. Lum, L. G., Thakur, A., Al-Kadhimi, Z., Colvin, G. A., Cummings, F. J., Legare, R. D. et al. Targeted T-cell therapy in stage IV breast cancer: a phase I clinical trial. *Clin. Transl. Oncol.* 21, 235–236 (2019).
38. Vaishampayan, U., Thakur, A., Rathore, R., Kouttab, N. & Lum, L. G. Phase I Study of anti-CD3 x anti-Her2 bispecific antibody in metastatic castrate resistant prostate cancer patients. *Prostate Cancer* 2015, 285193 (2015).
39. Thakur, A., Rathore, R., Kondadassua, S. V., Uberti, J. P., Ratanatharthorn, V. & Lum, L. G. Immune T cells can transfer and boost anti-breast cancer immunity. *Oncoimmunology* 7, 1500672 (2018).
Overcoming the challenges associated with CD3+ T-cell redirection in cancer
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