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Anti-Tumour Treatment

Novel TCR-based biologics: mobilising T cells to warm 'cold' tumours

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ABSTRACT

Immunotherapeutic strategies have revolutionised cancer therapy in recent years, bringing meaningful improvements in outcomes for patients with previously intractable conditions. These successes have, however, been largely limited to certain types of liquid tumours and a small subset of solid tumours that are known to be particularly immunogenic. Broadening these advances across the majority of tumour indications, which are characterised by an immune-excluded, immune-deserted or immune-suppressed ('cold') phenotype, will require alternative approaches that are able to specifically address this unique biological environment. Several newer therapeutic modalities, including adoptive cell therapy and T cell redirecting bispecific molecules, are considered to hold particular promise and are being investigated in early phase clinical trials across various solid tumour indications. ImmTAC molecules are a novel class of T cell redirecting bispecific biologics that exploit TCR-based targeting of tumour cells; providing potent and highly specific access to the vast landscape of intracellular targets. The first of these reagents to reach the clinic, tebentafusp (IMCgp100), has generated demonstrable clinical efficacy in an immunologically cold solid tumour with a high unmet need. Here, we highlight the key elements of the ImmTAC platform that make it ideally positioned to overcome the cold tumour microenvironment in an off-the-shelf format.

Introduction

The T cell repertoire evolved to distinguish self from foreign antigens, enabling the recognition and elimination of pathogen-infected cells, while avoiding reactions to healthy organs and tissues. Although the T cell system monitors almost all types of body cells and eliminates aberrant ones, for example if they display viral antigens, it is nevertheless often completely or, at least, partly ineffective against cancer cells. The T cell repertoire is selected so as to not recognise self-antigens and, as malignant cells display an almost normal antigen selection, they are often 'invisible' to T cell-mediated immunity. While the accumulation of genetic mutations during oncogenesis and the subsequent aberrant expression of antigens can result in some cancer cells being detected and targeted by antigen-specific T cells, cancer cells develop multiple mechanisms to evade T cell surveillance and establish an immunosuppressive micro-environment [1,2]. Therefore, overcoming both the limited natural T cell response to cancer cells and the resistance to T cell immunity within the tumour micro-environment (TME) are key challenges to any anti-tumour T cell therapy.

The last decade has seen significant advances in the field of immuno-oncology (I-O) with the development of a range of agents that exploit both the innate and adaptive arms of the immune system to fight cancer [3–5]. A key milestone was the approval of monoclonal antibodies that target the regulatory molecules CTLA-4 and PD-1/PD-L1 (so called 'checkpoint inhibitors') which relieve T cell immune suppression [6]. Despite encouraging results in the clinic, response rates to checkpoint inhibitors remain variable as their activity principally relies on pre-existing tumour-specific T cell populations, which are often restricted in size and fitness within the TME [7]. This is illustrated by the response rates seen with anti-PD-1/PD-L1 monotherapy, which are typically around 25% and can be as low as 5% in immune-deserted (or immunologically 'cold') tumours, such as uveal melanoma where T cells are almost completely absent from the tumour [8,9]. While immune-deserted tumours are associated with a poor prognosis, multiple studies demonstrate a correlation between the abundance of pro-inflammatory T cells within tumour infiltrating lymphocytes (TILs) and prolonged

Abbreviations: ACT, adoptive cell transfer; AFP, alpha-feta protein; APC, antigen presenting cell; B-ALL, B cell acute lymphoblastic leukaemia; BCMA, B cell maturation antigen; CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; CMTA, commonly mutated tumour antigens; CRES, CAR-T cell related encephalopathy syndrome; CTA, cancer testis antigen; CRS, cytokine release syndrome; HLA, human leukocyte antigen; ImmTAC, immune mobilizing monoclonal T-cell receptors against cancer; scFv, single-chain variable fragment; sTE, soluble T cell engager(s); TCR, T cell receptor; TIL, tumour infiltrating lymphocytes; TME, tumour micro-environment

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survival, particularly in response to immunotherapy [10–15].

Accumulating clinical evidence supports the need to not only relieve T cell suppression, but to also develop strategies that promote the engagement and accumulation of cancer specific pro-inflammatory T cells within the TME [16]. Several approaches to direct T cells with anti-tumour activity within the TME are gaining traction (reviewed by [17]). Among these approaches, adoptive T cell therapy and soluble T cell redirecting molecules show considerable promise. This review will focus on these approaches, with special attention to soluble T cell redirectors and in particular the ImmTAC[1] (Immune mobilising monoclonal TCRs Against Cancer) platform. The fundamental features of this new class of bispecific T cell engager, the biology that supports the technology, and the potential it presents in delivering efficacy against 'cold' tumours is considered.

Adoptive T cell therapy

Adoptive T cell therapy works on the paradigm of delivering increased numbers of antigen-specific T cells to the tumour by expanding the patient's own T cells *ex vivo* before adoptively transferring them back to the patient. Methods to enrich tumour-specific T cells for adoptive cell transfer (ACT) include expansion and selection of TILs or engineering the patient's T cells to express either antibody-based chimeric antigen receptors (CARs) or antigen-specific T cell receptors (TCRs) (Fig. 1).

Cellular therapy has generated much interest and success in the I-O field fuelled by FDA approval of two anti-CD19 CAR-T cell therapies in 2017, Kymriah (tisagenlecleucel) and Yescarta (axicabtagene ciloleucel) for second-line treatment of adults with certain types of relapsed or refractory large B cell lymphomas and Kymriah for second-line treatment of patients up to 25 years of age with B cell precursor acute lymphoblastic leukemia (B-ALL). However, to date, efficacy of CAR-T cell therapies has been restricted to select haematopoietic lineages and they have shown little efficacy in solid tumours [18]. Limitations in tumour-specific target antigen identification as well as the nature of the ACT approach (single dose transfer and consequential irreversible onset of immune activity together with intensive preconditioning regimens) have restricted broad utility of this therapeutic approach. Also, severe cytokine release syndrome (CRS) and neurological toxicity manifesting as CAR-T cell related encephalopathy syndrome (CRES) have been observed in CD19 CAR-T cell therapy clinical trials [19–21]. A myriad of factors is thought to contribute to the exacerbation of symptoms, including the nature of the disease indication, CD19-antigen expression profile, preconditioning regimen and the nature of the CAR construct [20,21]. Encouragingly, recent targeting of B cell maturation antigen (BCMA) by a second generation CAR construct for treatment of multiple myeloma reported both a manageable safety profile and promising efficacy data [22]. Outside of CAR-T cell therapy, T cells engineered to express affinity-enhanced antigen-specific TCRs to the NY-ESO-1 antigen have demonstrated the first example of T cell adoptive therapy responses in solid tumours for ACT second-line treatment of patients with soft tissue sarcomas [23].

Fuelled by a growing enthusiasm in the field due to encouraging early clinical results, significant efforts are underway to overcome the current hurdles for adoptive T cell therapy, including the requirement for patient preconditioning and the elevated costs associated with specialised sites for T cell expansion and engineering.

T cell engaging bispecific molecules

An attractive and promising alternative to adoptive T cell therapy is the redirection of native T cells to target cells within the tumour. T cell redirectors are soluble chimeric proteins with an antigen recognition domain and a T cell engaging domain. The combination of antigen recognition and T cell engaging domains enables polyclonal activation of T cells independently of their TCR specificity. In contrast to ACT, T cell redirectors offer the possibility of using off-the-shelf molecules in a doseable manner, which presents fewer challenges for both manufacturing and administration to patients.

T cell redirector molecules almost universally employ an anti-CD3 scFv as the T cell engaging moiety, due to its ability to efficiently trigger T cell activation and to only do so when immobilised on a target cell. Generally, the affinity of the anti-CD3 scFv is tuned to maintain monomeric specificity so as to avoid non-specific crosslinking of CD3. However, bivalent CD3 binding is also being explored [24,25].

While an almost universal T cell engaging moiety is utilised, two distinct classes of targeting domains (TCR or antibody-based domains) have been applied to date to target specific antigens (Figs. 1 and 2).

Antigen recognition

TCRs are expressed on the T cell surface in complex with CD3 signalling components and show specificity toward linear peptide antigens (typically 8–20 amino acids in length) derived from intracellular, cell surface or extracellular proteins in complex with human leukocyte antigen (HLA). Specificity is mediated by six hypervariable loops (complementary determining regions (CDRs)) that form the antigen binding site. TCRs dock onto peptide-bound HLA (pHLA) complexes using a conserved canonical binding mode, forming a large binding interface between the TCR and pHLA. This large binding interface enables broad contacts across both the peptide backbone and the HLA heavy chain to be achieved. TCRs are selected in the thymus to bind weakly to self-pHLA, ensuring HLA-restriction and limited self-reactivity of T cells in the periphery [26]. Natural TCRs typically have affinities in the 0.1–500 μM range, where 1 μM is considered 'high affinity'. Even within this range, the cumulative strengths of the TCRs (i.e. avidity) allows the T cell to recognise cells presenting just a small number of a particular pHLA molecule [27].

ImmTAC molecules are first-in-class soluble T cell engagers (sTE) that use TCRs to target tumour cells. The ImmTAC platform utilises an affinity-enhanced soluble TCR, incorporating proprietary TCR technology, fused to an anti-CD3 scFv T cell engaging domain (Fig. 1) [28]. TCR affinity to a tumour-specific antigen is enhanced up to several-million-fold (K_D ~ pM) to enable T cell redirection to target cells presenting as few as 10–50 pHLA antigens, (Table 1) [29–31]. By combining a high affinity TCR and an optimised anti-CD3 scFv in a soluble format, ImmTAC molecules are able to bind their intended target with a long half-life, and therefore efficiently redirect T cells to tumour cells displaying low target density on their surface. A key advantage of employing a high affinity TCR moiety is that it provides ImmTAC molecules with a unique ability to target the vast pool of intracellular antigens that cannot currently be accessed by antibody-based bispecific molecules (Fig. 2).

The structural and biophysical mechanisms used by antibodies to recognise protein antigens are fundamentally distinct from that of the TCR-pHLA interaction. The antibody-antigen binding interface is formed of two polypeptide chains contributing six hypervariable loops that come together to form the antibody binding fragment (Fab) which bind only cell surface and extracellular antigens *in vivo*. Compared to the large binding interface formed between TCR-pHLA, the antibody binding footprint tends to be more focussed and recognises diverse epitopes of different shapes and structures [32].

Engineering TCRs as soluble entities is a significant technical challenge, not least because of their inherent instability when not embedded within a membrane but also due to their propensity to aggregate in solution [33]. The technical challenges associated with the development of soluble TCR technology is reflected by the limited number of TCR-based T cell redirectors in clinical studies (currently only three derived TCR-based T cell redirectors (all from the ImmTAC

[1] ImmTAC is a registered trademark of Immunocore Ltd.

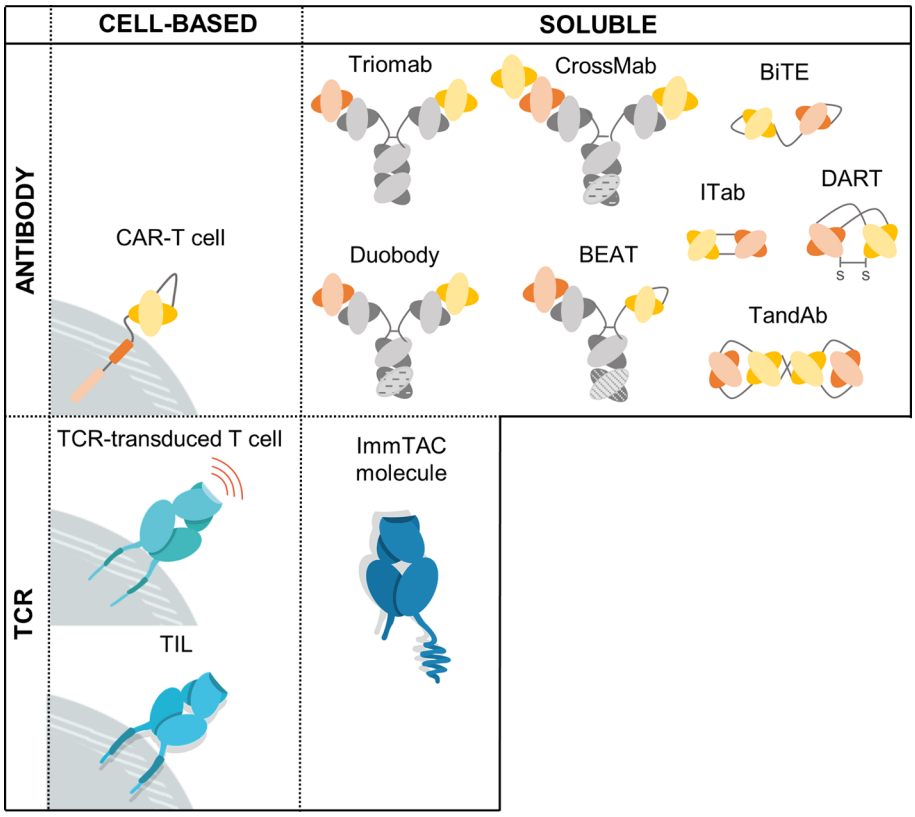

Fig. 1. Schematic overview of the different platforms of T cell redirecting immuno-oncology agents categorised on the use of a cell-based versus soluble approach and the use of an antibody versus TCR antigen targeting system. Chimeric antigen receptor (CAR)-T cells are generated by expanding a patient's T cells *ex vivo* to present a CAR formed of an extracellular single-chain variable fragment (scFv) tumour antigen-targeting domain (yellow) followed by a variable length spacer/hinge, transmembrane region and T cell signalling domains (e.g. CD28, 4-1BB or ζ-chain) before ACT back to the patient. Examples of soluble bispecific antibodies that have reached clinical development are shown, where yellow domains depict tumour antigen binding sites, orange domains depict CD3 antigen binding sites and grey domains depict heavy chain components. Triomab; IgG format with two antigen-targeting domains formed of variable heavy (VH) and variable light (VL) chains, Duobody; IgG format with two VH/VL antigen-targeting domains and a silenced Fc domain, CrossMAb (2:1 format); IgG format with three VH/VL antigen targeting domains and a silenced Fc domain, Bispecific Engagement by Antibodies based on T cell receptor (BEAT); IgG format with one VH/VL, one scFv targeting domain and the Fc region silenced and engineered to mimic the TCR, Dual Affinity Re-Targeting (DART); two VH/VL targeting domains linked as shown and stabilised by a disulphide bond, Bispecific T cell Engagers (BiTE); two VH/VL regions engineered as scFvs and connected by flexible linker peptides. TandAb; four VH/VL targeting domains linked as shown and ImmunoTherapy antibodies (Itab) two VH/VL targeting domains linked as shown. TCR-transduced T cells are generated by expanding a patient's T cells *ex vivo* to present engineered affinity-enhanced TCRs for ACT. Tumour infiltrating lymphocytes (TILs) are selected and expanded from a patient *ex vivo* before ACT. ImmTAC molecules are soluble TCRs stabilised by a disulphide bond and fused to an anti-CD3 scFv. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

platform) are in clinical trials compared to more than 30 antibody-based molecules [24,34–36].

To date, two antibody-based soluble bispecific T cell engagers have received market approval. The first to receive market approval (since withdrawn), Removab (catumaxomab), adopts a tri-functional antibody format (Triomab) replacing one tumour antigen binding domain with an anti-CD3 T cell binding domain to deliver a three-armed anti-tumour response against EpCAM-positive solid tumours in treatment of malignant ascites (Fig. 1) [37]. Catumaxomab was delivered locally (intraperitoneal) to avoid broad systemic immune activation by the Fc domain. Platforms that remove the Fc domain face greater technical challenges in purification, solubility and stability, but have the potential to be more broadly applicable and deliver higher penetrance into the solid tumour microenvironment based on their smaller size [36]. The second approved soluble bispecific T cell engager, Blincyto (blinatumomab), was developed as a bispecific T cell engager (BiTE) composed of two scFv domains, one targeting CD19 on B cell-derived tumours and the other targeting CD3 to engage T cells (Fig. 1) [38,39]. Blinatumomab was licensed in 2015 for the treatment of refractory B cell precursor Acute Lymphoblastic Leukemia (R/R B-ALL) [19,40,41]. Despite response rates against R/R B-ALL of up to 42.9%, BiTE's have yet to demonstrate efficacy against other tumours in large patient cohorts [34]. Other examples of anti-CD3 T cell engaging bispecific antibody platforms to progress to clinical development for treatment of various haematopoietic and solid tumours indications include Duobody [42], CrossMab [43,44], Bispecific Engagement by Antibodies based on T cell receptor (BEAT) molecules [45], dual-affinity re-targeting (DART) antibodies [46], Tandem diabodies (TandAb) [47], and ImmunoTherapy antibodies (ITab) [48] (Fig. 1).

The most advanced ImmTAC molecule, tebentafusp (IMCgp100), redirects T cells toward HLA-A*02:01-positive melanoma cells presenting a specific peptide derived from the melanoma-associated antigen gp100. Early trial data using this molecule has demonstrated clinical efficacy as a monotherapy against the gp100-positive tumour, uveal melanoma [49].

Targetable cancer antigens

An ideal cancer antigen is expressed at sufficiently high levels on cancer cells to trigger tumour recognition, and is absent or presented at lower levels on normal cells such that no response is induced [50]. Three key groups of cancer targets include the cancer testis antigens (CTAs), lineage marker antigens, and overexpressed antigens that show upregulated expression on cancer cells over normal cells [50].

Therapeutic antibodies, designed to bind to cells presenting > 10,000 epitopes, can selectively target overexpressed tumour antigens with low-level expression on normal tissues. An example is the CEA-targeted BiTE, where preclinical evaluation showed that a threshold of 10,000 CEA-binding sites was required for efficient tumour cell killing, with no T cell-mediated killing observed in normal cells expressing < 2000 CEA-binding sites [43]. Similarly, specific targeting of HER-2- and EGFR-over expressing tumours using a HER-2 BiTE, EGFR monoclonal antibody (nimotuzumab) or EGFR BiTE was critically dependent on identifying the optimal nM affinity to deliver efficacy in the absence of on-target, off-tumour activity on normal cells [51–53].

ImmTAC molecules are designed to redirect T cells toward tumour cells displaying low levels of antigens and are therefore uniquely placed to target both intracellular and extracellular protein antigens presented by HLA. More than 90% of the 19,613 protein coding genes have validated peptide targets that could be targeted by ImmTAC molecules. In comparison, only 7.6% (1492) of proteins are validated as being represented on the cell surface and potentially could be accessed by

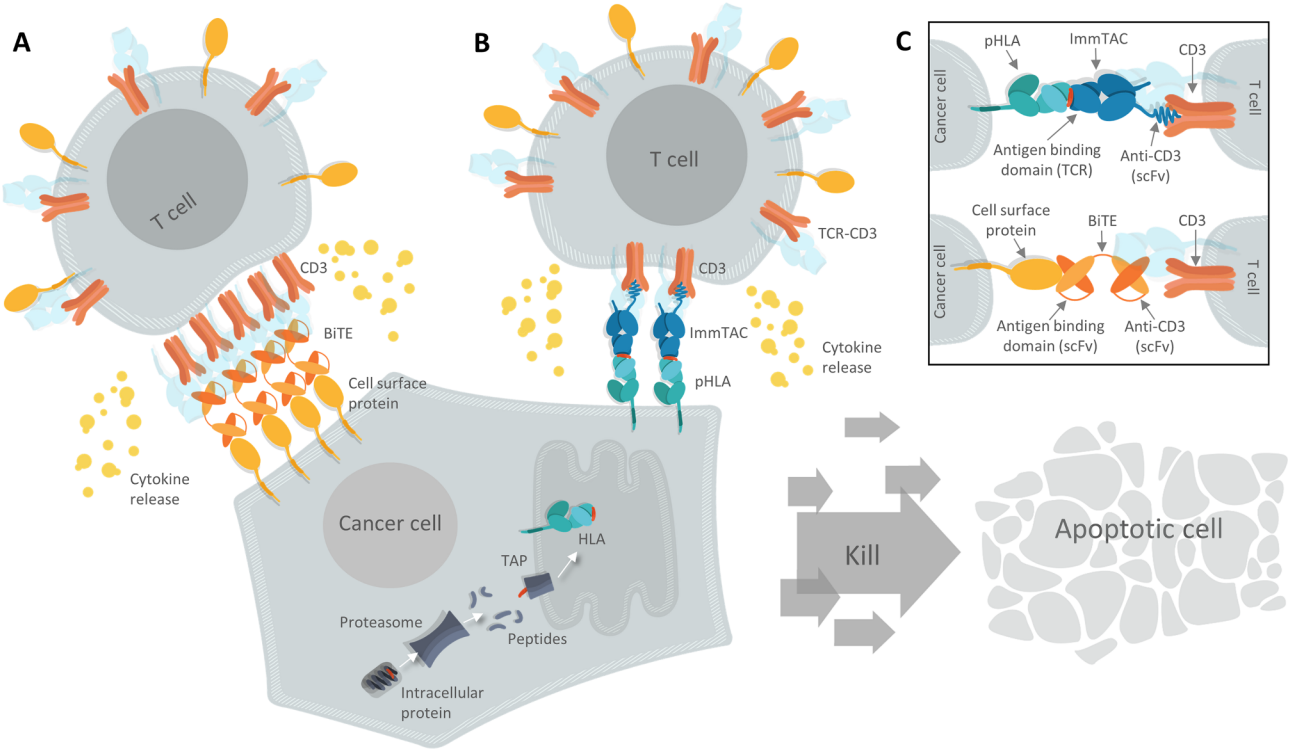

Fig. 2. Recognition of tumour antigens displayed as protein (A) or as peptide on the surface of HLA (pHLA) (B) for recognition by antibody-based BiTE molecules or TCR-based ImmTAC molecules, respectively. (A) BiTE molecules recognise cell surface proteins via their scFv antigen targeting domain and recruit T cells via their anti-CD3 scFv domain. Anti-CD3 mediated T cell engagement induces downstream signalling events that activate mechanisms of T cell cytotoxicity (including cytokine release) causing target cell killing. (B) Intracellular proteins are processed and presented on the cell surface by HLA. ImmTAC molecules recognise pHLA via their high-affinity TCR targeting domain and recruit T cells via their anti-CD3 scFv domain. (C) Higher magnification schematic to demonstrate the bispecific targeting of both ImmTAC molecules and BiTE molecules.

Table 1
Characteristics of BiTE, TCR mimic antibodies and ImmTAC molecules.

	BiTE's	TCR-mimic antibodies	ImmTAC molecules
Targeting cell surface antigens	Yes	No	No
Targeting intracellular antigens	No	Yes	Yes
HLA-restricted	No	Yes	Yes
Affinity for target antigen	Med (K_D nM)	High (K_D nM	Very High (K_D pM)
Lowest number of epitopes shown for specific target cell lysis	~10,000	–*	~10–50
Specificity for target antigen	High	Low	High

* Undetermined due to low reported specificity. BiTE; Bispecific T cell Engager, TCR; T cell receptor, ImmTAC; Immune mobilising monoclonal TCR Against Cancer, HLA; human leukocyte antigen.

antibody-based bispecific T cell redirectors [54]. This ability of TCRs to access dysregulated intracellular proteins in tumours substantially extends the gamut of druggable targets for I-O, with clear clinical potential.

CTAs are an attractive class of antigen with a unique tumour expression pattern, for which expression on normal tissues is generally restricted to male germ cells that are devoid of HLA-class I and therefore cannot present antigens to T cells [55]. The first ImmTAC molecule to target a CTA, IMCnyeso, entered the clinic in 2018, followed by IMC-C103C in 2019 for treatment of various solid tumour indications (NCT03515551 & NCT03973333). Both molecules target their respective antigens, NY-ESO-1 and MAGE-A4, respectively, with low pM affinity, enabling potent and specific activation against a low-level target, as validated by a comprehensive *in vitro* preclinical testing package [56].

In addition to CTAs, lineage-restricted proteins represent attractive targets for the ImmTAC platform. Efficacy has been observed for an ImmTAC molecule (IMCgp100) targeting gp100 (a melanocyte lineage antigen overexpressed in melanoma) in patients with both metastatic cutaneous and uveal melanoma. Normal tissue expression of gp100 found in melanocytes was consistent with clinically manageable skin rash observed in responding patients as predicted from preclinical studies [49,56,57].

Beyond CTAs and lineage marker antigens, commonly mutated tumour antigens (CMTA) offer an additional opportunity to expand the repertoire of novel cancer targets available. CMTAs are derived from tumour-specific somatic mutations that, due to their *de novo* generation, are not presented during thymic selection and thus have the potential to exhibit heightened immunogenicity and act as model targets for cancer immunotherapy [58]. Importantly, CMTAs are distinct from patient-specific neoantigens as they are found in a larger population of patients. While neoantigens are attractive targets for personalised

immunotherapeutic approaches, their utility for universal off-the-shelf therapies is challenging [59,60]. CMTAs generated by recurrent mutations arising from 'driver' genes, such as BRAF and KRAS, that are specific to certain indications and expressed in broad patient populations, represent promising targets for these off-the-shelf therapies [61].

Targeting intracellular antigens

By utilising a TCR targeting domain for recognition of intracellular antigens, ImmTAC molecules have access to > 10 fold more potential cancer targets than current soluble antibody-based bispecific molecules, thus broadening opportunities across haematological malignancies and solid tumours.

The potential to access intracellular antigens is so attractive that several approaches have been explored to utilise antibodies to tap into the vast landscape of TCR-restricted intracellular antigens, including the development of antibodies against specific pHLA complexes (TCR-mimic antibodies). However, because antibodies are not naturally selected to recognise pHLA, engineering them to do so is challenging [32].

In an early attempt to generate antibodies that recognise a pHLA complex, the binding affinity of the TCR-mimic antibody to MAGE-$1_{161–169}$–HLA-A*01:01 was shown to focus on the HLA α1 helix with no contact between the antibody and N-terminal MAGE-A1 peptide residues [62]. A similar phenomenon was reported nearly a decade later, where the binding affinity of a TCR-mimic antibody engineered to recognise WT-$1_{126–134}$–HLA-A*02:01 was again focussed on the HLA, this time making contacts with only the N-terminal peptide residues [63]. In an encouraging study by Steward-Jones et al., a TCR-mimic antibody engineered to bind to NY-ESO-$1_{157-165}$–HLA-A*02:01 epitope was shown to adopt a TCR-like canonical binding geometry [64]. However, functional cellular analysis of the antibody as a CAR-construct showed specificity could only be achieved using lower affinity variants reminiscent of a natural TCR and thus was not relevant as a soluble therapeutic [65]. Specificity analysis of a TCR-mimic antibody to $PRAME_{300-309}$–HLA-A*02:01 was inconclusive. The molecule delivered mixed results in *in vitro* cellular assays with good specificity towards a panel of cell lines and whole blood but had a high degree of tolerance for alanine substitution at all peptide positions with the exception of positions 8 and 9 (indicating a potential for cross reactivity) [66]. More promising was the report describing a TCR-mimic antibody towards the $EBV_{426-434}$CLGGLLTMV–HLA-A*0201 antigen, which displayed a bell-shaped distribution of contacts across the peptide backbone. However, cross-reactivity analysis of the lead higher affinity variant (~34 nM) indicated that this molecule was not sufficiently specific to progress for further development [67]. To date, the only TCR-mimic antibody to progress for clinical development is the 8F4 antibody targeting the myeloid protein epitope PR1 (VLQELNVTV)–HLA-A*0201 for the treatment of acute myeloid leukemia (AML) [68].

Taken together, advances in hybridoma and phage display technologies have enabled the production of dozens of high affinity TCR-mimic antibodies targeting diverse cancer and viral antigens [69]; yet, while antigen binding affinities in the picomolar range have been reported [70], the achievable specificity of TCR-mimic antibodies towards the linear peptide target remains questionable (Table 1). In the CAR-T cell therapy format, where the affinity requirements appear lower, the clinical development of TCR-mimic antibodies has shown greater progress, with a TCR-mimic antibody recognising alpha-feta protein (AFP) entering clinical trials for hepatocellular carcinoma with early data showing response in 3 of 6 treated patients with no reported CRS or drug-related neurotoxicity [71,72].

To advance the development of soluble bispecific T cell engagers, TCR-mimic antibodies developed to shift the binding focus away from HLA and establish broader contacts across the peptide would be attractive [73]. This remains an appreciable challenge given the complex underlying dynamics governing TCR-peptide affinity and specificity that remain to be fully elucidated [74,75].

Immunology of T cell redirection

Antibody-mediated T cell redirection was first proposed over 30 years ago [76], and in 2009, the first antibody-bispecific T cell redirector (Triomab; catumaxomab), employing an anti-CD3 effector function, entered clinical trials [37]. Anti-CD3 remains the most widely used T cell engaging effector function due to its ability to mimic TCR-mediated T cell activation.

Our current understanding of the biology and in particular the molecular and cellular mechanisms downstream of CD3 engagement comes predominantly from studies performed *in vitro* and in preclinical models. As yet, there is limited information from the clinic regarding the processes that promote or limit the therapeutic activity of sTE in patients. Dissecting the positive and negative contribution of the individual immunological machinery involved in the response will require substantially more clinical data. Developing technologies that enable the required depth of analysis of clinical data is critical to advance our understanding of both the mode of action of T cell redirector molecules and resistance mechanisms that limit their efficacy.

Evidence to date supports the notion that sTE induce polyclonal T cell responses by promoting activation of CD4+ and CD8+ T cells, associated with the release of pro-inflammatory cytokines (such as IFN-γ, TNF-α, IL-2 and IL-6) and killing of tumour cells, predominantly mediated by the perforin/granzyme system [34,77]. IMCgp100, the most clinically advanced ImmTAC molecule, has been shown *in vitro* to redirect CD8+ T cells, and to a lesser extent CD4+ T cells, to tumour cells displaying very low levels of targets on their surface [78]. In addition, redirected T cells, including effector and memory cells, released inflammatory cytokines (TNF-α, IFN-γ, IL-2 and IL-6) and chemokines (macrophage inflammatory protein-1a-b, interferon-c-inducible protein-10, monocyte chemoattractant protein-1), but failed to secrete Th2 (i.e.IL-4) and immune-suppressive (i.e. IL-10) cytokines, which have been shown to impair and counteract anti-tumour immunity [79]. These data suggest that IMCgp100 administration to patients might result in: (i) activation of TILs, (ii) recruitment of circulating T cells within the TME and (iii) stimulation/mobilisation of other immune cells. Thus, IMCgp100 might promote polyfunctional and complementary responses in patients, the coordinated occurrence of which are considered essential for effective anti-tumour immunity [80]. Consistent with this hypothesis are clinical observations of lymphocyte mobilisation, serum cytokine increase, evidence of CD8+ PD-1+ T cell infiltration into the tumour bed that were associated with prolonged survival and tumour shrinkage in a significant fraction of advanced uveal melanoma patients treated with IMCgp100 [49].

Manageable skin toxicity closely associated with clinical response is also found in these patients. Whether this is a consequence of an on target / off-tumour effect, due the concomitant expression of gp100 in normal melanocytes, or results from a target independent systemic immune response driven by IMCgp100 and homing to the skin, warrants further investigation. Strikingly, metastatic uveal melanoma is characterised by a very poor prognosis and a significant unmet need, with survival largely unchanged in the last 50 years and no proven standard of care treatment for metastatic patients. Initial results with IMCgp100 suggests that it can trigger a systemic and clinically relevant immune response to target a solid tumour classified as poorly inflamed and non-immunogenic, highlighting the therapeutic potential of the ImmTAC platform and its anticipated applicability in multiple cancer indications.

In vitro, IMCgp100 promotes the cross-presentation of tumour antigens by antigen presenting cells (APCs) [81]. In patients, this could lead to the priming of endogenous T cells specific for antigens released by dying cancer cells, a phenomenon known as epitope spreading [82]. Epitope spreading is considered necessary to stimulate self-sustaining and long-lasting anti-tumour immune responses [83]. Several I-O

treatments, including checkpoint inhibitors, sTE, ACT and oncolytic viruses have reported evidence of epitope spreading in both preclinical models and clinical studies [73,83]. Whether epitope spreading occurs in advanced uveal melanoma patients treated with IMCgp100 and contributes to the clinical response observed in these patients is currently under investigation.

Challenges and perspectives

T cell engaging bispecific molecules have significantly advanced the I-O field. However, clear challenges exist, which if overcome, will enable the full potential of this therapy class to be realised. Some challenges are platform-specific (i.e. overcoming HLA restriction for TCR-based therapies), while others are platform-agnostic and include achieving optimal dosing, selecting the best targets, obtaining greater efficacy and overcoming tumour resistance.

Overcoming HLA restriction

Currently, all TCR-based therapies reliant on binding a pHLA complex are restricted to a single (or small number of) HLA allelic variants. This HLA-restriction limits the relevant patient populations with any single TCR-based therapeutic as these are fragmented by HLA allele expression. For example, the commonly targeted HLA-A*02:01 allele is represented at its highest frequency (~45%) in the Caucasian population, meaning ~45% of this ethnic group would be eligible for therapy [84]. To overcome HLA-restriction and retain the ability to access the pHLA antigenic landscape, multiple strategies may be employed, each bringing a unique set of challenges and opportunities. Coverage of multiple HLA sub-groups may be achieved by the generation of multiple TCRs for a given target or by achieving HLA subgroup tolerance in a single TCR. While overcoming restriction is important, it is also essential that each selected TCR is assessed for potential HLA alloreactivity through robust allo-screening in preclinical assays [56,85].

An attractive alternative approach is to exploit non-polymorphic HLA molecules such as HLA-E, HLA-G, CD1 and MR1 [86,87]. CD1 and MR1, in particular, have recently attracted interest in this space, following the identification of T cell clones able to recognise CD1- or MR1-presented antigens expressed by tumour cells [88]. Interestingly, CD1 and MR1 present lipids and metabolites, respectively, which show distinct chemistry, cellular biosynthetic and catabolic pathways, *in vivo* distribution and abundance from peptides. Thus, targeting CD1 and/or MR1, in addition to circumventing HLA restriction, might extend the landscape of druggable targets to additional classes of molecules, some of which might be components of metabolic pathways essential for tumour cell growth and survival.

Half-life, dosing and toxicity

sTE are available in different formats and sizes, which substantially influence their individual pharmacokinetic properties and therefore the administration doses and regimens. Sustained exposure is generally required for the therapeutic activity of sTE and it depends on two main factors: the plasma half-life of the molecule and its capacity to diffuse and penetrate in tissues; the latter feature being particularly important for solid tumours. Plasma half-life is determined by the size, the capacity to bind blood proteins, and the ability to recycle after cell internalisation. Generally, large molecules (100–150 KDa) have longer half-life in blood (several days), while smaller agents (< 60 KDa) show a faster clearance from circulation (minutes to hours) primarily due to renal filtration. For the latter molecules, diverse strategies of half-life extension have been successfully exploited, such as introducing either domains that bind carrier protein in the serum (i.e. albumin) or the FcRn receptor on cells and tissues (which allows recycling of the molecules once internalised) [89]. Continuous infusion is another example of an administration strategy employed to maintain the sTE's concentration above a threshold level required to achieve efficacy.

Prolonged drug exposure and systemic administration, however, can also be associated with toxicity, caused by the persistence of agents in the circulation, which may favour off-tumour/off-target responses (i.e. uncontrolled cytokine release by over-stimulated T cells). To achieve higher drug exposure in solid tumours, without the disadvantages of potential severe toxicity associated with systemic administration, additional strategies are being considered including intratumoural injection or vehicles able to preferentially deliver these sTE agents to the TME (liposomes, nanoparticles, oncolytic viruses).

An ideal sTE would penetrate solid tumours with high efficiency and persist within the TME long enough to achieve a high tumour/blood ratio. Achieving this would facilitate the maintenance of a high drug concentration at the tumour site without the need for prolonged systemic exposure and would be compatible with administration in cyclic doses.

ImmTAC molecules have a molecular weight in the range of 75 kDa, and as such, are above the molecular cut-off for renal filtration. Furthermore, once bound to their targets on the tumour cell surface, ImmTAC molecules have a binding half-life of several hours [28]. This combination of characteristics is attractive as it makes ImmTAC molecules unlikely to be cleared by renal filtration, small enough to access solid tumours and be retained within tumours for a significant amount of time after binding their targets.

IMCgp100 is currently in clinical trials for metastatic uveal melanoma. Preliminary data from first in human Phase 1 study in both uveal and cutaneous melanoma estimates a circulating half-life of 6–8 h. In this and ensuing studies (Phase 1/2 study in metastatic uveal melanoma) IMCgp100 was well tolerated and demonstrated a manageable safety profile. Promising clinical signs of therapeutic activity and immune activation, including lymphocyte trafficking with the first 3 weeks dosing, were observed in a significant fraction of treated patients [49,90].

Target selection and validation

Appropriate target selection is central to the success of sTE-based therapies, and more broadly, all I-O approaches. Unfortunately, ideal targets expressed in cancer cells that are completely absent in normal cells have not been identified to date. An acceptable therapeutic compromise strictly depends on the differential expression levels of targets between healthy and malignant tissues. This differential expression allows the identification of a therapeutic window dictated by doses at which the therapeutic agent is able to induce significant on-tumour reactivity without triggering off-tumour responsiveness in critical organs. Generally, a wider therapeutic window reduces the risk of treatment-related severe toxicities.

When considering potential therapeutic targets, a key advantage for ImmTAC molecules and TCR-mimic antibodies is the ability to target peptides derived from intracellular proteins, which cannot be accessed by other targeting systems. Intracellular proteins are far more numerous than surface ones and include most of the oncogenic drivers (e.g. TP53, PIK3CA), which are often mutated in cancer, thereby increasing the number of targets that fall within the restrictive target selection criteria. Cross-reactivity, however, might become a hurdle in this context and requires comprehensive assessment and quantification using appropriate tools, methods and preclinical models.

Differential quantification of target expression between tumour and normal cells is a key target selection criterion. The analysis of RNA and relative protein expression levels are important in determining the therapeutic potential of a target protein; however, when considering TCR-based therapies, the target is the pHLA complex, and as such, quantifying peptide presentation is the ultimate measure. Validation at the peptide and HLA level is instructive not only for defining the therapeutic window but also for patient selection. Quantification of target pHLA presentation requires reliable methods to quantitatively

analyse the HLA-bound peptidome, as well as to discriminate HLA expression at allelic levels, in healthy and malignant cells and tissues [56]. This is especially critical given that some cells within a tumour may acquire defects in their antigen processing or presenting machinery resulting in heterogeneous levels of pHLA [91]. However, ImmTAC molecules have been engineered to overcome low level expression of pHLA, with IMCgp100 showing efficacy against tumour cell lines expressing as few as 10 epitopes (Table 1) [30].

Heterogeneity of target expression, both across the patient population and within the same patient, adds another layer to the complexity to target selection. The abundance of a target can be influenced by a myriad of factors including the intrinsic biological variability of individual tumour cells and the environment to which they are exposed. In addition, primary, metastatic and relapsed tumours might show substantially different expression of target proteins. The technological advancement achieved in the high throughput 'omics' analysis of different biomolecules at population and individual cell levels will be instrumental in informing on these target selection considerations.

Activating the 'right' T cell response

sTE elicit polyclonal T cell activation independently of native T cell specificities. This is important to consider as different T cell populations contribute differently to cell-mediated immunity. It is well recognised that cytotoxic and helper T cells both contribute in different ways to support effective anti-tumour immune responses [92,93]. However, it is also known that certain T cell populations with immune-regulatory properties can directly and indirectly inhibit T cell reactivity, contribute to establishing an immune-suppressive TME, and even promote tumour progression [94–96]. An ideal sTE would not engage these T cell populations, thereby preventing them from exerting their immune-suppressive and tumourigenic functions. In this context, understanding the impact that specific T cell effector phenotypes may have on the efficacy of sTE could enhance the therapeutic potential of sTE. Moreover, identifying unique T effector cells associated with sTE responses will be instrumental in developing strategies to specifically support, expand and maintain these T cell populations.

In vitro, IMCgp100 preferentially redirects CD8+ T cells [28], which may be due to the unique capacity of IMCgp100 to target an HLA-A2 presented epitope, whose natural recognition by T cells requires the CD8 co-receptor. Consequently, at the therapeutic doses used in patients, IMCgp100 might only induce full activation of CD8+ cytotoxic T cells, while engaging CD4+ T cells sub-optimally. This could limit recruitment of CD4+ T-helper cells, which are major producers of inflammatory mediators (such as IL-6 and TNF-α) previously associated with severe sTE toxicity [97,98], as well as CD4+ regulatory T cells, which have been linked with poor responses to different I-O agents [99–102].

The engagement and recruitment of T cells from the circulation to the tumour following systemic administration of any sTE is also an important feature to consider. Circulating T cells show high expression of CD3 and at least a proportion of systemic sTE may bind these T cells. However, this interaction is generally characterised by a fast off-rate, allowing the molecule to bind for only a short time (minutes) [25]. When tested *in vitro* in the absence of tumour targets most sTE, including ImmTAC molecules, do not induce T cell activation (assessed by proliferation, cytokine release or cytotoxicity) [25,28]. However, whether the initial weak contact with CD3 could trigger signalling in T cells resulting in a change of their migratory behaviour needs further investigation using more sensitive tools and read-outs.

While sTE redirect T cells to the tumour, immunosuppressive factors within the TME lead to T cell dysfunction [103,104]. Ideally, to achieve long-lasting efficacy, a therapy will need to not only recruit T cells to tumours but also to prevent their exhaustion and reactivate dysfunctional tumour-specific T cells already present. While sTEs could reactivate TILs, to prevent exhaustion there is good rationale for combining sTE with checkpoint inhibitors, which together could enhance targeted T cell responses and alleviate the immunosuppressive TME.

Concluding remarks

The TCR-based ImmTAC platform constitutes a first-in-class biologic therapy that shows high potential for targeted tumour therapy, and promising capacity to overcome T cell tolerance and promote durable anti-tumour responses. IMCgp100 monotherapy is showing clinical promise in a 'cold' tumour setting with a high unmet need (uveal melanoma), including prolonged overall survival, a manageable safety profile, and T cell infiltration into tumours; supporting the value and potential of the ImmTAC platform within the I-O space [49].

As our understanding of the mechanisms of tumour resistance and interplay of the tumour micro-environment increases, it will inform optimal selection of cancer therapies [105–107]. Strategies that induce a multifaceted attack of cancers, while concomitantly restraining the major cellular and environmental factors responsible for tumour resistance show the highest potential for future I-O approaches. The unique characteristics and versatility of ImmTAC molecules make them attractive as monotherapies as well as appealing partners for other immunotherapy agents. In particular, combining tumour-targeted therapies, such as ImmTAC molecules, with more systemic approaches, including checkpoint inhibitors and/or other agents that are able to promote a favourable immunological environment by supporting *de novo* endogenous T cell responses (such as innate immune cell engagers, oncolytic viruses or low dose chemotherapies) and limiting suppressive factors (for example myeloid and stromal immune-suppressive cells). The challenge moving forward is to envisage which combinations will be most effective, and this requires a deeper knowledge of the tumour-immune system interactions and insight from clinical data and patient outcomes from ongoing clinical investigations.

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All authors contributed to manuscript writing and review.

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