The T Cell Promise

New immunotherapeutic methods are being developed with the help of T cells. These strategies look set to evolve the cancer therapy field significantly

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The potential of T cells to eradicate tumours has inspired considerable effort towards the development of novel T cell-based immunotherapeutic strategies. Such approaches range from cell-based adoptive therapy using autologous T cells, to engineered bi-specific reagents that combine targeted antigen recognition with T cell recruitment and activation. This article will discuss the benefits and potential risks of T cell-based therapies and describe some of the most promising examples of this type of therapeutic modality. Furthermore, we highlight how recent and emerging clinical data are beginning to corroborate the pre-clinical findings, with evidence of both potent and durable responses *in vivo*, while also revealing the need for improved pre-clinical toxicity testing.

The cellular arm of the adaptive immune system is thought to be a critical component in the eradication of tumour cells. Indeed, there is considerable evidence indicating that CD8+ cytotoxic T lymphocytes (CTLs) are able to mediate potent killing of tumour cells both *in vitro* and *in vivo*. Interaction of the T cell receptor (TCR) with major histocompatibility complex (MHC) restricted peptide antigens (pMHC) presented by tumour cells is central to this process. Nonetheless, cancers can, and do, evade immune detection by subverting immune recognition; this can be through secretion of immuno-suppressive factors, altered or inefficient processing of antigenic peptides, and/or down-regulation of MHC presentation.

However, perhaps the most salient impediment to the generation of an anti-tumour immune response is that naturally occurring TCRs bind too weakly to tumour-associated antigens (TAAs) to trigger CTL activation. Since the majority of TAAs are derived from self-antigens, T cells with high affinity for TAAs are removed from the repertoire during thymic selection to prevent autoimmunity. Of the TAA-specific T cells that have been isolated to date, the vast majority possess much weaker affinity for antigen than their viral-antigen-specific counterparts (1).

Therapeutic strategies that harness the potent cancer-killing ability of CTLs and overcome the immune evasion tactics employed by cancers are likely to be of substantial value (see Figure 1). Approaches based on the re-direction of T cells are beginning to generate promising clinical results, including evidence of efficacious and durable responses post-treatment, although they are not without risk. Of particular note are cell-based therapies, including adoptive transfer of tumour-infiltrating lymphocytes (TILs), or genetically engineered T cells, and T cell-engaging soluble bi-specific reagents, such as BiTEs and ImmTACs. Despite its popularity, vaccination using antigenic peptides has not proved particularly successful thus far, probably because of the TCR affinity hurdle imposed by thymic selection on naturally ocurring T cells.

Cell-Based Therapy

The biological rationale for this approach is to augment the number of TAA-specific T cells in the patient. This involves *ex vivo* expansion of autologous T cells and their adoptive transfer back into the patient under lymophodepleting conditions. So far, adoptive transfer of TILs has shown some success in the treatment of late-stage melanoma; however, the scarcity of TAA-specific T cells and the low affinity of the TCRs they possess remains a limiting factor to broader application of this approach (2). Recent advances in T cell engineering and gene transfer have enabled investigators to work around this issue, and this has led to the development of two distinct types of gene-modified T cells, both of which express novel engineered receptors capable of recognising TAAs with high affinity.

Chimeric antigen receptors (CARs) couple target cell recognition in a non-MHC-restricted manner with immune effector function. The use of antibody-mediated TAA recognition avoids the problems of thymic selection, as well as MHC down-regulation and altered peptide processing. Furthermore, antibody-antigen affinity is several times stronger than natural TCR-mediated recognition. The majority of first-generation CARs mediate signalling through the zeta subunit of the TCR signalling complex. A major limitation of these CARs is the poor persistence of the CAR-engineered T cells *in vivo*; CAR persistence correlates with tumour regression in patients with advanced metastatic cancer (3,4). Second- and third-generation CARs now include additional co-stimulatory signalling domains, such as those from CD28, CD27 and 41-BB, all designed to enhance immune activation and T cell persistence.

Among the most notable positive clinical data are trials of CAR-engineered T cells targeted to the B cell antigen CD19, in which two out of three patients with chronic lymphocytic leukaemia showed complete remission (5). Nevertheless, since CARs only recognise surface-exposed antigens, they potentially have access to only 10-20 per cent of all coding genes in the human genome (6). Furthermore, the unnatural molecular spacial arrangement imposed by antibody binding may impact optimal T cell functionality.

Accessing intracellular-derived antigens, which comprise the majority of TAAs, requires a TCR-based targeting system and, although targeting is MHC-restricted, the interaction mimics

natural T cell engagement. While adoptive transfer of autologous T cells engineered to express a TAA-specific TCR can be effective, the scarcity of high affinity TAA-specific TCRs favours a strategy whereby low affinity TCRs are first affinity-enhanced by specifically mutating the complementarity determining regions (7,8). The precise level of affinity improvement requires careful investigation. Nevertheless, interim results from an ongoing Phase 2 trial in multiple myeloma patients, reported a "very good partial response", or better, in 7 out of 11 patients treated with TCR affinity-enhanced T cells specific for an epitope of NY-ESO-1, which correlated with prolonged T cell persistence (9).

Soluble Bi-Specific Molecules

The manufacture of soluble biologics can be considerably less expensive and time-consuming than cell-based therapies. Typically, T cell-engaging biologics are bi-specific fusion proteins that combine high-affinity TAA recognition – either antibody or TCR-based – with T cell activation, (usually via an anti-CD3 scFv antibody fragment), resulting in an activation that is independent of the T cells' natural specificity.

Of all the antibody-based approaches, Triomabs are the most advanced, with catumaxomab recently on the market. These reagents incorporate an additional fragment crystallisable (Fc) component to enable the formation of a bridge between three cells: tumour target cell, T cell and accessory cell (macrophage, dendritic cell or natural killer (NK) cell). Triomabs targeting EpCam or Her2 are currently in Phase 1-3 clinical trials in various solid tumour indications (10). T cell-engaging antibodies (BiTEs) contain two single-chain variable fragments (scFv) produced as a single polypeptide chain. The most advanced BiTE – a CD19 targeting agent – resulted in an 80 per cent response rate in a Phase 2 trial in patients with acute lymphoblastic leukemia (11). Also in early phase clinical development are the closely related dual affinity retargeting antibodies (DARTS), which are produced as separate polypeptides joined by a stabilising interchain disulphide bond, and tetravalent tandem diabodies (TandAbs), in which the antibody fragments are produced as non-covalent homodimers folded in a head-to-tail arrangement (12,13).

Unlike antibodies, TCR-based biologics potentially access the entire repertoire of target antigens; however, progress has historically been limited because of the challenges of producing soluble TCRs. Immune-mobilising monoclonal TCRs against cancer (ImmTACs) provide a solution. ImmTACs comprise a soluble TCR, stabilised by a novel interchain disulphide bond and engineered to possess picomolar affinity for pMHC, fused to anti-CD3 scFv, to trigger potent T cell-mediated tumour cell killing *in vitro* and *in vivo* (14). ImmTACs represent the first generation of TCR-based soluble agents with picomolar affinities for their pMHC, overcoming one of the most pertinent obstacles to T cell tumour recognition. The furthest advanced ImmTAC is a gp100 peptide targeting agent which is currently being tested in a Phase 1/2 clinical trial in metastatic melanoma patients: some promising preliminary data is emerging from these studies (15).

Safety

For all the promise and, in some cases, stunning clinical effects of T cell-engaging treatments, there are safety risks associated with such strategies. Both on-target and off-target reactivity against healthy tissue is a particular concern. Clinical trials using MAGE-A3/A9/A12 and Her2-directed T cells provide examples of the possible effects of target toxicity against normal tissues, where adoptively transferred T cells resulted in patient deaths (16,17). Alternatively, cross-recognition of unintended targets expressed by normal tissues has manifested severe adverse events using adoptively transferred T cells directed to MAGE-A3 or MART-1 (18,19). Other types of toxicities triggered by the introduction of non-human sequences into patients have been observed. Indeed, a CAR derived from a murine antibody resulted in a serious adverse event attributed to the generation of IgE antibodies specific to the CAR (18). The adverse events recorded for bi-specific soluble agents have, in general, been less severe due to the inherently transient exposure of patients to these biologics. Nonetheless, some severe toxicities have been observed (11).

Future Perspectives

Advances in a few key areas would further improve the safety and clinical success of redirected T cell therapies. Firstly, choosing a suitable target, coupled with an improved understanding of target expression in cancers and normal tissues, is of paramount importance, particularly for TCR-based

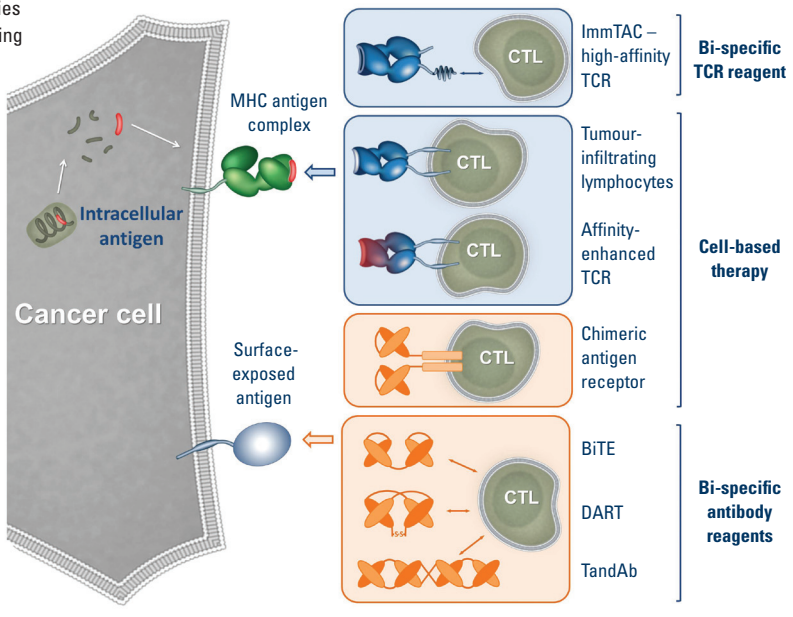

Figure 1: Strategies used for redirecting T cells to target cancer cells

targeting systems that can access a vast array of potential antigens. Secondly, the application of comprehensive pre-clinical tests using more elaborate *in vitro* and *in silico* tools to predict clinical safety and toxicity is crucial to ensure the progression of the most promising pipeline candidates. Indeed, an effective preclinical pathway has recently been described for TCR-based therapeutics (20). Thirdly, the implementation of an 'abort' mechanism for adoptively transferred T cells, such as an inducible suicide system, could improve clinical safety.

Finally, as it is becoming increasingly evident that tumour eradication may require a combination approach, it is probable that redirected T cell therapies will be combined with other treatments, such as chemotherapies or antibodies, in an attempt to improve efficacy and response durability. Conceptually, there is huge potential for synergy with an approach such as this. For example, combined with immune checkpoint antibody inhibitors like anti-PD1, redirected T cells may prove persistently more active as a consequence of inhibiting T cell negative regulation.

In conclusion, there is little doubt that redirection of T cell effector functions for cancer therapy is showing great promise, and perhaps we are now at a tipping point for the field.

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