Background

The goal of T cell therapies is to harness the cytotoxic and other immunomodulatory capabilities of T cells to eliminate cancers or viral infections without eliciting undesired side effects\textsuperscript{1,2,3}. The function of T lymphocytes as orchestrators and effectors of the adaptive immune response is directed by the specificity of their T cell receptors. Early clinical trials showed that the adoptive transfer of autologous or allogeneic isolated CD8+ T cells that are specific for antigens expressed only or at much higher levels in cancer or virally infected target cells can produce therapeutic benefit. However, essential to this strategy is the ability to isolate T cells with high functional avidity. Although it may be possible to isolate such T cells for viral targets, it is typically not the case for many tumour associated antigens (TAAs) which are derived from self and therefore poorly immunogenic. In addition, tumours use several immune subversive approaches to render themselves hostile to immune attack. Such strategies include reduced expression of HLA molecules and target antigens, the establishment of an inhibitory microenvironment\textsuperscript{4} and the ability of the cancer cells to dedifferentiate to evade detection in response to inflammatory cues provided by tumour-specific T cells.

A number of strategies have been employed to isolate or genetically modify T cell receptors. In this way the functional activity of large populations of T cells can be redirected against defined targets. The potential of therapeutic T cells to traffic to sites of disease, to expand and to persist after a single treatment has the prospect of providing major advantages over currently available immunotherapies. There are two main T cell therapy approaches being explored in the clinic: adoptive transfer (T cell reconstitution) and genetically modified T cell therapies

Adoptive Transfer

Profound immunodeficiency such as that associated with allogeneic hematopoietic stem cell transplantation (HSCT) is permissive to uncontrolled replication of latent human herpesviridae such as cytomegalovirus. Morbidity and mortality associated with viral dissemination or its treatment are significant. Adoptive cellular therapy with virus-specific T cells offers the potential for accelerating pathogen-specific immune reconstitution. Since the source of T cells is typically an allogeneic donor there is a risk of induction of graft-versus-host disease (GvHD). In addition the logistics of production of clonal T cell populations may restrict application.

Attempts to restore antiviral immunity following allogeneic HSCT with cell-based immunotherapies initially focused on strategies that required prolonged culture in vitro, and translation into routine clinical practice was limited by the logistics and costs associated with such cell expansion processes. Subsequent approaches have focused on more rapid generation of cellular therapy products, either with more efficient short term culture techniques, direct selection of antigen specific T cells from the donor, or a combination of these strategies\textsuperscript{5}. A theoretical concern with the abbreviated protocols is the potential for persistence of residual T cells with primary alloreactivity and the risk of GvHD. The traditional protocols, with longer-term culture, diluted this risk through repetitive rounds of virus specific stimulation but this mitigation is removed in the shorter term cultures. The limited available clinical data from trials employing the shortened adoptive T cell selection protocols, however, has been
encouraging with no toxicity attributable to reactive T cells and immunological responses against infecting viruses being reported\(^5\).

**Genetically Modified T cell Therapies**

There are two classes of genetically enhanced T cell therapies; gene modified T cell receptor (TCR) therapies and chimeric antigen receptor (CAR) therapies. The defining difference between the two classes of T cell therapy is the type of antigen target; CAR therapies directly recognise the antigen with which they interact (external antigens) whilst TCR therapies require presenting elements such as HLA molecules (internal antigens). This difference is reflected in the respective active moieties. TCR therapy specificity is determined by the transfer of specific T cell receptor \(\alpha\) and \(\beta\) chains whereas a CAR therapy is a fusion receptor coupling antibody-like recognition of the target with T cell activating signals (see Box 1 for summary). For both technologies persistence of a given therapy is linked to the properties of the T cell from which the cells were derived as well as the immune environment into which they are infused.

<table>
<thead>
<tr>
<th>Box 1 Summary of Gene Modified T Cell Therapies: Structural and Functional Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TCR</strong></td>
</tr>
<tr>
<td>Specificity of T cell redirected by transferring (\alpha) and (\beta) chains of a specific TCR</td>
</tr>
<tr>
<td>High avidity achieved by minimising mispairing with native TCRs, e.g. addition of a disulphide bond in extracellular domain</td>
</tr>
<tr>
<td>2nd generation incorporate intracellular domains of costimulatory molecules</td>
</tr>
<tr>
<td>Both</td>
</tr>
</tbody>
</table>

- Expansion and persistence related to intrinsic property of T cell from which infused cells derived
- Efficacy can be increased by patient conditioning

**Gene Modified T Cell Receptor (TCR) Therapies**

By transferring genes for the \(\alpha\) and \(\beta\) chains of a specific TCR, the specificity of a T cell can be redirected (Figure 1). Using this technology, large populations of antigen-specific T cells can be generated with high avidity for the antigen of interest. The most commonly used delivery vehicles are gammaretroviral and lentiviral systems. These integrate into the genome (providing the potential long-term stable expression of transgene), encode no vector proteins (therefore are not immunogenic) and have relatively large packaging capability. Gene modified TCR therapies are based on a single TCR. Since the cognate peptide that is recognised by the receptor will be presented by major histocompatibility complex (MHC) these therapies are MHC restricted.

Crucial to this technology is the minimisation of mixed TCR dimer formation between the introduced TCR and endogenous TCR of the T cell, as mispairing will reduce the number of CD3 (TCR co-receptor) molecules available to form properly paired TCR complexes. Mispairing can significantly decrease functional avidity of the cells expressing the introduced TCR and potentially result in the acquisition of specificities that pose a significant risk for autoimmunity. Numerous strategies have been employed to increase interchain affinity and minimise the risk of mispairing; strategies include murinised TCRs, addition of a second disulphide bond into the extracellular domain through the introduction of an additional cysteine residue into both the \(\alpha\) and \(\beta\) chain C domains or the insertion of point mutations into the \(\alpha\) and \(\beta\) chain C domains.

Gene modified T cell therapy is often limited by the ability of transferred T cells to expand and persist in vivo after transfer and the intrinsic properties of the T cell from which infused cells are derived contributes to their fate in vivo. The introduction of TCR genes into central memory T (T\(_{CM}\)) cells is an attractive strategy for TCR gene therapy\(^5\), because T\(_{CM}\)-derived cells appear not only to function and
survive better after transfer, but can be expanded in vivo which may facilitate the generation and maintenance of large numbers of persistent, target-specific, gene-modified T cells. Patient conditioning can also increase the efficacy of adoptive T cell therapy\(^3\). Lymphodepletion before transfer of T cells, using fludarabine or cyclophosphamide, has been shown in clinical trials to increase the efficacy of infused T cells. Lymphodepletion may promote both the in vivo expansion of transferred cells by increasing the availability of cytokines such as interleukin (IL)-7 and IL-15 that promote the homeostatic proliferation of the existing T cell compartment, and by decreasing the number of T-regulatory cells present\(^6\).

**Figure 1 Gene Modified T cell Therapies Structural and Functional Differences**

A) Generic structure of an endogenous T cell receptor. TCR \(\alpha/\beta\) heterodimers associate with the chains of the CD3 complex. The TCR-CD3 complex is comprised of four dimeric modules: TCR\(\alpha\beta\), CD3\(\delta\epsilon\), CD3\(\gamma\epsilon\), and \(\zeta\zeta\), which associate through intramembrane contacts to form the intact complex. In the T cell receptor construct the \(\alpha\) and \(\beta\) chains are modified to change recognition of the peptide. An additional disulphide bond (ss) in the extracellular domain has been employed to increase interchain affinity and minimise mispairing with endogenous T cell receptors. (Adapted from Stauss et al\(^7\) 2007)

B) Generic structure of a chimeric antigen receptor (CAR). A CAR is a fusion receptor comprised of a number of elements. In this example the CAR is comprised of antibody single chain variable fragments (scFv) conferring target specificity coupled via a hinge and trans-membrane to the CD3\(\zeta\) signalling sequences together with co-stimulation provided by CD28 and a tumour necrosis factor receptor (TNFr). Both the original antibody and the CAR recognise the same cellular target (adapted from Maher\(^2\) 2012).

**Chimeric Antigen Receptor (CAR) Therapies**

CARs are bespoke fusion receptors engineered as chimeric cDNAs and couple the recognition of a designated molecular species on the cell surface (e.g. TAA) to the delivery of a tailored T cell activating signal\(^2\). The principle was identified 25 years ago when it was shown that antibody variable light and heavy gene segments can transfer specificity for native antigen when substituted for the corresponding elements within the TCR \(\alpha\beta\) heterodimer. T cell activation is coupled to antibody like recognition. CAR
specificity is frequently determined by a single chain variable fragment (scFv – the targeting domain) formed by the self-association of cloned variable regions of heavy and light chains of a monoclonal antibody. The scFv is linked via a flexible spacer region to an intracellular signalling domain typically one of the TCR complex molecules such as the CD3ζ transmembrane and endodomain. Unlike αβ T cell receptors, antigen recognition by CARs is direct and is therefore, not dependent upon HLA status so in principle the same CAR-based approach can be used in all patients who express the target of interest. A range of macromolecules can also be targeted using this system, including proteins, carbohydrates, and glycolipids. A disadvantage however, is that CARs cannot recognise intracellular antigens unless specifically designed to recognize defined HLA-peptide combinations.

There are currently 3 generations of CAR therapies. First generation CARs contain heavy and light chain immunoglobulin variable regions fused as a single chain to the ε, γ or ζ signalling sequences of the TCR or the signalling region of the Fcγ domain. First generation CAR T cells typically have limited survival in patients and limited anti-tumour responses in the clinic because of incomplete activation of T cells with limited expansion and persistence in vivo. Normal T cell activation is dependent on receipt of two classes of signal: one through engagement of the TCR with antigen presented by MHC and a second through engagement of co-stimulatory molecules such as CD28, OX40 and CD40L. As tumours often do not express appropriate ligands for co-stimulatory molecules, engagement of a first generation CAR in the absence of co-stimulation leads to anergy and failure of in vivo expansion. To overcome these restrictions second generation CARs were developed incorporating the intracellular domains of co-stimulatory molecules (e.g. CD28). An alternative strategy is to provide co-stimulatory molecules by delivery of CAR transgenes to virus-specific T cells allowing for the induction of the co-stimulatory cascade upon cognate TCR ligation. So called “Third generation” CARs are now in development which deliver more than one type of co-stimulatory molecule. Inclusion of the additional co-stimulatory sequences such as OX40 as well as CD28 appears to reduce the resulting increased inhibitory cytokine IL-10 production observed with the second generation CARs, while preserving or enhancing the production of pro-inflammatory cytokines. However, since many in vivo studies have not included a comparison of available second and third generation CARs definitive conclusions on improved function are limited.

Although the majority of CARs are expressed in autologous patient-derived T cells functional CAR-based fusions have been demonstrated in other leukocytes including NK cells, dendritic cells and neutrophils. Antigen targeting by CAR molecules most commonly involves the use of scFv from monoclonal antibodies but several other cellular targets can also serve this purpose, including ligands, peptides, receptor derivatives and single domain antibodies. CAR functionality may be compromised by immunogenicity. Early fusions contained murine sequences but the move now is towards humanized or fully human molecules. This is likely to ameliorate the problem but immunogenicity may still arise due to the presence of idiotype sequences and fusion junctions between CAR components. Current research into the development of improved CAR molecules is focused on strategies to optimise the tumour microenvironment (e.g. modifying cells to secrete IL-12), modifications to enhance T cell trafficking (e.g. co-expression of chemokines), improving cell survival (e.g. provision of cytokine support) and investigating delivery of CARs to more immature T cell populations as such cells may exhibit less effector function but have greater capacity for in vivo survival and proliferation. To maximise therapeutic benefit it is likely that future CARs may also need to activate other strands of the host immune response.
Safety of T Cell Receptor Therapies

Gene modified T cell therapies require vectors capable of sustained high levels of expression and the ability to package large inserts. TCR therapies require the transfer of the variable and constant regions of the α and β genes of interest and for CAR therapy, the targeting moiety is coupled in series to hinge, transmembrane and T cell activating domains. Retroviral vector mediated gene transfer has, therefore, been central to the development of gene modified T cell therapies. However, retroviral vectors come with generally accepted risks; the production of replication competent viruses (see Box 2 for summary) and insertional mutagenesis, specifically oncogenic activation (see Box 3 for Summary). Advances in vector design have minimised the risk of replication competent viruses and there is no evidence to date of their detection in the clinical setting. Oncogenic activation, however, has been observed, with leukaemias or pre-leukaemias reported in four gene therapy clinical trials that used first generation γ-retroviral vectors to modify haematopoietic stem cells⁹,¹⁰,¹¹. However, numerous clinical trials targeting mature T cells utilizing γ-retroviral vectors have not yielded evidence for insertional adverse events despite long-term persistence of transduced cells¹². These data suggest that disease background factors and cell-intrinsic mechanisms may modify the risk of insertional mutagenesis.

The majority of genetically modified T cell therapies are tumour immunotherapies and the targets are therefore self-antigens. On target-off tumour activity is a clear risk and one that has been realised in the clinic. There have been reported serious adverse events following administration of T cell receptor therapies. Two patients died shortly after adoptive transfer of CAR T cell therapies¹³ and four patients have died following administration of genetically engineered TCR T cells¹⁴,¹⁵ and serious adverse
events, severe transient inflammatory colitis\textsuperscript{16} and melanocyte destruction in skin, ears and eyes\textsuperscript{17,18}, have also been reported in two other TCR T cell trials. Evidence is emerging of recognition of antigen expressed on non-tumour cells by some T cell therapies. This may manifest as immediate toxicity or late sustained toxicity. This is especially of concern with T cell therapies directed against untested and/or endogenously prevalent TAAs, as T cells with high avidity receptors may respond to cells that express their targets at levels currently too low for detection. New technologies are being investigated to better understand the expression pattern of TAA genes in normal tissues such as deep sequencing and large scale immunohistochemical analysis. In addition cautious approaches are employed in the design of initial clinical trials, particularly with respect to starting cell dose\textsuperscript{6}.

**Clinical Efficacy of T cell Therapies**

Virus specific T cell therapies have been successfully applied in the clinic (Table 1) and have been shown to prevent and treat a range of post-transplant viral infections, with long term protection being reported\textsuperscript{32}. Strategies are now being implemented to move towards the larger scale trials needed to progress these therapies into routine clinical use\textsuperscript{32}. There has also been significant progress in the development of gene modified T cell therapies and the first reports of benefits to patients are being reported in clinical trials (Table 1) with complete responses being reported in both trials of TCR and CAR T cell therapies\textsuperscript{33}.

<table>
<thead>
<tr>
<th>Target Antigen</th>
<th>Target Disease</th>
<th>T Cell Therapy</th>
<th>Patients</th>
<th>Responses</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adv</td>
<td>Adenoviral infection reactivation post HSCT</td>
<td>Adoptive transfer</td>
<td>5</td>
<td>3 patients infections resolved</td>
<td>19</td>
</tr>
<tr>
<td>CMV</td>
<td>Cytomegalovirus infection reactivation post HSCT</td>
<td>Adoptive transfer</td>
<td>16</td>
<td>12 patients infections resolved</td>
<td>20</td>
</tr>
<tr>
<td>EBV, CMV, Adv</td>
<td>Viral reactivation post HSCT</td>
<td>Adoptive transfer</td>
<td>10</td>
<td>4 single infection and 4 dual infection patients infections resolved</td>
<td>21</td>
</tr>
<tr>
<td>GP100</td>
<td>Melanoma</td>
<td>TCR</td>
<td>16</td>
<td>1 CR and 2 PR</td>
<td>18</td>
</tr>
<tr>
<td>MAGEA3</td>
<td>Melanoma, oesophageal and synovial sarcoma</td>
<td>TCR</td>
<td>9</td>
<td>1 CR and 4 PR</td>
<td>22</td>
</tr>
<tr>
<td>NYESO-1</td>
<td>Melanoma and sarcoma</td>
<td>TCR</td>
<td>17</td>
<td>2 CR and 7 PR</td>
<td>23</td>
</tr>
<tr>
<td>NYESO-1/LAGE1</td>
<td>Multiple Myeloma</td>
<td>TCR</td>
<td>11</td>
<td>3 CR, 4VGPR, 3PR (interim data)</td>
<td>24</td>
</tr>
<tr>
<td>CD19</td>
<td>Lymphoma and CLL</td>
<td>CAR</td>
<td>7</td>
<td>1 CR, 5 PR and 1 SD</td>
<td>25</td>
</tr>
<tr>
<td>CD19</td>
<td>CLL and B-ALL</td>
<td>CAR</td>
<td>8</td>
<td>3 SD to 6 months</td>
<td>26</td>
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<tr>
<td>CD19</td>
<td>NHL</td>
<td>CAR</td>
<td>6</td>
<td>2 SD to 10 months</td>
<td>27</td>
</tr>
<tr>
<td>CD19</td>
<td>CLL</td>
<td>CAR</td>
<td>3</td>
<td>2 CR and 1 PR</td>
<td>28</td>
</tr>
<tr>
<td>CD19</td>
<td>ALL</td>
<td>CAR</td>
<td>2</td>
<td>1 CR and 1 CR followed by relapse after 2 months</td>
<td>29</td>
</tr>
<tr>
<td>CD19</td>
<td>ALL</td>
<td>CAR</td>
<td>27</td>
<td>24 CR (89%) within 28 days. 18 ongoing CR (64%) at a median of 2.6 months after treatment</td>
<td>30, 31</td>
</tr>
</tbody>
</table>

There is still a high degree of variability in success in the reported gene modified T cell therapy trials, not just between trials but also within a trial. Recently however, in a trial run by the Children’s Hospital of Philadelphia and the University of Pennsylvania, 89% of children and adults with a highly aggressive form of acute lymphoblastic leukemia showed no evidence of cancer after receiving a CD19 CAR T cell therapy. Although 6 patients subsequently relapsed from complete response, 64% of patients remained in complete response at a median of 2.6 months demonstrating the potential clinical benefit of this class of therapies. Patients who have shown complete responses typically exhibit greater persistence and survival of the gene modified T cells. Consistency of manufacturing process, patient conditioning regimens, cytokine support and the T cell subset transduced are all variables that may impact efficacy and are the subject of ongoing research. Another factor is cell dose. T cell therapies are typically administered as number of cells per kilogram body weight but T cells are replicative and delivered dose may not bare any resemblance to final steady state number of cells and will be patient specific. It is not yet known if tumour antigen loss variants will have a significant impact on long term efficacy but such an outcome has been reported in one clinical trial of a CD19 CAR therapy for the treatment of acute lymphoblastic leukaemia. Although the patient initially showed a complete response they subsequently relapsed due to the presence of blast cells that no longer expressed CD19. Other patients, however, have reported complete remissions sustained for a number of years. Clinical trials targeting optimal tumour target and clinical regimens are now warranted.

UK Future Directions

The UK has an established commercial and research base with active clinical and research programmes in all three T cell therapy classes. The field of T cell therapies is poised for significant clinical advances and a phase III trial for an adoptive T cell therapy has already been initiated by one UK commercial organisation. Research efforts are focused on addressing the key challenges of T cell avidity, persistence and ability to exert the desired anti-tumour effects as well as identifying new target antigens. These programmes are advancing the clinical translation of T cell receptor therapies.

References


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