T cell engineering as therapy for cancer and HIV: our synthetic future

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It is now well established that the immune system can control and eliminate cancer cells. Adoptive T cell transfer has the potential to overcome the significant limitations associated with vaccine-based strategies in patients who are often immune compromised. Application of the emerging discipline of synthetic biology to cancer, which combines elements of genetic engineering and molecular biology to create new biological structures with enhanced functionalities, is the subject of this overview. Various chimeric antigen receptor designs, manufacturing processes and study populations, among other variables, have been tested and reported in recent clinical trials. Many questions remain in the field of engineered T cells, but the encouraging response rates pave a wide road for future investigation into fields as diverse as cancer and chronic infections.

1. Introduction

It is widely accepted that the immune system has evolved cellular and humoral mechanisms that can evoke natural immune responses to tumours [1]. However, in most instances, vaccines fail to induce rejection of established tumours [2]. Adoptive T-cell transfer, a term coined by Billingham et al. [3], has the potential to overcome one of the significant limitations associated with vaccine-based strategies, and specifically the requirement to de novo activate and expand a tumour antigen-specific T-cell response in patients, who are often immune compromised. Mitchison [4] first reported the targeting of cancer through the adoptive transfer of lymphocytes in rodent models over 50 years ago.

Application of the emerging discipline of synthetic biology to cancer, which combines elements of genetic engineering and molecular biology to create new biological structures with enhanced functionalities [5], is the focus of this volume. In 1989, Eshhar and co-workers [6] reported the first synthetic receptor expressed in lymphocytes. Shortly thereafter, Irving & Weiss [7] reported that a chimeric antigen receptor (CAR) comprised CD8, and the CD3ζ chain was sufficient to activate T cells. A coalescence of pre-clinical and clinical data supports the premise that the principles of gene transfer combined with adoptive cellular therapy are poised to overcome the fundamental limitations associated with central and peripheral tolerance and enable the potent and efficient at-will targeting of tumours.

There are many mechanisms that prevent the immune system from eliminating tumours in most patients [8]. One major issue is the relatively low affinity of T-cell receptors (TCRs) for self-antigens compared with foreign antigens. In humans, comparative analyses have revealed that the TCRs from T cells that recognize self-tumour antigens have a substantially lower affinity (approx. 1.5 logs) for cognate major histocompatibility complex (MHC) : peptide complexes compared with their virus-specific TCRs [9]. Adoptive transfer using engineered TCRs and CARs is a promising approach to overcome this obstacle (figure 1). The adoptive transfer of T cells with endogenous TCRs is an effective therapy for virally induced tumours. As reviewed by Rooney and co-workers [10], the fraction of cancer known to be caused by tumour-associated viruses continues to increase. Because cytomegalovirus (CMV) appears to infect...
Adoptive transfer of tumour-infiltrating lymphocytes (TILs)

2. Tumour-infiltrating lymphocytes

Despite anti-retroviral therapies (ARTs), HIV-1/AIDS continues to cause a considerable medical and economic burden, and there continues to be a pressing need for an HIV-1 cure. The goal of engineered T-cell therapy for HIV is to generate an immune system that can resist HIV-1 infection, control viral replication below the limit of detection and persist at high functional competency in the absence of ART. A number of recent technical, conceptual and clinical trial advances now make the goal of a HIV-1 cure tangibly reachable.

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A number of recent technical, conceptual and clinical trial advances now make the goal of a HIV-1 cure tangibly reachable. Our group has recently infused CD4 T cells rendered HIV-1 resistant by deletion of CCR5 using zinc finger nucleases into HIV-1-infected individuals [13]. These modified T cells not only persisted during ART interruption, but also exerted some control of HIV-1 replication in vivo. In an unpublished follow-up study supported by Sangamo Biosciences, two subjects controlled HIV-1 replication off ART, one maintaining control for 48 weeks.

2. Tumour-infiltrating lymphocytes

Adoptive transfer of tumour-infiltrating lymphocytes (TILs) following harvest from tumour and ex vivo expansion was pioneered by a group at the National Cancer Institute, under the premise that lymphocytic infiltrates at tumours are enriched for tumour antigen-specific T cells. As reviewed by Hinrichs & Rosenberg [14], many factors influence the success of this approach, including culture technology and host conditioning with chemotherapy and ionizing radiation. TIL cultures for adoptive transfer typically are generated via short-term ex vivo expansion and screening for anti-tumour activity. TIL-based approaches have been primarily evaluated in the setting of melanoma, in part because melanoma biopsies are readily obtainable and in part because melanoma has long been considered to be an ‘immunogenic’ tumour. TIL therapy has been shown to result in durable tumour regression in a subset of patients with advanced metastatic melanoma [15]. As reviewed by Linnemann et al. [16], the mechanisms of responses of patients treated with TILs are the result of T cells reacting to shared antigens as well as neo-antigens created by tumour-specific mutations or by epitopes that are encoded by alternative open reading frames [17,18]. Preliminary data suggest that some T-cell responses against neo-antigens may be of a higher magnitude than T-cell responses against shared self-antigens [19,20]. We believe that the major issue facing the field that prevents the widespread use of TIL therapy has been the infusions of high dose IL-2 and the attendant off target toxicities. A secondary obstacle is the challenging logistics of tumour harvest and TIL culture that has prevented investigators from conducting randomized clinical trials analysed with intent to treat endpoints.

3. Chimeric antigen receptors

CARs are modular polypeptides typically consisting of three distinct modules: an extracellular target-binding module, a transmembrane module anchoring the CAR into the cell membrane and an intracellular signalling module. The extracellular target-binding module is usually derived from scFv determinants isolated from antibodies, linked in a single chain through linker polypeptide sequences. Transmembrane modules are usually derived from molecules involved in T-cell function such as the CD8 and CD4 coreceptor molecules [21]. Recent contributions by Chmielewski et al. [22], Cheadle et al. [23], Ruella & Kales [24] and Jensen & Riddell [25] focus on the status of CARs in clinical trials. The principal advantage of CAR-based strategies is that the target-binding moiety is derived from antibodies with affinities several orders of magnitude higher than TCRs. In addition, because CARs recognize intact cell surface proteins, targeting of target cells is neither MHC restricted nor dependent on processing and effective presentation of target epitopes, and therefore, CAR-based approaches are insensitive to tumour escape mechanisms related to MHC loss variants. At this point, many groups have shown that CAR T cells have potent anti-tumour effects against a variety of advanced haematologic malignancies of the B-cell lineage. The central issue facing the field is whether
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T cells, expanded from both cancer patients and healthy volun-
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a
b
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endogenous TCR and to target the reservoir of HIV-1 by
infected CD19, because it has also been observed with blinatumomab [29].
The management of cytokine release syndrome has recently
been reviewed [30].

4. T-cell receptor engineering

The feasibility of transferring T-cell specificity into primary
T cells through transfer of TCR α and β chains was demon-
strated almost 20 years ago [31,32]. Tumour-antigen-specific
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implemented to expand such T cells. Because of the low fre-
cuency of such T cells in peripheral blood, the lack of effective
culture and expansion methodologies, and the impact of central
tolerance on the repertoire, T cells have only be isolated with
considerable difficulty using these approaches; furthermore,
such T cells are in general of low affinity and demonstrate
weak anti-tumour activity. A number of approaches to over-
come these issues and generate more potent tumour antigen-
specific T cells have been developed. One recent and promising
approach to overcome the issue of the intrinsically low-affinity of
TCR to self-antigens has been to enhance the affinity of the
TCR isolated from such T cells by mutagenesis of α and β recep-
tor chains. Recent technological advances have facilitated
elegant molecular and rational high-throughput genetic
approaches to affinity enhance TCRs [33–35], and such efforts
have resulted in the ability to reproducibly generate TCR with
substantially higher affinities for target antigens [36]. An
alternative strategy to enhance TCR affinity follows from
observations of enhanced functional avidity and improved rec-
ognition of tumour cells following introduction of mutations
that reduced N-glycosylation on TCR chains [37].

As reviewed by Hinrichs & Rosenberg [14], and Ruella &
Kalos [24], there are promising early results in a variety of
tumours treated with T cells expressing TCRs engineered by
various approaches. However, there have also been on-target and off-target toxicities with engineered TCRs. In one trial, T
cells were engineered to express a TCR generated in HLA-
A*0201 transgenic mice (i.e. not subjected to selection by the
human immune system) and that recognized an epitope
shared between MAGE-A3, -A9 and -A12. Of nine patients
-treated, five demonstrated objective clinical responses, but
three patients demonstrated serious adverse events associated
with neural toxicity, including two deaths. Post-mortem anal-
ysis revealed rare and previously unrecognized expression of
MAGE-A12 in brain tissue [38]. Two trials that evaluated the
use of affinity enhanced HLA-A*01-restricted and MAGE-A3-
specific TCR to target melanoma and myeloma were reported
recently. The first treated patient in each of these trials experi-
ced severe cardiac toxicity, and each patient died within 7
days of T-cell infusion [39]. Retrospective analysis dem-
strated that the affinity enhancement of the TCR resulted in
the off target recognition of a related HLA-A*01-restricted epitope
from the protein titin expressed in cardiac cells [40]. These
results highlight the potency of adoptively transferred T cells
with redirected specificity and the need to develop improved
methods for pre-clinical screening of engineered TCRs. A
potential toxicity following the introduction of engi-
neered TCRs is the production of mixed dimers comprised
chains from the endogenous TCR with chains from the
transgenic TCR [41]. As reviewed by Torikai et al. [42], a
particularly elegant approach to prevent this complication
involves TCR gene editing with zinc finger nucleases.
Expression of the endogenous TCR α and β chains can be
permanently abrogated using this approach, resulting in
improved expression and function of the transgenic TCRs
and CARs [42,43].

5. Bridging success in cancer to HIV

It is interesting to note from a historical perspective that some of
the first forms of adoptive cell transfer (ACT) involving gene-
modified T cells were conducted two decades previously in
patients with advanced HIV-1/AIDS [44], and that many of
the results from trials conducted in patients with AIDS have
informed current concepts in the field of cancer [45]. The initial
trials were done in order to control drug-resistant forms of HIV-
1 infection. However, the current challenge in the field is to
develop cellular therapies with the potential to eliminate the
reservoir of HIV-1 that is resistant to current antiviral therapies
[46]. The field has been energized by an extraordinary exper-
iment conducted by Gero Hütter and co-workers in Berlin in a
patient who has apparently been cured of HIV infection follow-
ing an allogeneic haematopoietic stem cell (HSC) transplant
and ACT from a homozygous CCR5 delta32 donor [47]. There are a
number of approaches to induce a cell-intrinsic resistance to
HIV-1 infection and to target the reservoir of HIV-1 by
gene-modified ACT [48].
6. Cellular engineering

In addition to receptor engineering, optimizing the effector function of engineered T cells can also increase clinical efficacy. Previous disappointing results with adoptive transfer strategies were due to the use of cell culture approaches that resulted in a population of terminally differentiated effector cells. Recent results with CAR T cells indicate that proliferative capacity of the infused T cells is a predictive biomarker of clinical responses, as reviewed by Kalos and co-workers [24]. It is now well recognized that stimulation of T cells via their TCR without a second costimulatory signal induces tolerance and more recent CAR-based technologies have focused on overcoming this limitation. Thus, while first-generation CARs depended on intracellular transduction of the recognition signal via the CD3ζ chain alone, second- and third-generation CAR constructs have incorporated costimulatory signalling domains such as those derived from CD27, CD28, CD134 or CD137. In addition, culture systems that provide costimulation by immobilized ligands on beads have improved the function of adoptively transferred T cells [49]. Sophisticated artificial antigen-presenting cells that provide arrays of selected costimulatory molecules and cytokines have been developed [50,51], as reviewed by Butler & Hirano [52].

A major controversy in the field is defining the optimal cell product for infusion. At issue is whether to purify selected subsets of cells for culture and subsequent genetic engineering or, more straightforwardly, to use bulk cell products that contain mixtures of CD4⁺ helper, CD8⁺ cytotoxic, naive, central memory, effector memory and other subsets? For example, cell culture conditions can be optimized to promote the expansion of T-central memory cells using anti-CD3 and anti-CD28 coated beads with IL-7 and IL-15 [53]. As summarized by Fowler [54], the blockade of the mechanistic target of rapamycin (mTOR) during culture has the potential to enhance adoptive therapy approaches. Manipulation of metabolic pathways with rapamycin and other mTOR kinase inhibitors can change the fate and function of adoptively transferred T cells [55]. Furthermore, CAR T cells encoding a rapamycin-resistant mutant of mTOR have enhanced anti-tumour effects in pre-clinical models [56]. The factors related to the desired composition of the adoptively transferred cells are reviewed by Jensen & Riddell [25]. T cells with stem cell-like properties have been described [57,58]; however, it is not yet known if these cells are superior to central memory or naive T cells. Ghosh et al. [59] have focused on the development of T-cell-based immunotherapy for use in the context of allogeneic HSC transplantation. They have reviewed some recent studies on the development of ‘off the shelf’ cellular immunotherapies across MHC barriers, highlighting the key milestones in their development and use. In particular, they show that the adoptive transfer of precursor T cells enhances T-cell reconstitution after allogeneic stem cell transplantation [60].

A major issue with clinical adoptive cell transfer therapy is the avoidance of senescent and exhausted states in the infused cells. This issue was not predicted in mouse models because of substantial differences in telomere biology between the mouse and human immune systems [61]. With TIL therapy, the telomere length of the transferred lymphocytes correlates with in vivo persistence and tumour regression in melanoma patients receiving cell transfer therapy [62]. CD28 costimulation can augment telomerase activity and enhance telomere length during in vitro culture [63,64]. One approach to circumvent this issue is the use of HSCs or induced pluripotent stem cells [65,66], as reviewed by Gschweng et al. [67]. Another approach to prevent terminal differentiation during culture is to uncouple cell proliferation from effector differentiation. Crompton et al. [68] have reviewed the cellular mechanisms that lead to progressive differentiation during the physiologic immune response and they propose the use of synthetic biology to uncouple proliferation from differentiation.

A potential safety concern related to the infusion of engineered T cells is virus integration-related insertional mutagenesis and cellular transformation, which has been demonstrated with the genetic engineering of HSCs [69]. This issue may also occur with non-viral-based integration using sleeping beauty, as described by Cooper [42,43]. In patients with congenital and acquired immunodeficiency, genetically modified T cells have been shown to persist after adoptive transfer in humans for more than a decade without adverse effects [45,70], indicating that the approach to genetically modify mature human T cells is fundamentally safe, at least in part, because lentiviral integration sites are not random and do not favour proto-oncogenes [71]. Furthermore, unlike B cells, T cells are subject to clonal competition at the TCR level, which may explain the rarity of T-cell leukaemia and the relative resistance of T cells to transformation [72].

The development of mechanisms to control the lifespan of the transferred T cells is yet another challenge for the field. Initial approaches attempted to introduce ‘suicide genes’ such as the herpes simplex virus thymidine kinase (TK) gene; however, these efforts revealed the strong potential for immunologic rejection based on targeting of TK-derived sequences [73]. More recently, an elegant and potentially powerful inducible system based on the use of a modified human caspase-9 fused to a human FK506 binding protein permits conditional dimerization and delivery of apoptotic signals in response to small molecules that can permeate the T-cell plasma membrane is currently being evaluated in clinical trials [74]. Approaches to regulate the persistence of engineered T cells are discussed by Dotti and co-workers, Gottschalk, Savolodo and Brenner [75] and by Jensen & Riddell [25].

7. Conclusion

In this review, we have highlighted two basic gene-transfer approaches that are being pursued to bypass the effects of central and peripheral tolerance on the T-cell repertoire. Clinical data from the group at the University of Pennsylvania and elsewhere generated principally over the past 5 years suggest that we are at the threshold of a golden era for adoptive T-cell therapy, with a number of recent profound examples of the potency and promise of this approach to target cancer. Recent reports, using CAR T cells with CD137 and CD3ζ signalling domains, which documented long-term functional persistence of T cells engineered to target CD19, along with long-lasting clinical remissions and ongoing B-cell aplasia, have highlighted the potential for adoptive T-cell transfer to induce a profound long-term functional anti-tumour activity [76,77]. Despite these early successes, a number of fundamental and important questions still remain to be resolved for the broad, reproducible and
effective implementation of this approach to treat cancer beyond B-cell malignancies.

A few common themes have emerged as the principle challenges to the field. First, identification of the optimal composition of the transferred cellular product requires clarification. Second, in ongoing clinical studies with CAR-engineered cells that target CD19, patients remain disease free with persisting engineered T cells for more than 4 years post-treatment but also with ongoing B-cell aplasia owing to the practical necessity to eventually ablate engineered cells and enable normal B-cell reconstitution. Therefore, a central issue facing the field is the design and implementation of various approaches to control the fate of adoptively transferred cells. These findings are being translated into the clinic at a rapid pace, and it is likely that engineered T-cell transfer will become established as an effective cancer therapy during the next decade. Finally, a challenge for adoptive T-cell therapy will be the necessity and rationale to combine the therapy with other anti-tumour therapies. In particular, we will require information to rationally combine with therapeutic vaccination, checkpoint inhibition, agonistic antibodies, small molecule inhibitors of tumours and the targeting of tumour stroma and neo-vascularisation, as discussed by Yee [78].

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