

Review Article

Improving TCR Gene Therapy for Treatment of Haematological Malignancies

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Adoptive immunotherapy using TCR gene modified T cells may allow separation of beneficial Graft versus tumour responses from harmful GvHD. Improvements to this include methods to generate high avidity or high affinity TCR, improvements in vector design and reduction in mispairing. Following adoptive transfer, TCR transduced T cells must be able to survive and persist in vivo to give most effective antitumour responses. Central memory or naive T cells have both been shown to be more effective than effector cells at expanding and persisting in vivo. Lymphodepletion may enhance persistence of transferred T cell populations. TCR gene transfer can be used to redirect CD4 helper T cells, and these could be used in combination with CD8+ tumour specific T cells to provide help for the antitumour response. Antigen specific T regulatory T cells can also be generated by TCR gene transfer and could be used to suppress unwanted alloresponses.

1. Introduction

Allogeneic haematopoietic stem cell transplantation (HSCT) is an effective treatment for many haematological malignancies. In addition, unselected donor lymphocyte infusions (DLIs) can be utilized to successfully treat relapsed leukaemia after HSCT [1]. Depending on the degree of HLA mismatch, donor T cells recognize alloantigens derived from allogeneic MHC or from polymorphic minor histocompatibility antigens (mHags) expressed by the host. Whilst able to deliver beneficial Graft versus Tumour effects (GvT), alloreactive T cells may also direct their response against normal tissues resulting in Graft versus Host Disease (GvHD), and this is one of the leading causes of transplant-related morbidity and mortality. The incidence of GvHD can be reduced by utilizing T-cell-depleted transplants-but this also leads to an increase in disease relapse rate [2–4]. How best to deliver effective GvT responses whilst minimizing harmful GvHD remains a significant challenge.

Refining the concept of donor lymphocyte infusions by isolating donor lymphocytes that have known tumour reactivity may result in more effective GvT. Falkenburg et al. have

utilized donor-derived leukaemia reactive cytotoxic T lymphocytes (CTLs) to treat a patient with relapsed accelerated phase CML after HSCT [5]. The patient, who had previously been resistant to DLI, went on to achieve a complete remission as a result of this therapy. A phase I/II study looking at generating leukaemia reactive CTLs for patients with relapsed leukaemia after HSCT found that whilst this strategy was feasible, it was complex and time consuming requiring improvements before becoming a definitive treatment strategy [6].

As an alternative to isolating tumour reactive lymphocytes, the specificity of T cells can be redirected by retroviral gene transfer of T-cell receptor (TCR) genes. This allows T cells to be generated that are specific for a defined tumour antigen presented by MHC. TCR gene transfer using retroviruses was first demonstrated by Clay et al. who transduced human T cells with a TCR that was specific for a melanoma antigen presented by HLA-A2. These redirected T cells had demonstrable antitumour reactivity in vitro [7]. TCRs targeting a variety of tumour associated antigens (TAA) have now been used for retroviral transduction of T lymphocytes and shown to respond to specific tumour antigens

in vitro or provide tumour protection in vivo in murine models post adoptive transfer [8–12].

The first clinical trial using TCR gene modified T cells was for treatment of patients with metastatic melanoma. In this study, autologous peripheral blood lymphocytes were transduced with alpha and beta chains specific for a melanoma TAA, MART-1, and adoptively transferred to a cohort of patients with metastatic melanoma. This resulted in an objective response rate in 2/15 patients (13%) with both responders obtaining long-term disease remission [13]. A high-avidity TCR targeting the same MART-1 epitope has been developed, and this has led to anti tumour responses in 6/20 (30%) of patients treated. In the same trial, a high avidity TCR targeting the gp100 melanoma antigen resulted in tumour regression in 3/16 patients (19%) [14]. TCR-transduced autologous T cells targeting the cancer testis antigen, NYESO1, have been used to treat patients with metastatic melanoma and advanced synovial cell carcinoma resulting in response rates of 45% and 67%, respectively [15]. Despite this, the response rate is still far below that which has been achieved using antigen-specific tumour infiltrating lymphocytes (TILs). Currently the best objective response rate described using autologous TIL in patients with metastatic melanoma is 72%, with 16% achieving complete remission [16]. Clinical trials utilizing TCR-transduced cells for treatment of haematological malignancies are still awaited, but this may allow the delivery of effective GvT responses without harmful GvHD effects.

2. Generating High-Affinity and High-Avidity TCR-Transduced T Cells and Reducing Mispairing

Improving the therapeutic GvT effect may be achieved by an increase in the functional avidity of the transduced T cell for its specific tumour antigen. Increasing T cell avidity has been correlated with improved elimination of tumour cells in vivo [17]. Avidity of the TCR is a function of the level of expression of the TCR on the T-cell surface and the individual affinity of the TCR for its cognate peptide-MHC (pMHC). High-avidity CTLs specific for TAA may be absent from the peripheral T cell repertoire as a result of deletion during thymic selection or tolerance induction following encounter of TAA in the periphery.

One method to generate high-avidity TAA-specific CTLs is to immunize human HLA expressing transgenic mice with human TAA peptides thus eliciting an immune response against the human peptide. CTLs that express high-avidity TCRs can then be selected, and the TCR genes isolated and used to transduce peripheral human lymphocytes [18, 19]. Alternatively the allorestricted technique can be utilized: T cell tolerance is self MHC restricted, but peptides presented in the context of an allogeneic MHC molecule will be able to stimulate high-avidity CTLs. TAA peptides presented by MHC molecules of a known HLA haplotype can be used to stimulate lymphocytes in vitro from a HLA-mismatched

donor, and, from this, antigen specific high avidity T cells can be isolated [20, 21].

Naturally occurring TCRs are typically of low affinity, normally within the range of 1–100 μmol [22, 23]. TCRs can be modified to increase individual TCR affinity by introducing amino-acid substitutions into the CDR regions of the alpha and beta chains, in particular into CDR3 regions that bind to peptide. TCRs can be selected for increased affinity using yeast or bacteriophage display techniques [24–26]. This has led to the generation of high-affinity TCR which have affinity of up to 1 million times higher than that of wild-type TCR but still retain the ability to recognize specific peptide MHC. Some of the high affinity TCRs do lose antigen specificity as the affinity for pMHC increases, particularly if the TCR is expressed in CD8+ T cells [25]. Zhao et al. transduced CD8+ and CD4+ T cells with a class I-restricted TCR specific for NY-ESO. They found CD8+ cells transduced with high-affinity TCR lost their antigen specificity at very high ranges of affinity whilst CD4+ T cells transduced with high-affinity TCR had a marked increase in reactivity against specific pMHC and tumour cell lines with no evidence of cross-reactivity [27]. One other possible drawback of increasing TCR affinity is that there may be a threshold of affinity above which TCR function begins to plateau or even decrease. Higher-affinity TCRs have a longer dissociation time from pMHC, reducing the number of TCRs bound by any given pMHC which may lead to a reduction in T-cell activation, particularly if antigen is present at low concentration. Thomas et al. have recently demonstrated that TCR with affinity above the normal range trigger faster effector responses than wild-type TCR, but this is associated with a progressive loss of response to low-density antigen as affinity increased [28].

Higher surface expression of TCR has been shown to correlate with an increased responsiveness to specific antigen [29–31]. Improvements in vector design have led to an increased transduction efficiency of the introduced alpha and beta chains leading to an increased surface expression of the TCR. Commonly the TCR genes are inserted into a retroviral cassette although the use of lentiviral vectors has increased. The use of an IRES to link the alpha and beta chains allows transcription of a single mRNA initiating at the long terminal repeat (LTR) of the virus. The upstream gene is translated from the 5' cap and the downstream gene translated from the IRES element. However this can lead to differential expression of the two genes with the downstream gene being expressed at a lower level [32]. More commonly genes are now being linked by viral derived 2A oligopeptide linker sequences [33]. 2A sequences can be inserted between 2 genes within the one vector backbone. The upstream gene is translated fully, but, upon reaching the 2A sequence, an interaction between the 2A and the ribosome terminates translation. The first protein is released with the 2A sequence fused to its C terminus and the ribosome “skips” to the downstream gene and resumes translation. This can lead to the production of multiply expressed genes within one vector cassette [34]. Direct comparison of retroviral vectors expressing alpha and beta chains linked by IRES or 2A

sequences demonstrates that the 2A linker sequences result in higher expression of TCR genes and improved T-cell function [35]. Codon optimization of the insert by replacing rarely used codons with those in more frequent use has also been shown to increase expression level of introduced TCR which leads to an increase in tumour protection in vivo [31].

When introducing a new alpha and beta chain into a T cell, ideally they will pair only with one another. However, the introduced alpha and beta chains may form mixed heterodimers whereby the introduced alpha chain pairs with the endogenous beta chain and the introduced beta chain pairs with the endogenous alpha chain. This can lead to the expression of 2 new TCR specificities by the T cell with the potential to target normal tissues as they will not have undergone selection in the thymus. In addition, this will lead to lower expression of the correctly paired TCR and thus lower the functional avidity of the T cell. There are now a number of methods to reduce mispairing. The introduction of a new disulphide bond into the constant region of the introduced alpha and beta chain ensures that these chains will more efficiently bond with each other and not with the endogenous TCR chains. This has been shown to reduce mispairing and improve the functional avidity of T cells [36–38]. Replacing the constant region of human TCRs with the murine constant region also leads to preferential pairing of the modified alpha and beta chains leading to increased expression of the TCR. As well as preventing mispairing, it has been shown that murine constant region pairs more efficiently with human CD3 resulting in more efficient competition for binding to CD3 [37]. The combination of murinization of constant regions and an additional disulfide bond has an additive effect on reducing mispairing and increasing surface expression of the introduced TCR although neither completely eliminates mispairing [36]. Expression of the introduced TCR may also be increased by suppression of the expression of the endogenous TCR. This has been achieved by transducing human PBMC with a vector which encodes a tumour-specific TCR and a small interfering (siRNA) which specifically downregulates the endogenous TCR [39].

TCR gene transfer experiments have demonstrated that TCR cell surface composition following transduction is dependent on properties of both the introduced TCR and the existing endogenous TCR. This has been described as TCR strength, with “strong” TCRs being more efficiently expressed on the cell surface compared to “weak” TCRs. T-cell strength may be controlled by the intrinsic pairing properties of the alpha and beta chains and also by their ability to bind to CD3 [40]. The introduced TCR must compete with the endogenous TCR prior to expression on the T-cell surface for binding to CD3. TCR and CD3 assemble into a TCR-CD3 complex within the endoplasmic reticulum and once fully assembled, exported to the cell surface. The amount of CD3 within the T cell thus is rate limiting for expression of the introduced TCR. TCR and CD3 can be cotransduced in order to provide an excess of CD3 and thus increase expression of the introduced TCR. Recent data has suggested that the increased surface expression of TCR following cotransduction of CD3 and TCR leads to an increase in tumour protection in vivo (Ahmadi, Blood, in press).

3. Improving Survival and Persistence of TCR-Transduced T Cells Following Adoptive Transfer

The ability of transduced T cells to persist long-term, mount robust memory responses and migrate to the site of response following adoptive transfer is key to successful antitumour responses. Persistence of transferred tumour reactive T cells has been shown to correlate with an effective therapeutic effect in adoptive immunotherapy utilizing TIL for treatment of metastatic melanoma [41]. From the earliest trials of adoptive cellular therapy, it has been demonstrated that “younger” TIL- that is, those subjected to fewer rounds of stimulation delivered better antitumour responses. In mouse models, tumour-specific CD8+ T cells that had undergone a number of in vitro stimulations and acquired effector functions were less effective at mediating tumour regression after adoptive transfer [42, 43]. Cells that had a prolonged period in culture acquired a T effector cell phenotype with decreased expression of cell surface markers associated with trafficking to secondary lymphoid organs, for example, CCR7 and CD62L and also downregulation of costimulatory receptors such as CD27 and CD28 [44]. Shorter telomeres were also associated with a reduction in clinical response and reduced in vivo persistence [45]. It is becoming increasingly apparent that the transfer of end stage effector CD8 T cells may not be desirable for adoptive immunotherapy but that transfer of naive or memory cell subsets may be more effective.

Naive, central memory (CM) and effector memory (EM) populations each have distinct phenotypic and functional characteristics. In vitro stimulation of these subsets induces their proliferation and differentiation into cytolytic effector cells. EM T cells preferentially home to peripheral tissues, have immediate effector function on antigen rechallenge but show only poor numeric expansion. They have downregulated CD62L although maintain expression of CCR7. CM T cells have high expression of CD62L and CCR7 and thus can home to and recirculate through secondary lymphoid organs where they can interact efficiently with antigen-presenting cells. They have enhanced proliferative capacity upon antigen reencounter [43, 46]. A comparison of transfer of tumour-specific CM T cells and EM T cells in a murine model of melanoma showed that CM T cells had more efficient in vivo recall responses compared with EM T cells which were associated with superior antitumour ability [43].

Berger et al. looked at the persistence of EM T cells versus CM T cells following adoptive transfer of CD8+ CMV-specific effector cells into unconditioned primates. The effector T cells that were derived from EM T cells only persisted in the blood for a short period and were unable to persist within lymph node or bone marrow or peripheral tissues. They were also unable to differentiate back into an EM T-cell phenotype. In contrast the effector T cells derived from CM T-cells were able to persist in blood and could migrate to bone marrow and lymph nodes and had the ability to differentiate to both CM and EM T cell phenotype. Effector cells derived from CM T cells appeared to be more

plastic than EM T cells, retaining the ability to revert to both a CD62L⁻ and CD62⁺ memory cell phenotype [47].

However, this study did not address the role of naive T cells for use in adoptive immunotherapy. Hinrichs et al. utilizing a murine adoptive transfer model have suggested that naive T cells may be superior to T CM cells. T cells were isolated from pmel-1 TCR-transgenic mice which express TCR specific for self-antigen, gp100 that is also expressed by murine melanoma cells. Sublethally irradiated mice with established B16 melanoma tumours received adoptive transfer of CD8⁺ naive T cells (CD62L⁺ CD44^{low}) or CM T cells (CD62L⁺CD44^{high}). All mice also received specific peptide vaccination and IL-2. Both naive and CM T cells were able to mediate tumour regression following adoptive transfer, but effector cells derived from naive T cells had superior antitumour activity. Adoptively transferred naive T cells also showed greater expansion within spleen and draining LN and also produced higher amounts of IFN- γ and IL-2 following ex vivo restimulation [48].

A novel way to try to prevent T cells acquiring a T effector phenotype prior to adoptive transfer is to target the Wnt/Beta Catenin pathway which is thought to be important in the generation and maintenance of T-cell memory [49]. The Wnt/beta catenin pathway can be activated artificially in vitro and in vivo utilizing inhibitors of glycogen synthase kinase 3 β (Gsk-3 β). Gsk-3 β inhibitors lead to an accumulation of beta catenin which mimics Wnt signaling [50]. Utilizing pmel-1 transgenic T cells, Gattonini et al. primed naive T cells in the presence of Gsk-3 β inhibitors. This inhibited cells from acquiring a T effector phenotype and also generated a CD44 low CD62L high T-cell population which had properties of stem cells, that is, robust self-renewal and the capacity to generate CM, EM, and effector T cells. They also had a characteristic phenotype, with high expression of Sca1, CD122, and Bcl2, previously described by Zhang et al. as CD8 memory stem cells [51]. Following adoptive transfer in a tumour model they had superior proliferative potential and superior antitumour function when compared to CM T cells or EM T cells [50]. Targeting of Wnt/beta catenin signaling pathways to generate CD8 memory stem cells in combination with TCR transduced T cells represents an attractive strategy for adoptive immunotherapy.

Lymphodepletion as a result of conditioning therapy pre-adoptive transfer has been shown to increase persistence of transferred T cells. Trials in melanoma patients conditioned with chemotherapy or chemotherapy plus total body irradiation demonstrated that inducing lymphopaenia improved the persistence of the transferred T-cell population [16, 41, 52]. Increasing the level of lymphodepletion in mouse models also demonstrated an increase in persistence of T cells and improvement in antitumour effect [53]. Lymphodepletion results in a spontaneous homeostatic proliferation of the remaining peripheral T cells which can lead to reconstitution of the peripheral lymphocyte pool [54, 55]. In addition T cells that are adoptively transferred into lymphopaenic hosts undergo the same homeostatic proliferation [56]. This process is dependent on homeostatic cytokines, IL-7 and IL-15, and also interaction between TCR and self-peptide/MHC and is similar to normal homeostatic mechanisms that

maintain the peripheral T-cell repertoire [22, 57–60]. The degree of expansion of T cells following adoptive transfer is likely to be controlled by access to IL-7 and IL-15 and also the availability of self-peptide/MHC ligands. T cells which have a higher affinity for self-peptide/MHC may have a selective advantage in the lymphopaenic environment and undergo more rapid and efficient expansion [61–63]. This may benefit TCR-transduced T cells specific for tumour-associated antigens which are self-antigens, leading to increased proliferation and persistence following adoptive transfer. It may also be possible to augment this proliferation by increased exposure to specific antigen which could be achieved by peptide vaccination at the time of adoptive transfer which may also give transferred cells an advantage over non-antigen-specific residual host T cells. [64]. It has been shown that CD4⁺ CD25⁺ FoxP3⁺ T regulatory cells also undergo homeostatic proliferation and thus may act to control cell expansion in the lymphopaenic environment [51, 65].

4. The Role of TCR-Transduced CD4⁺ T Cells in the Treatment of Haematological Malignancies

Most adoptive immunotherapy protocols have focused on the transfer of CD8⁺ tumour-specific T cells. However, in the absence of CD4 help, CD8⁺ T cells have impaired function, persistence, and cannot mount effective memory responses [66–68]. The generation of both TCR-transduced CD8⁺ and CD4⁺ T helper cells may have a beneficial effect on sustained tumour control (Figure 1).

Quezada et al. explored the role of classical class II-restricted CD4⁺ T cells in a murine model of advanced melanoma. Adoptive transfer of small numbers of naive class II-restricted tumour specific CD4⁺ T cells into irradiated recipients led to in vivo differentiation and expansion of CD4⁺ T cells and tumour regression. These CD4⁺ T cells acquired cytotoxic activity and directly rejected the tumour in a class II-dependent manner. The efficacy of CD4⁺ T cells was increased by concurrent blockade of CTLA-4 resulting in greater expansion of CD4⁺ T cells and decreased numbers of T regulatory cells [69].

The use of CD4⁺ T cells for adoptive immunotherapy is limited due to a lack of well-characterized tumour antigens presented by class II MHC. In addition the majority of tumour cells are class II negative and therefore cannot directly present antigen to CD4⁺ T cells. To overcome these limitations, CD4⁺ T cells expressing class I restricted TCR have been generated by gene transfer. It has been demonstrated that CD4⁺ T cells transduced with class I-restricted TCR can provide antigen-specific helper functions. Some class I-restricted TCRs are independent of the CD8 coreceptor and function in CD4⁺ T cells in the absence of additional CD8 [19]. However the majority of class I-restricted TCRs are CD8 dependent and thus require cotransduction of CD8 to be fully functional in CD4⁺ T cells. CD8 stabilizes the interaction between the TCR and peptide-MHC and increases the duration of this interaction. Kessels et al. transduced CD4⁺ T cells with the class I-restricted OT1

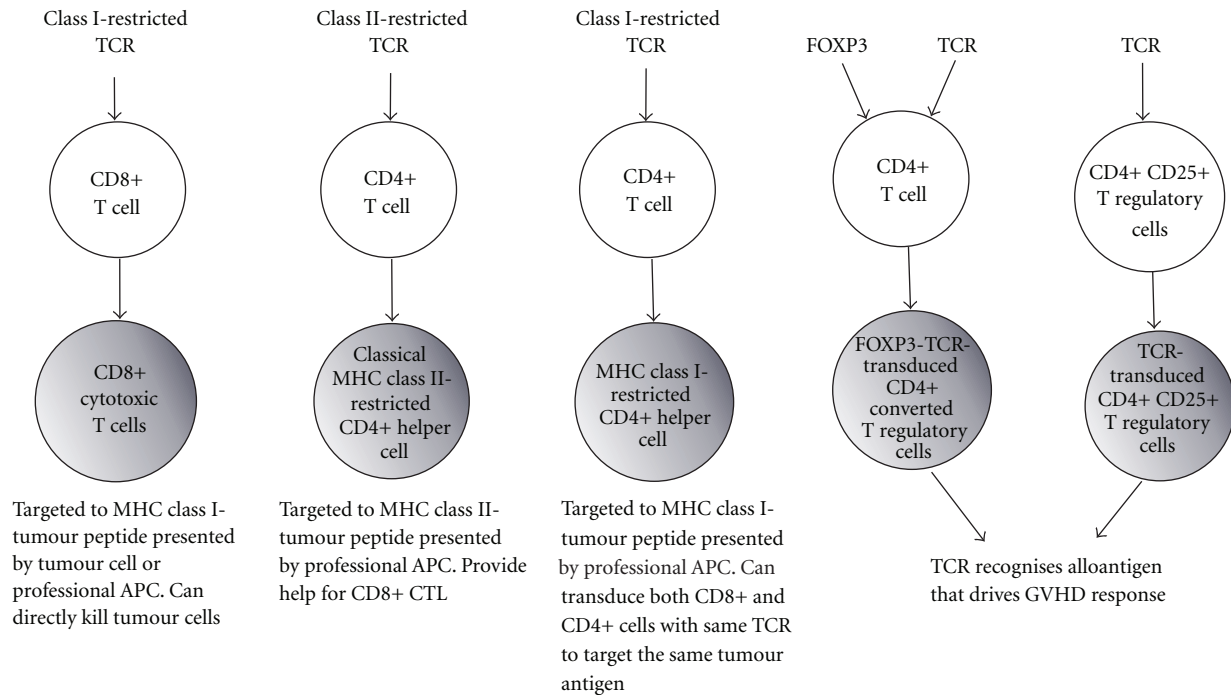


FIGURE 1: Populations of antigen specific T cells that can be generated by TCR gene transfer for use in adoptive immunotherapy. Retroviral gene transfer can be used to generate different populations of T cells for use in adoptive immunotherapy in the setting of haematological malignancy. Both CD8 and CD4 T cells can be transduced with class I-restricted TCR of the same specificity, targeting the same tumour antigen. Alternatively CD4 T cells can be transduced with class II-restricted TCR-specific tumour antigen presented by class II MHC. Antigen specific T regulatory cells can be generated by TCR transduction of CD4+ CD25+ T regulatory cells or CD4+ T cells can be cotransduced with TCR and FOXP3 resulting in production of antigen-specific converted CD4+ T regulatory cells. Antigen-specific T regulatory cells could be adoptively transferred in the context of HSCT or DLI to reduce harmful GvHD responses of T effector cells.

TCR [70]. OT1 TCR CD4+ T cells required cotransduction of the alpha and beta chains of CD8 in order to respond to specific antigen, and the transduction of just the alpha chain of CD8 was not sufficient to provide full CD8 coreceptor function. Morris et al. transduced CD4+ T cells with a class I-restricted TCR which is specific for NP peptide and is partially CD8 dependent [9]. CD4+ TCR-transduced T+ cells were able to produce IL-2 and proliferate in vitro in response to class II-negative tumour cells expressing specific peptide, but these cells were not able to generate an IFN- γ response. In vivo, CD4+ T cells could provide help for CTL-mediated tumour eradication, and these cells persisted in vivo for up to 90 days after tumour regression and were able to reexpand following tumour challenge. The generation of class I-restricted TCRs is a promising strategy with the advantage that tumour-specific CD4+ and CD8+ T cells targeting the same epitope can be generated.

CD4+ CD25+ FOXP 3+ T regulatory cells (CD4+ T regs) play a crucial role in the control of GvHD. This has been demonstrated via the depletion of CD4+ T regs from donor cells in murine MHC-mismatched transplant models. These transplants were done using a variety of different strain combinations, and the depletion of CD4+ T regs resulted in an increase in GVHD and an increase in mortality [71]. Adoptive transfer of additional CD4+ T regs with T effector cells is one possible strategy of reducing GvHD burden

after allogeneic transplant. The infusion of freshly isolated polyclonal CD4+ T regs in a 1:1 ratio with T effector cells has been shown to lead to a delay in development of GvHD in a MHC-mismatched murine transplant [72, 73]. Polyclonal CD4+ T regs can be expanded dramatically ex vivo using high-dose IL-2 and anti-CD3/CD28 beads or can also be expanded ex vivo utilizing irradiated allogeneic splenocytes prior to adoptive transfer. Trenado et al. cultured CD4+ T regs in the presence of allogeneic irradiated splenocytes which led to a 1000-fold expansion. These alloreactive CD4+ T regs were infused to lethally irradiated mice in a 1:1 ratio with T effector cells in a murine MHC-mismatched transplant model. This led to complete protection from GvHD. CD4+ T regs that were expanded using third-party splenocytes were of much lower efficacy than alloreactive CD4+ T regs. They also demonstrated that the reduction in GvHD did not lead to reduction in GvT responses mediated by CD8+ effector T cells following the injection of A20 leukaemia cell lines to recipient mice on the day of transplant [74]. Other groups have also demonstrated the adoptive transfer of murine CD4+ T regs in addition to HSCT can reduce GVHD whilst maintaining GVT effects [75, 76].

The use of antigen-specific CD4+ T regs is more effective at reducing GvHD than a polyclonal population [77]. CD4+ T regs require to meet specific antigen in order to be activated although once activated can mediate suppression of

T effector cells regardless of specificity. However, if CD4+ T regs are antigen specific, this has the advantage that they can be targeted to the same antigen as the T effector cell, targeting the suppressive response directly to the site of the harmful effector response. It has been shown that the recruitment of T regulatory cells to the site of GvHD is critical for the suppression of alloreactive T cells [78].

Gene transfer of antigen-specific CD4+ T cells with FOXP3 is one possible mechanism of generating large numbers of antigen-specific CD4+ T regs. The ectopic expression of FOXP3 had been shown to convert CD4+ T cells to regulatory T cells that are phenotypically and functionally the same as CD4+ T regs. Albert et al. produced CD4+ T regs by transducing CD4+ CD25- TCR-transgenic cells with FOXP3 and found that FOXP3-transduced T regs were as effective as wild-type CD4+ T regs at suppressing GvHD [77]. Antigen-specific CD4+ T regs may also be generated by the transduction of a specific TCR into CD4+ T regs or also by the cotransduction of both TCR and FOXP3 into conventional CD4+ T cells. In a mouse model of inflammatory arthritis, both TCR-transduced T regulatory cells and FOXP3 and TCR-co-transduced CD4+ T cells were able to mediate suppressive effects following adoptive transfer leading to reduction in progression of inflammatory arthritis [79]. There is some concern that CD4+ T regs may convert following adoptive transfer to TH17 cells thus abrogating their suppressive function [80]. However FOXP3 and TCR-co-transduced CD4+ T cells should be a more stable population due to the persistent and stable expression of FOXP3 from a strong retroviral promoter.

5. Tumour Antigen Selection

In order to develop effective adoptive immunotherapy protocols, it is important that the correct TAA or epitope is targeted. The use of high throughput screening of cancer genomes can identify large numbers of novel TAA, and it is important that research is focused, leading to more effective translation of TCR gene therapy and vaccine strategies into clinical practice. An ideal tumour antigen would be one that is widely expressed in different tumour types and not unique to individual patients. Targeting tumour antigens that are essential to the oncogenic process or cancer cell survival may induce sustained tumour regression. Specifically targeting a single epitope, however, may lead to the selection of tumour variants that lack the target antigen as a result of antigen loss or aberrant presentation via MHC loss [81–83]. Selecting a target that does not lead to autoimmune damage will also be of paramount importance. This has been recently demonstrated in a study where lymphocytes transduced with a high-avidity TCR specific for carcinoembryonic antigen (CEA) were administered to 3 patients with metastatic colorectal cancer. All patients in the study developed severe inflammatory colitis as a result of the TCR gene-modified T cells recognizing CEA expressed within normal colonic epithelium [84].

A National Cancer Institute Pilot project has been developed to try to prioritize antigens for further research and

development [85]. This utilized a methodology termed Analytic Hierarchy Process (AHP), which breaks down complex problems into a hierarchy of subproblems allowing comparison on a pairwise basis. 75 cancer antigens were ranked and scored based on criteria that had been defined and given differential weightings by a panel of experts. The criteria used for ranking (and in order of weighting) were demonstrable therapeutic function of antigen, known immunogenicity, oncogenic function, whether antigen is expressed widely or uniquely by tumour cells, expression level of antigen within tumours, expression by tumour stem cells, the number of patients with antigen positive cells, the number of epitopes of the tumour antigen and whether it is able to bind to multiple MHC molecule, and finally the cellular location of the antigen. The three top ranked antigens were WT1, MUC1, and LMP2. The antigens that have already been extensively researched score higher as there is more supportive evidence of therapeutic function and immunogenicity. For some of the subcriteria, there exists only limited information, but this process is dynamic, allowing reanalysis as further evidence accrues.

6. Safety Considerations of TCR-Transduced T Cells

In addition to reducing the efficacy of TCR transduced T cells, mispairing is also a concern with regards to safety of TCR-transferred T cells for clinical use. Bendle et al. have demonstrated that TCR-transduced T cells can induce GVHD following adoptive transfer. This is not a result of the on-target toxicity of the introduced TCR but is a result of the formation of new heterodimers that have off-target toxicity directed against normal tissues [86]. This stresses the importance of utilizing mechanisms to prevent mispairing of the introduced alpha and beta chains.

Lymphocytes can be transduced with suicide genes which enables selective elimination of transduced cells should GvHD develop following adoptive transfer. The most extensively studied to date is herpes simplex thymidine kinase (HSV-TK). HSV-TK phosphorylates ganciclovir to a toxic metabolite which interrupts DNA elongation resulting in the selective death of cells transduced with HSV-TK following the infusion of ganciclovir. When administered to patients with relapsed haematological malignancy after allogeneic HLA-matched HSCT, rates of GvT using HSV-TK-transduced lymphocytes were comparable to that of DLI using unmanipulated lymphocytes. The administration of ganciclovir for treatment of GVHD or for CMV reactivation selectively eliminated HSV-TK-transduced cells and improved GvHD [87]. HSV-TK-transduced lymphocytes have also been investigated as a coinfection at the time of T-depleted HLA identical HSCT and also as incremental add back infusion following haploidentical transplant [88, 89]. Although a promising strategy for control of GVHD after DLI, the efficacy may be limited due to the immunogenicity of HSV-TK leading to a decrease in persistence of transduced cells [90]. In addition the administration of ganciclovir for treatment of CMV reactivation results in the premature

destruction of HSV-TK-transduced cells leading to loss of efficacy.

Another suicide gene strategy in development is based on human apoptosis proteins. A fusion protein consisting of a late stage apoptosis molecule, caspase 9 fused to a FK506-binding protein (FK506BP) analogue has been transduced into lymphocytes. Apoptosis of the transduced cells is induced following the administration of a chemical inducer of dimerization (CID) which results in aggregation and activation of caspase 9. The CID which has been used is a non-toxic FK506 analog which has been modified so that it has much higher affinity for the modified FK506BP than the endogenous FK506BP. A single dose of CID resulted in death of >99% of transduced cells both *in vitro* and *in vivo*. These molecules are human derived and therefore should in theory be less immunogenic than HSV-TK [91].

An additional safety concern of gene therapy is insertional mutagenesis secondary to gene insertion into the host chromosome leading to disruption or activation of cellular genes. Out of the 19 X-linked severe combined immunodeficiency patients treated with retrovirally transduced haematopoietic stem cells there have been 5 cases of T-cell leukaemia reported [92–94]. In 4 of the 5 cases, the leukaemia arose secondary to retroviral integration in the region of a T-cell oncogene, LMO2, resulting in its deregulated expression. In these clinical trials, the vector expressed the IL-2 receptor gamma (IL-2R- γ) chain, and there is some controversy over whether this gene itself had a role as a cooperating oncogene. Insertions near both the LMO2 and IL-2R- γ chain gene have been described in murine T-cell leukaemia [95]. In addition, adoptive transfer of HSC transduced with lentiviral vectors expressing very high levels of the IL-2R- γ chain led to a high incidence of lymphoma in a murine model system [96]. Pike-Overzet et al., however, have shown that the overexpression of IL-2R- γ chain did not have any effect on haematopoietic cell development whilst overexpression of LMO2 led to a block in T-cell development leading to a preleukaemic phenotype [97]. Modlich et al. also found that vector-mediated expression of IL-2R- γ chain was not sufficient to lead to induction of leukaemia following HSCT in mouse models using both lentiviral and retroviral vectors [98].

All cases of leukaemia arising secondary to insertional mutagenesis have involved the use of retroviral vectors which have LTRs with strong enhancer/promoter elements. Strong LTRs may cause the transcription of cellular genes in addition to the transgene which can lead to unregulated gene expression. By using self inactivating vectors (SIN), it may be possible to reduce the risk of insertional mutagenesis. SIN vectors contain a deletion within the U3 region of the 3'LTR which leads to inactivation of the 5'LTR following reverse transcription, thus, inactivating both LTRs [99, 100]. Any expression of the transgene is then driven by an internal promoter. The use of SIN in preclinical models suggests that they have a lower oncogenic potential [101–103].

Both lentiviral and gamma retroviral vectors integrate in a semirandom fashion with a bias towards insertion into transcriptional units. Gamma retroviral vectors tend to integrate close to transcription start sites or DNA regulatory

areas such as CpG islands. Insertions of viral LTR at these points will have a higher probability of causing aberrant gene expression [104, 105]. In contrast, lentiviral vectors tend to integrate within active transcription units and as a result may have a safer insertion profile [106, 107]. Cattoglio et al. analyzed gammaretroviral and lentiviral vector insertion “hot spots” in human cord blood and bone marrow derived CD34+ HSC which had been transduced *in vitro*. Gamma retroviral vector integration (but not lentiviral) occurred at high frequency (i.e., >20%) in “hot spot” areas enriched in protooncogenes and genes involved in control of cell proliferation [108]. Using a tumour prone mouse model, Montini et al. have compared the oncogenic potential of lentiviral and gamma retroviral vectors, both of which had equivalent strength LTRs and found that lentiviral vectors required a 10-fold higher integration load than retroviral vectors to induce oncogenesis [101]. Only lentiviral vectors that had an active LTR led to insertional transformation of HSCs whereas SIN lentiviral vectors did not lead to transformation above background levels.

The risk of insertional mutagenesis may also be affected by the cell type being transduced, and there is a lower risk of transformation by transducing terminally differentiated cells as opposed to HSC. A study looking at long-term followup of patients receiving T cells transduced with thymidine kinase genes in the context of HSCT demonstrated that up to 20% of gene insertions resulted in altered gene expression of neighbouring genes, but there had been no incidence of clonal selection in these cells [109]. To date, there have been no cases of leukaemia or malignancy arising as a result of adoptive transfer of gene-modified T lymphocytes.

7. Conclusion

TCR gene transfer to produce antigen-specific T cells represents a targeted approach to treatment of haematological malignancies which may generate more specific GvT responses whilst reducing harmful GvHD responses. In addition to using TCR-transduced T cells to enhance GvT, we can also generate antigen-specific T regs to directly suppress harmful GvHD responses. Experience from clinical trials of melanoma antigen-specific TCR-transduced T cells has highlighted areas where this technique can be further refined and improved. Improvements in vector design, generation of high-avidity TCR, and reduction of TCR mispairing has all led to improvements in preclinical models. The importance of phenotype and subtype of the transferred population and the host environment into which they are transferred is becoming increasingly evident. Clinical trials utilizing WT1 TCR-transduced lymphocytes for treatment of haematological malignancies are due to commence in the near future hopefully leading to progress translating this technique into wider clinical use.

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