# Monoclonal T-Cell Receptors: New Reagents for Cancer Therapy

Hans J Stauss[1], Michela Cesco-Gaspere[1], Sharyn Thomas[1], Daniel P Hart[1], Shao-An Xue[1], Angelika Holler[1], Graham Wright[1], Mario Perro[1], Ann-Margaret Little[2], Constantina Pospori[1], Judy King[1] and Emma C Morris[1]

[1]*Department of Immunology and Molecular Pathology, University College London, Hampstead Campus, Royal Free Hospital, London, UK;* [2]*The Anthony Nolan Trust, The Royal Free Hospital, London, UK*

**Adoptive transfer of antigen-specific T lymphocytes is an effective form of immunotherapy for persistent virus infections and cancer. A major limitation of adoptive therapy is the inability to isolate antigen-specific T lymphocytes reproducibly. The demonstration that cloned T-cell receptor (TCR) genes can be used to produce T lymphocyte populations of desired specificity offers new opportunities for antigen-specific T-cell therapy. TCR gene-modified lymphocytes display antigen-specific function *in vitro*, and were shown to protect against virus infection and tumor growth in animal models. A recent trial in humans demonstrated that TCR gene-modified T cells persisted in all and reduced melanoma burden in 2/15 patients. In future trials, it may be possible to use TCR gene transfer to equip helper and cytotoxic T cells with new antigen-specificity, allowing both T-cell subsets to cooperate in achieving improved clinical responses. Sequence modifications of TCR genes are being explored to enhance TCR surface expression, while minimizing the risk of pairing between introduced and endogenous TCR chains. Current T-cell transduction protocols that trigger T-cell differentiation need to be modified to generate "undifferentiated" T cells, which, upon adoptive transfer, display improved *in vivo* expansion and survival. Both, expression of only the introduced TCR chains and the production of naïve T cells may be possible in the future by TCR gene transfer into stem cells.**

*Received 5 April 2007; accepted 26 April 2007; published online 17 July 2007; doi:10.1038/sj.mt.6300216*

## ADOPTIVE T-CELL THERAPY

Adoptive T-cell transfer can cure leukemia patients following allogeneic stem cell transplantation.[1] The infused donor-derived T lymphocytes recognize allo-antigens expressed by leukemia cells, resulting in T cell–mediated leukemia killing.[2] Unfortunately, T cell recognized allo-antigens are also expressed in normal tissues which can lead to graft-versus-host disease, a condition where allo-reactive donor T cells attack and damage normal tissues such as gut, skin and liver. Hence, the lack of specificity for defined tumor antigens limits the usefulness of adoptive T-cell therapy for the management of leukemia.

The adoptive transfer of tumor antigen-specific T cells has been used successfully in melanoma patients.[3] Although anti-melanoma effects were initially unimpressive, two recent developments substantially improved the clinical benefit of adoptive T-cell therapy in melanoma.[4] First, co-transfer of $CD4^+$ T-helper lymphocytes together with $CD8^+$ cytotoxic T lymphocytes (CTL) was found to be more effective than previous protocols using only $CD8^+$ CTL. Second, conditioning of patients is now used to create a lymphopenic environment that favors the engraftment and expansion of adoptively transferred T cells. While T-cell transfer into normal recipients typically resulted in poor T-cell survival and persistence, transfer into lymphopenic hosts leads to substantial expansion and long-term survival of injected T cells. The combination of conditioning and transfer of $CD4^+$ and $CD8^+$ T cells resulted in disease control in approximately 50% of melanoma patients, an impressive clinical response rate that probably cannot be achieved with alternative therapy options.[5]

Adoptive T-cell therapy is also effective in the management of latent infection by Epstein-Barr virus and cytomegalie virus in immuno-suppressed individuals.[6–13] In immuno-competent individuals, latent Epstein-Barr virus and cytomegalie virus infection is well tolerated as antigen-specific T cells efficiently control virus load. In contrast, the break down of T-cell surveillance, often seen after allogeneic stem cell or solid organ transplantation, is associated with a high risk of virus reactivation and the development of clinical symptoms such as Epstein-Barr virus-driven lymphoproliferative disorders and cytomegalie virus disease. In this case, the transfer of relatively small numbers of virus-specific donor T lymphocytes can reverse disease progression and establish long-term protection.

The inability to generate antigen-specific T cells is a serious limitation of adoptive T-cell therapy of cancer. Tumor antigens are often poorly immunogenic and patients are frequently immunocompromised as a consequence of tumor burden or as a side effect of radiation treatment and chemotherapy. In a similar way, adoptive T-cell therapy of Epstein-Barr virus and cytomegalie virus is typically based on the isolation of virus-specific T cells

***Correspondence:*** *Hans J. Stauss, Department of Immunology and Molecular Pathology, University College London, Hampstead Campus, Royal Free Hospital, Rowland Hill Street, London NW3 2PF, UK. E-mail: h.stauss@medsch.ucl.ac.uk*

from latently infected healthy donors, followed by transfer to patients. At present, there is no reliable strategy to isolate virus-specific T cells from uninfected naïve individuals, as the precursor frequency is low and the *in vitro* priming of T-cell responses is inefficient. T-cell receptor (TCR) gene transfer offers a strategy to produce antigen-specific T cells independent of precursor frequency and without the need for T-cell priming.

## GENE MODIFICATION TO PRODUCE ANTIGEN-SPECIFIC T CELLS

Retroviral transfer of chimeric single chain antibody constructs (scFv) has been used as a strategy to produce T cells with defined antigen-specificity.[14] For the most part, chimeric scFv constructs were linked to the intracellular signaling domains of FcR-gamma or CD3-zeta to trigger T-cell effector function.[15] More recently, the CD3-zeta domain has been combined with the signaling domains of co-stimulatory molecules such as CD28,[16–20] 4-1BB[21] or OX40.[22] In these constructs, antibody engagement can trigger effector T-cell function and also deliver co-stimulatory signals. An attractive feature of antibody-based receptors is that antigen recognition is HLA-independent, whereas TCR recognition is HLA-restricted. A disadvantage of antibody receptors is that they can only recognize cell surface antigens, whereas TCRs can recognize intracellular antigens that are cleaved into peptide fragments and presented in the context of major histocompatibility complex class I and class II molecules on the surface of antigen-presenting cells or tumor cells.

For a detailed discussion of strategies to improve chimeric scFv receptors, we refer to the review by Rossig and Brenner.[23] Here, we will discuss in more detail the challenges related to TCR gene therapy.

## TCR GENE TRANSFER

In the last years, several groups have demonstrated the feasibility of retroviral TCR gene transfer to produce antigen-specific T lymphocytes for adoptive immunotherapy.[24–37] Retroviral TCR gene transfer was first demonstrated using a melanoma antigen-specific TCR, although the efficiency was low in these initial studies.[24] In the last few years, the efficiency of TCR gene transfer has been improved substantially, providing an opportunity to produce relatively large numbers of antigen-specific T cells rapidly. Detailed analyses demonstrated that the fine specificity and the avidity of TCR-transduced CTL is similar to that of the parental CTL from which the TCR was isolated.[32] In addition, murine experiments have demonstrated that adoptive transfer of TCR-transduced T cells can protect against virus infection and mediate rejection of tumors expressing the TCR-recognized antigen.[27,36] Recently, the first clinical trial by Rosenberg's group demonstrated that TCR gene-modified T cells can have antitumor effects in melanoma patients.[38] In this trial, patient T cells were transduced with a retroviral construct encoding the alpha and beta chains of a MART1-specific TCR. Fifteen patients showed high level engraftment of the TCR-transduced autologous T cells, and two showed a clinical response. Although this milestone study demonstrated the feasibility and potential of TCR gene therapy, it also indicated that further modifications are required to improve the clinical benefit of this approach.

To date, bulk T-cell populations were used in most TCR gene transfer protocols. The polyclonal activation using anti-CD3 antibodies alone or in combination with anti-CD28 antibodies efficiently trigger the proliferation of $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells, rendering them susceptible to retroviral infection. It is currently not known to what extent these activation protocols also trigger the proliferation of $CD4^+25^+$ regulatory T cells. In the setting of cancer immunotherapy, TCR gene transfer into regulatory T cells is highly undesirable, as these T cells would be expected to suppress the anti-cancer activity of TCR gene-modified cytotoxic and helper T cells. The depletion of the $CD4^+25^+$ T-cell population before retroviral transduction is an efficient strategy to minimize the risk associated with TCR gene transfer into regulatory T cells.

There is good evidence that for an optimal and efficient CTL response help derived from $CD4^+$ T cells is required.[39] The relative lack of defined epitopes that are recognized by tumor-reactive helper T cells may limit the ability to provide help for effective antitumor immunity. An attractive strategy to overcome this limitation and to produce tumor-reactive helper T cells is by redirecting their specificity using major histocompatibility complex-class I restricted TCR with defined antitumor specificity. The feasibility of this approach was demonstrated when tumor-reactive helper T cells were generated by retroviral transduction with major histocompatibility complex-class I restricted, CD8-independent TCR.[40–43] In contrast, the generation of tumor-reactive helper T cells using TCR that require the function of the CD8 co-receptor requires retroviral co-transfer of TCR and CD8 genes.[44,45] Although TCR affinity for major histocompatibility complex-class I-peptide complexes can determine the level of CD8-independence, structural features of TCR that are currently poorly understood can also determine CD8-dependent/independent function.[46]

## OPTIMIZING TCR EXPRESSION

To date, the majority of TCR gene transfer protocols have used retroviral vectors. For efficient infection of human T cells, viral particles are usually packaged with amphotropic envelopes or with gibbon ape leukemia virus envelopes. For efficient infection of mouse T cells, viral particles with ecotropic envelopes are most commonly used. However, efficient infection does not necessarily lead to efficient expression of the introduced TCR on the surface of infected T cells.

TCR alpha and beta chains form heterodimers that require association with the CD3 gamma, delta, epsilon, and zeta chains before they can be expressed as functional receptor on the cell surface of T cells (**Figure 1**). Without CD3 molecules, TCR alpha/beta heterodimers cannot assemble properly and are degraded in the endoplasmic reticulum.[47] As CD3 molecules are required for the cell surface expression of the endogenous TCR, there is little or no free CD3 available for the introduced TCR. Hence, the endogenous and the introduced TCR compete for association with the CD3 molecules, which sets a limit to the amount of total TCR that can be expressed in transduced T cells.

Frequently, the introduced TCR chains are expressed at lower levels than the endogenous TCR (**Figure 2**), which is a concern as it may reduce the ability of transduced T cells to respond to low

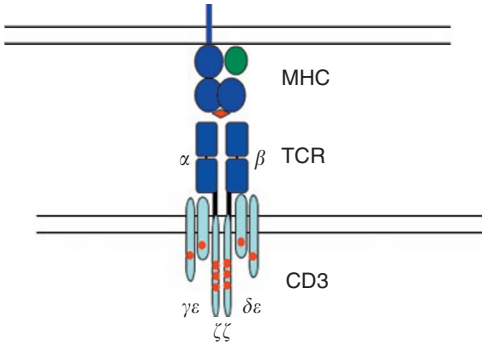


**Figure 1 Diagram showing T-cell receptor (TCR) surface expression and the interaction with a peptide-presenting major histocompatibility complex (MHC) class I molecule.** TCR alpha/beta heterodimers associate with the chains of the CD3 complex. This association is essential for expression of the TCR on the cell surface. The CD3 chains are also essential for signal transduction. The red dots represent immunoreceptor tyrosine-based activation motifs that can be phosphorylated when the TCR recognizes a peptide presented by an MHC molecule.

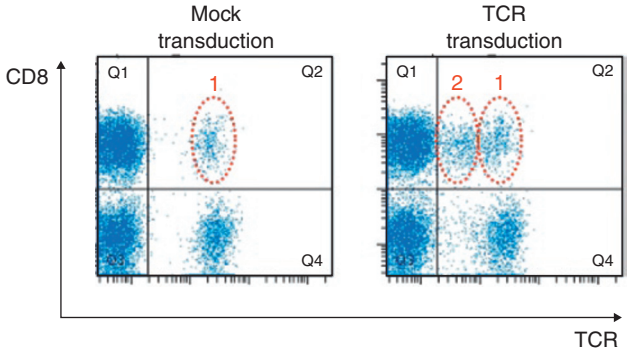


**Figure 2 Low T-cell receptor (TCR) expression in gene transduced T cells.** Human T lymphocytes were mock transduced or transduced with a retroviral vector encoding the human TCR Vα1.5 and Vβ2.1 chains. Lymphocytes were stained with antibodies specific for human CD8 and Vβ2.1. The expression of the endogenous Vβ2.1, indicated in circle 1, is substantially higher than the expression of the introduced TCR chain, indicated in circle 2.

concentrations of the TCR-recognized antigen. Recent studies have demonstrated that the expression levels of the introduced TCR can depend on the "strength" of the endogenous TCR.[48,49] TCR transfer into T cells expressing a "strong" TCR resulted in poor expression of the introduced TCR, whereas transfer into T cells expressing a "weak" TCR resulted in high level expression of the introduced TCR. At present, the molecular basis that determines whether TCR expression on the surface of T cells is efficient or inefficient (*i.e.*, whether a TCR is "strong" or "weak") is not clear, although sequences in the variable region of the TCR play an important role.[48]

Poor TCR expression may impair the therapeutic efficacy of transduced T cells in adoptive immunotherapy of tumors, as high avidity T cells are more likely to control tumor growth than low avidity T cells.[50] One parameter that is likely to affect the competitiveness of the introduced TCR is the level of expression that can be achieved from the retroviral vector. Hence, retroviral vectors designed for high levels of gene expression were developed for efficient surface expression of human TCR genes in human T lymphocytes.[51,52]

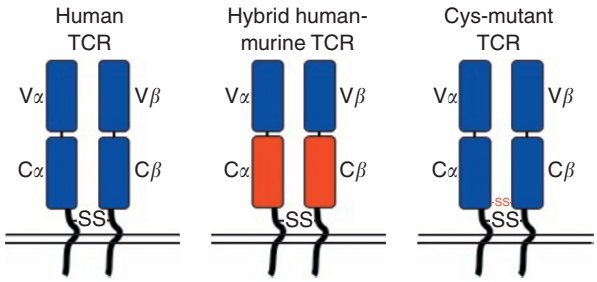


**Figure 3 T-cell receptor (TCR) constructs to reduce mis-pairing and increase surface expression.** Human TCRs were modified by replacing the constant regions with murine sequences, and by introducing and additional disulfide bond into the constant region.

Surprisingly, murine TCRs were found to be more efficiently expressed in human T cells than human TCRs, and hybrid TCR constructs demonstrated that the murine constant region was responsible for this effect.[28] Hybrid TCR constructs (**Figure 3**) were recently exploited to enhance the expression of human TCRs that were poorly expressed in human T cells. Replacing the human constant region with murine sequences resulted in efficient expression of the hybrid TCR, without altering TCR specificity.[53] It is likely that this strategy can be employed for all poorly expressed human TCRs, while murine constant region sequences may be less effective in enhancing the expression of well-expressed human TCRs. A drawback of this approach is that murine protein sequences are likely to trigger immune responses in patients treated with TCR-transduced T cells, which may lead to the rejection of the transferred T cells. However, the conditioning regimens used to prepare patients for adoptive T-cell therapy,[38] not only causes lymphodepletion but may also result in sufficient immunosuppression to allow the engraftment of T cells expressing murine sequences.

In a simplistic two-step model of TCR assembly and expression, it may be useful to consider the pairing between the TCR alpha and beta chains as a first step, the efficacy of which is affected by both the constant and the variable TCR domains (**Figure 4**). The association of the paired TCR chains with the CD3 components can be considered as the second step, which involves interactions between the TCR constant region (including the transmembrane and cytosolic portions) and the CD3 molecules. This would predict that murine TCR constant regions bind to human CD3 molecules more efficiently than human TCR constant regions, which was observed in a recent study of human/murine hybrid TCRs.[53]

Codon optimization is an alternative strategy to enhance the expression of TCR genes. A recent study demonstrated that improved expression of human TCR genes can be achieved by altering the sequence of rare codons to frequently used codons, and by removing messenger RNA instability motifs and cryptic splice sites. Human T cells transduced with the optimized genes displayed substantially higher TCR expression and improved functional activity compared to T cells transduced with the wild type sequence.[54]

Increasing the supply of CD3 molecules is likely to increase TCR expression in gene-modified T cells. Recently, it was shown that the genes for CD3-gamma, CD3-delta, CD3-epsilon and CD3-zeta, linked via 2A self-cleaving sequences, could all be

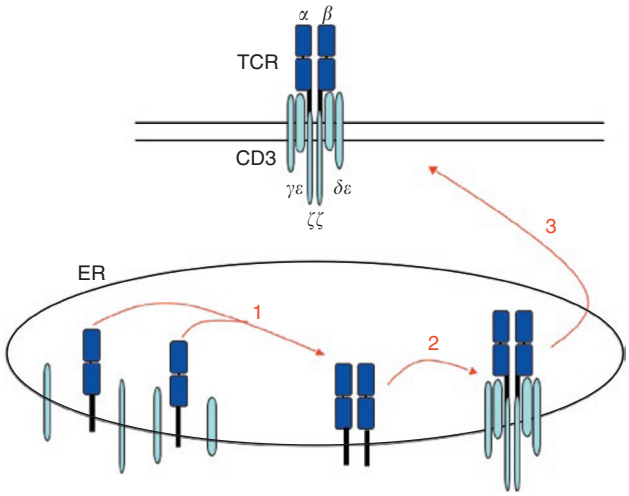


**Figure 4 Schematic diagram of T-cell receptor (TCR) assembly.** In this simplistic model, the efficiency of TCR expression is determined by the efficiency of TCR alpha/beta heterodimer formation (step 1), and by the efficiency of the TCR heterodimer to assemble with CD3 chains (step 2). Fully assembled TCR/CD3 complexes are released from the endoplasmatic reticulum (ER) and migrate to the cell surface (step 3).

accommodated in one retroviral vector. This vector drives the expression of CD3 chains that were able to associate with TCR chains to form a functional receptor on the surface of infected cells.[55] Although it is possible to express four genes encoding CD3 components from a single retroviral vector, it is very difficult to achieve equimolar expression levels of each chain or to mimic the relative expression levels of endogenous CD3 chains. Nevertheless, co-transfer of the genes for the CD3 chains and the TCR chains should improve TCR surface expression by alleviating the fierce competition between endogenous and introduced TCR for limited amounts of CD3.

Interestingly, there is evidence that reduced amounts of the CD3-zeta chain in patients with chronic infection and cancer can impair T-cell function.[56,57] Hence, it is conceivable that CD3-zeta is a rate-limiting factor in TCR assembly and that co-transfer of the gene for this chain is sufficient to improve TCR expression in gene-modified T cells.

A combination of the strategies described above is possibly required for optimal TCR expression in human T cells. For example, sequence optimization to enhance the levels of protein expression combined with the introduction of murine constant region sequences to enhance the efficiency of CD3 association may work synergistically and allow introduced TCRs to compete with endogenous TCR successfully for expression on the cell surface. In this scenario, the expression of additional CD3 molecules in gene-modified T cells would further benefit the introduced TCR and improve its surface expression.

## REDUCING TCR MIS-PAIRING

TCR gene transduced T cells express at least two TCR alpha and two TCR beta chains. While the endogenous TCR alpha/beta chains form a receptor that is self-tolerant, the introduced TCR alpha/beta chains form a receptor with defined specificity for a tumor target antigen. However, mis-pairing between endogenous and introduced chains form novel receptors, which might display unexpected specificities for self-antigens and cause autoimmune damage when transferred into patients. Hence, several strategies have been explored to reduce the risk of mis-pairing between endogenous and introduced TCR chains. The introduction of murine constant region sequences described above (**Figure 3**) not only enhanced the association with CD3 molecules, but it also decreased the level of mis-pairing with TCR chains expressed in Jurkat cells.[53] This indicates that human/murine hybrid TCR chains with murine constant region sequences preferentially pair with each other and have a reduced ability to pair with full-length human TCR chains. Interestingly, we found that human TCR chains that only differed in the variable region showed large differences in their ability to pair with human/murine hybrid chains. This shows that the TCR variable region has an important role in TCR alpha/beta pairing, and it also indicates that the murine constant region only reduces the frequency of mis-pairing, rather than eliminating this risk completely.

Mutations of the TCR alpha/beta interface is an alternative strategy to reduce unwanted mis-pairing. For example, the introduction of an additional cysteine in the constant domains of the alpha and beta chain (**Figure 3**) allowed the formation of an additional disulfide bond and enhanced the pairing of the mutant chains while reducing the efficiency of pairing with wild type chains.[58] Again, the reduction of pairing is not absolute, as a substantial number of wild type TCR chains can still pair with the cysteine mutant chains.

The generation of single chain TCR constructs is a strategy that can completely eliminate mis-pairing between endogenous and introduced TCR chains. Although single chain TCR molecules have been produced and are expressed in transduced T cells, they tend to be functionally impaired as suggested by the increased peptide concentrations required for stimulation of T cells expressing a single chain TCR construct compared with a normal TCR alpha/beta heterodimer.[59]

## TCR TRANSFER INTO STEM CELLS

The transfer of TCR alpha and beta genes into hematopoietic stem cells offers an attractive strategy that can lead to the production of mature T cells that express only the introduced TCR and no additional endogenous TCR chains.[60–62] Gene transfer into hematopoietic stem cells does not lead to TCR expression on the cell surface as stem cells do not express the CD3 molecules. However, when stem cells differentiate into lymphoid precursors that migrate to the thymus, the initiation of CD3 expression leads to the surface expression of the introduced TCR in thymocytes. The expression of functional TCR chains suppresses, by a mechanism called allelic exclusion, the rearrangement of endogenous TCR gene segments to form functional TCR alpha and beta genes. Although allelic exclusion is not complete, it is expected to impair the formation of endogenous TCR chains allowing a proportion of developing T cells to express only the TCR genes that were introduced into the stem cells. This would decrease the risk of TCR mis-pairing and also increase surface expression of the introduced TCR as it does not compete for CD3 molecules with the endogenous TCR (**Figure 5**).

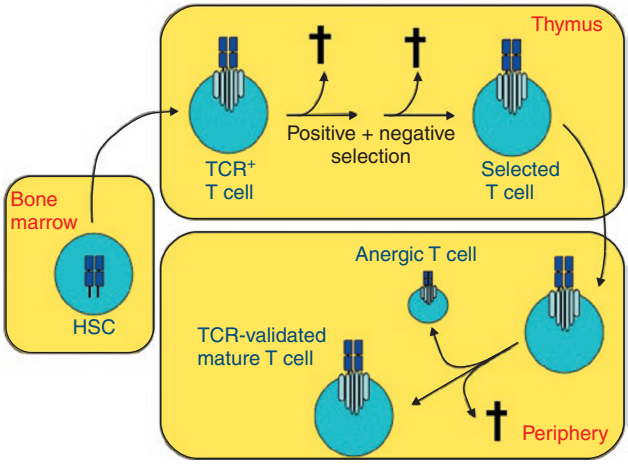


**Figure 5 The concept of T-cell receptor (TCR) validation by gene transfer into stem cells.** TCR gene transduced hematopoietic stem cells (HSC) in the bone marrow can express the TCR chains inside the cell, but not on the surface due to lack of CD3 molecules. CD3 expression in the thymus results in TCR surface expression, followed by positive and negative selection, which results in T-cell deletion if the TCR fails to interact with major histocompatibility complex (MHC) or recognizes self-antigens with high affinity. Selected T cells migrate to the periphery where encounter of self-antigens that are not expressed in the thymus may lead to deletion or the induction of anergy. Functional mature T cells are generated when the TCR has successfully completed all selection steps and is therefore able to interact with self-MHC without high avidity recognition of peptides derived from self-antigens.

The introduced TCR is responsible for further T-cell differentiation in the thymus, which involves positive selection provided the TCR can recognize self-antigens with low affinity. It also involves negative selection, which triggers deletion of developing T cells if the TCR recognizes self-antigens with high affinity. Therefore, mature T cells derived from TCR gene transduced stem cells express a validated TCR that is functional and free of auto-reactivity. A further benefit of this approach is that the gene-modified stem cells are a continuous source of mature T cells with defined antigen-specificity. A disadvantage of this approach is that TCRs with specificity for tumor-associated antigens may get deleted during T-cell development in the thymus, or may induce tolerance when expressed in peripheral T cells. This is because tumor-associated antigens represent normal self-proteins that are expressed in some normal tissues, which may include the thymus and/or peripheral tissues. Another disadvantage of this approach is the risk of retroviral insertional mutagenesis in stem cells. Insertional mutagenesis has been implicated in the development of T-cell leukemia in 4 out of 20 children treated for X-linked severe combined immunodeficiency disease by retroviral transfer of the gene encoding the interleukin-2 (IL-2) receptor common gamma chain into hematopoietic stem cells.[63–65] Proviral integration in the LMO2 locus, leading to up-regulation of LMO2 expression contributed to leukemogenesis in all affected patients. Whether the common gamma chain itself contributed to the development of T-cell leukemias is still under debate.[66–68]

To date, no side effects related to insertional mutagenesis in clinical trials with gene-modified mature T cells have been reported. A recent study showed that retroviral integration pattern in mature T cells is different from that in stem cells.[69] A follow-up of patients treated with retrovirus transduced T cells showed that the transferred cells displayed normal T-cell function *in vivo*, with no evidence of clonal selection 9 years after T-cell infusion.[69] These observations suggests that retroviral transduction of mature T cells is relatively safe, although larger trials are required to judge the risk more accurately.

Recent murine studies have clearly shown that TCR gene transfer into stem cells is an effective strategy to produce mature T cells that are functionally active, and that can protect against tumors expressing an "artificial" model antigen that is not expressed in any normal tissue.[61,62] TCR gene transfer into stem cells and differentiation into T cells can also be achieved *in vitro*.[70] Although the functional activity of such *in vitro* generated T cells has not yet been fully tested, this provides a strategy to avoid thymic and peripheral tolerance induction with TCRs that are specific for tumor-associated antigens.

## AVOIDING *IN VITRO* T-CELL ACTIVATION

Retroviral gene transfer involves T-cell activation and proliferation, usually achieved with anti-CD3 antibodies alone or in combination with anti-CD28 antibodies. This extensive *in vitro* stimulation triggers T-cell differentiation into effector cells, which is associated with a loss of expression of certain chemokine receptors and adhesion molecules that are involved in T-cell migration to lymphoid tissues. The lack of homing to lymphoid tissues is associated with a reduced ability of adoptively transferred T cells to control tumor growth in murine model experiments.[71] Hence, it is desirable to limit *in vitro* T-cell activation and to produce gene-modified T cells that can migrate to lymphoid tissues when injected into patients.

The antibody stimulation protocol used for retroviral transduction can trigger the down-regulation of the chemokine receptor CCR7 and the adhesion molecule CD62L, both of which are involved in T-cell migration to lymphoid tissues. Recently, it was demonstrated that the cytokines IL-15 and IL-21 can maintain the expression of CD62L in activated human T cells.[72] Therefore, it is possible that the addition of these cytokines to retroviral transduction protocols may generate transduced T cells with higher levels expression of lymphoid homing molecules, which would be expected to improve their therapeutic efficacy after adoptive transfer into patients.

An alternative strategy is to infect resting, non-proliferating T cells. This is achievable with lentiviral vectors, which can infect human T cells without the need for full activation and proliferation. Although quiescent human T cells were not infected with lentiviral vectors, T cells exposed to IL-2 and IL-7 progressed from $G_0$ to $G_1$, which rendered the cells susceptible to infection with lentiviral vectors containing the green fluorescent protein marker.[73] Infection did not require cell proliferation and did not alter the phenotype of the gene-modified T cells. Although lentiviral vectors containing TCR genes have not yet been successfully used to direct TCR expression in the absence of T-cell activation, this is an attractive strategy to produce TCR gene-modified T lymphocytes with minimal changes of the T-cell phenotype. Although fully activated effector T cells are less effective in providing tumor protection than less differentiated T cells,[71] it is not clear if minimally activated naïve T cells, transduced with a lentiviral TCR

**Table 1 The frequency of the pair HLA-A locus alleles found in individuals of the North European Caucasoid population**

| HLA-A alleles | | Frequency % | HLA-A alleles | | Frequency % |
|---|---|---|---|---|---|
| A*02 | A*01 | 10.8 | A*01 | A*03 | 5.58 |
| A*02 | A*03 | 8.46 | A*01 | A*01 | 5.04 |
| A*02 | A*02 | 7.26 | A*01 | A*24 | 3.54 |
| A*02 | A*11 | 4.68 | A*01 | A*11 | 2.76 |
| A*02 | A*14 | 4.32 | A*01 | A*29 | 1.56 |
| A*02 | A*29 | 3.12 | A*01 | A*68 | 1.38 |
| A*02 | A*31 | 1.86 | A*01 | A*32 | 1.26 |
| A*02 | A*32 | 1.68 | A*01 | A*31 | 0.90 |
| A*02 | A*68 | 1.62 | A*01 | A*26 | 0.84 |
| A*02 | A*30 | 1.62 | A*01 | A*30 | 0.84 |
| A*02 | A*25 | 1.38 | A*01 | A*25 | 0.54 |
| A*02 | A*26 | 1.26 | A*01 | A*23 | 0.48 |
| A*02 | A*23 | 0.96 | A*03 | A*24 | 2.40 |
| A*02 | A*33 | 0.54 | A*03 | A*11 | 1.98 |
| A*11 | A*24 | 0.96 | A*03 | A*03 | 1.80 |
| A*11 | A*32 | 0.66 | A*03 | A*29 | 1.38 |
| A*11 | A*26 | 0.48 | A*03 | A*68 | 1.02 |
| A*11 | A*68 | 0.48 | A*03 | A*30 | 0.78 |
| A*24 | A*68 | 0.54 | A*03 | A*23 | 0.72 |
| A*24 | A*24 | 0.48 | A*03 | A*31 | 0.66 |
| | | | A*03 | A*32 | 0.6 |
| | | | A*03 | A*25 | 0.48 |

construct, are effective when adoptively transferred *in vivo*. It is possible that lentiviral transduction of central memory T cells is a more promising approach, as this T-cell population has proven potential for long-term *in vivo* survival and is more easily activated by antigen than naïve T cells.

## FEW TCR CAN SERVE A LARGE PATIENT POPULATION

To date, most proof of principle studies were performed with TCR that recognize tumor-derived peptides in the context of HLA-A*02. This HLA allele was selected by most investigators because of its prevalence of 40–50% in the Caucasian population. We have explored how many TCR with defined HLA-restriction are required to cover 90% of the Caucasian population. **Table 1** shows the frequency of 5 HLA-A locus alleles, HLA-A*01, A*02, A*03, A*11 and A*24 in individuals of the north European Caucasoid population. These 5 alleles are found in 89.7% of individuals, indicating that a relatively small set of 5 TCR restricted by these alleles would provide a panel of therapeutic reagents for the majority of patients. If successful, TCR gene therapy will need to target several epitopes in one patient, which will reduce the risk of tumor escape due to loss a HLA and/or target antigen expression. It is most desirable to use TCR gene therapy in the adjuvant setting in patients with minimal tumor burden following conventional therapy. T-cell control of small numbers of tumor cells is expected to be more effective than control of large tumors, which are likely to contain variant tumor cells that can resist T-cell attack.

## CONCLUSION

The first TCR gene therapy trial in patients was recently completed, demonstrating the feasibility of this approach. In the near future, additional trials with TCRs of different specificities in different disease settings will provide valuable information about the potential benefits and the risks of this approach. TCR mis-pairing resulting in accidental recognition of self-antigens remains a risk, which can be reduced by modifications of the TCR sequences. An attractive option to reduce the risk associated with mis-pairing is the transfer of TCR genes into hematopoietic stem cells. The drawback of this approach is the increased risk of insertional mutagenesis of stem cells, which can give rise to leukemia. Co-transfer of TCR genes together with suicide genes is a possible strategy of risk management, allowing selective elimination of the gene modified cells when unwanted side effects occur. The development of optimized TCR constructs that can be safely delivered into minimally manipulated T lymphocytes holds great promise for antigen-specific adoptive T-cell therapy.

## ACKNOWLEDGMENTS

This work was supported by the Leukemia Research Fund and by the Medical Research Council and by the ATTACK project funded by the European Union.

## REFERENCES

1. Kolb, HJ, Mittermuller, J, Clemm, C, Holler, E, Ledderose, G, Brehm, G *et al.* (1990). Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood* **76**: 2462–2465.
2. Horowitz, MM, Gale, RP, Sondel, PM, Goldman, JM, Kersey, J, Kolb, HJ *et al.* (1990). Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* **75**: 555–562.
3. Rosenberg, SA, Packard, BS, Aebersold, PM, Solomon, D, Topalian, SL, Toy, ST *et al.* (1988). Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. *N Engl J Med* **319**: 1676–1680.
4. Dudley, ME, Wunderlich, JR, Robbins, PF, Yang, JC, Hwu, P, Schwartzentruber, DJ *et al.* (2002). Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science* **298**: 850–854.
5. Rosenberg, SA and Dudley, ME (2004). Cancer regression in patients with metastatic melanoma after the transfer of autologous antitumor lymphocytes. *Proc Natl Acad Sci USA* **101** (suppl 2): 14639–14645.
6. Rooney, CM, Smith, CA, Ng, CY, Loftin, S, Li, C, Krance, RA *et al.* (1995). Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. *Lancet* **345**: 9–13.
7. Walter, EA, Greenberg, PD, Gilbert, MJ, Finch, RJ, Watanabe, KS, Thomas, ED *et al.* (1995). Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N Engl J Med* **333**: 1038–1044.
8. Rooney, CM, Smith, CA, Ng, CY, Loftin, SK, Sixbey, JW, Gan, Y *et al.* (1998). Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. *Blood* **92**: 1549–1555.
9. Haque, T, Taylor, C, Wilkie, GM, Murad, P, Amlot, PL, Beath, S *et al.* (2001). Complete regression of posttransplant lymphoproliferative disease using partially HLA-matched Epstein Barr virus-specific cytotoxic T cells. *Transplantation* **72**: 1399–1402.
10. Einsele, H, Roosnek, E, Rufer, N, Sinzger, C, Riegler, S, Loffler, J *et al.* (2002). Infusion of cytomegalovirus (CMV)-specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy. *Blood* **99**: 3916–3922.
11. Peggs, KS, Verfuerth, S, Pizzey, A, Khan, N, Guiver, M, Moss, PA *et al.* (2003). Adoptive cellular therapy for early cytomegalovirus infection after allogeneic stem-cell transplantation with virus-specific T-cell lines. *Lancet* **362**: 1375–1377.
12. Bollard, CM, Aguilar, L, Straathof, KC, Gahn, B, Huls, MH, Rousseau, A *et al.* (2004). Cytotoxic T lymphocyte therapy for Epstein-Barr virus+ Hodgkin's disease. *J Exp Med* **200**: 1623–1633.
13. Cobbold, M, Khan, N, Pourgheysari, B, Tauro, S, McDonald, D, Osman, H *et al.* (2005). Adoptive transfer of cytomegalovirus-specific CTL to stem cell transplant patients after selection by HLA-peptide tetramers. *J Exp Med* **202**: 379–386.
14. Eshhar, Z (1997). Tumor-specific T-bodies: towards clinical application. *Cancer Immunol Immunother* **45**: 131–136.
15. Eshhar, Z, Waks, T, Gross, G and Schindler, DG (1993). Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proc Natl Acad Sci USA* **90**: 720–724.
16. Hombach, A, Wieczarkowiecz, A, Marquardt, T, Heuser, C, Usai, L, Pohl, C *et al.* (2001). Tumor-specific T cell activation by recombinant immunoreceptors: CD3 zeta signaling and CD28 costimulation are simultaneously required for efficient

IL-2 secretion and can be integrated into one combined CD28/CD3 zeta signaling receptor molecule. *J Immunol* **167**: 6123–6131.
17. Haynes, NM, Trapani, JA, Teng, MW, Jackson, JT, Cerruti, L, Jane, SM *et al.* (2002). Single-chain antigen recognition receptors that costimulate potent rejection of established experimental tumors. *Blood* **100**: 3155–3163.
18. Maher, J, Brentjens, RJ, Gunset, G, Riviere, I and Sadelain, M (2002). Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta/CD28 receptor. *Nat Biotechnol* **20**: 70–75.
19. Friedmann-Morvinski, D, Bendavid, A, Waks, T, Schindler, D and Eshhar, Z (2005). Redirected primary T cells harboring a chimeric receptor require costimulation for their antigen-specific activation. *Blood* **105**: 3087–3093.
20. Willemsen, RA, Ronteltap, C, Chames, P, Debets, R and Bolhuis, RL (2005). T cell retargeting with MHC class I-restricted antibodies: the CD28 costimulatory domain enhances antigen-specific cytotoxicity and cytokine production. *J Immunol* **174**: 7853–7858.
21. Imai, C, Mihara, K, Andreansky, M, Nicholson, IC, Pui, CH, Geiger, TL *et al.* (2004). Chimeric receptors with 4-1BB signaling capacity provoke potent cytotoxicity against acute lymphoblastic leukemia. *Leukemia* **18**: 676–684.
22. Pule, MA, Straathof, KC, Dotti, G, Heslop, HE, Rooney, CM, Brenner, MK *et al.* (2005). A chimeric T cell antigen receptor that augments cytokine release and supports clonal expansion of primary human T cells. *Mol Ther* **12**: 933–941.
23. Rossig, C and Brenner, MK (2004). Genetic modification of T lymphocytes for adoptive immunotherapy. *Mol Ther* **10**: 5–18.
24. Clay, TM, Custer, MC, Sachs, J, Hwu, P, Rosenberg, SA, Nishimura, MI *et al.* (1999). Efficient transfer of a tumor antigen-reactive TCR to human peripheral blood lymphocytes confers anti-tumor reactivity. *J Immunol* **163**: 507–513.
25. Cooper, LJ, Kalos, M, Lewinsohn, DA, Riddell, SR and Greenberg, PD (2000). Transfer of specificity for human immunodeficiency virus type 1 into primary human T lymphocytes by introduction of T-cell receptor genes. *J Virol* **74**: 8207–8212.
26. Fujio, K, Misaki, Y, Setoguchi, K, Morita, S, Kawahata, K, Kato, I *et al.* (2000). Functional reconstitution of class II MHC-restricted T cell immunity mediated by retroviral transfer of the alpha beta TCR complex. *J Immunol* **165**: 528–532.
27. Kessels, HW, Wolkers, MC, van den Boom, MD, van der Valk, MA and Schumacher, TN (2001). Immunotherapy through TCR gene transfer. *Nat Immunol* **2**: 957–961.
28. Stanislawski, T, Voss, RH, Lotz, C, Sadovnikova, E, Willemsen, RA, Kuball, J *et al.* (2001). Circumventing tolerance to a human MDM2-derived tumor antigen by TCR gene transfer. *Nat Immunol* **2**: 962–970.
29. Heemskerk, MH, Hoogeboom, M, de Paus, RA, Kester, MG, van der Hoorn, MA, Goulmy, E *et al.* (2003). Redirection of antileukemic reactivity of peripheral T lymphocytes using gene transfer of minor histocompatibility antigen HA-2-specific T-cell receptor complexes expressing a conserved alpha joining region. *Blood* **102**: 3530–3540.
30. Morgan, RA, Dudley, ME, Yu, YY, Zheng, Z, Robbins, PF, Theoret, MR *et al.* (2003). High efficiency TCR gene transfer into primary human lymphocytes affords avid recognition of melanoma tumor antigen glycoprotein 100 and does not alter the recognition of autologous melanoma antigens. *J Immunol* **171**: 3287–3295.
31. Clay, TM and Nishimura, MI (2003). Retroviral transfer of T-cell receptor genes into human peripheral blood lymphocytes. *Methods Mol Biol* **215**: 227–234.
32. Schaft, N, Willemsen, RA, de Vries, J, Lankiewicz, B, Essers, BW, Gratama, JW *et al.* (2003). Peptide fine specificity of anti-glycoprotein 100 CTL is preserved following transfer of engineered TCR alpha beta genes into primary human T lymphocytes. *J Immunol* **170**: 2186–2194.
33. Tahara, H, Fujio, K, Araki, Y, Setoguchi, K, Misaki, Y, Kitamura, T *et al.* (2003). Reconstitution of $CD8^+$ T cells by retroviral transfer of the TCR alpha beta-chain genes isolated from a clonally expanded P815-infiltrating lymphocyte. *J Immunol* **171**: 2154–2160.
34. Chamoto, K, Tsuji, T, Funamoto, H, Kosaka, A, Matsuzaki, J, Sato, T *et al.* (2004). Potentiation of tumor eradication by adoptive immunotherapy with T-cell receptor gene-transduced T-helper type 1 cells. *Cancer Res* **64**: 386–390.
35. Heemskerk, MH, Hoogeboom, M, Hagedoorn, R, Kester, MG, Willemze, R, Falkenburg, JH *et al.* (2004). Reprogramming of virus-specific T cells into leukemia-reactive T cells using T cell receptor gene transfer. *J Exp Med* **199**: 885–894.
36. Morris, EC, Tsallios, A, Bendle, GM, Xue, SA and Stauss, HJ (2005). A critical role of T cell antigen receptor-transduced MHC class I-restricted helper T cells in tumor protection. *Proc Natl Acad Sci USA* **102**: 7934–7939.
37. Xue, SA, Gao, L, Hart, D, Gillmore, R, Qasim, W, Thrasher, A *et al.* (2005). Elimination of human leukemia cells in NOD/SCID mice by WT1-TCR gene-transduced human T cells. *Blood* **106**: 3062–3067.
38. Morgan, RA, Dudley, ME, Wunderlich, JR, Hughes, MS, Yang, JC, Sherry, RM *et al.* (2006). Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science* **314**: 126–129.
39. Gerloni, M and Zanetti, M (2005). CD4 T cells in tumor immunity. *Springer Semin Immunopathol* **27**: 37–48.
40. Kuball, J, Schmitz, FW, Voss, RH, Ferreira, EA, Engel, R, Guillaume, P *et al.* (2005). Cooperation of human tumor-reactive $CD4^+$ and $CD8^+$ T cells after redirection of their specificity by a high-affinity p53A2.1-specific TCR. *Immunity* **22**: 117–129.
41. Roszkowski, JJ, Lyons, GE, Kast, WM, Yee, C, Van Besien, K, Nishimura, MI *et al.* (2005). Simultaneous generation of $CD8^+$ and $CD4^+$ melanoma-reactive T cells by retroviral-mediated transfer of a single T-cell receptor. *Cancer Res* **65**: 1570–1576.
42. Tsuji, T, Yasukawa, M, Matsuzaki, J, Ohkuri, T, Chamoto, K, Wakita, D *et al.* (2005). Generation of tumor-specific, HLA class I-restricted human Th1 and Tc1 cells by cell engineering with tumor peptide-specific T-cell receptor genes. *Blood* **106**: 470–476.
43. Zhao, Y, Zheng, Z, Robbins, PF, Khong, HT, Rosenberg, SA, Morgan, RA *et al.* (2005). Primary human lymphocytes transduced with NY-ESO-1 antigen-specific TCR genes recognize and kill diverse human tumor cell lines. *J Immunol* **174**: 4415–4423.
44. Kessels, HW, Schepers, K, van den Boom, MD, Topham, DJ and Schumacher, TN (2006). Generation of T cell help through a MHC class I-restricted TCR. *J Immunol* **177**: 976–982.
45. Willemsen, R, Ronteltap, C, Heuveling, M, Debets, R and Bolhuis, R (2005). Redirecting human $CD4^+$ T lymphocytes to the MHC class I-restricted melanoma antigen MAGE-A1 by TCR alphabeta gene transfer requires CD8alpha. *Gene Ther* **12**: 140–146.
46. McNicol, AM, Bendle, G, Holler, A, Matjeka, T, Dalton, E, Rettig, L *et al.* (2007). CD8 alph/alpha homodimers fail to function as co-receptor for a CD8-dependent TCR. *Eur J Immunol* (Epub ahead of print).
47. Call, ME and Wucherpfennig, KW (2005). The T cell receptor: critical role of the membrane environment in receptor assembly and function. *Annu Rev Immunol* **23**: 101–125.
48. Heemskerk, MH, Hagedoorn, RS, van der Hoorn, MA, van der Veken, LT, Hoogeboom, M, Kester, MG *et al.* (2007). Efficiency of T-cell receptor expression in dual-specific T cells is controlled by the intrinsic qualities of the TCR chains within the TCR-CD3 complex. *Blood* **109**: 235–243.
49. Sommermeyer, D, Neudorfer, J, Weinhold, M, Leisegang, M, Engels, B, Noessner, E *et al.* (2006). Designer T cells by T cell receptor replacement. *Eur J Immunol* **36**: 3052–3059.
50. Zeh, HJ 3rd, Perry-Lalley, D, Dudley, ME, Rosenberg, SA and Yang, JC (1999). High avidity CTLs for two self-antigens demonstrate superior *in vitro* and *in vivo* antitumor efficacy. *J Immunol* **162**: 989–994.
51. Weijtens, ME, Willemsen, RA, Hart, EH and Bolhuis, RL (1998). A retroviral vector system 'STITCH' in combination with an optimized single chain antibody chimeric receptor gene structure allows efficient gene transduction and expression in human T lymphocytes. *Gene Ther* **5**: 1195–1203.
52. Engels, B, Cam, H, Schuler, T, Indraccolo, S, Gladow, M, Baum, C *et al.* (2003). Retroviral vectors for high-level transgene expression in T lymphocytes. *Hum Gene Ther* **14**: 1155–1168.
53. Cohen, CJ, Zhao, Y, Zheng, Z, Rosenberg, SA and Morgan, RA (2006). Enhanced antitumor activity of murine-human hybrid t-cell receptor (TCR) in human lymphocytes is associated with improved pairing and TCR/CD3 stability. *Cancer Res* **66**: 8878–8886.
54. Scholten, KB, Kramer, D, Kueter, EW, Graf, M, Schoedl, T, Meijer, CJ *et al.* (2006). Codon modification of T cell receptors allows enhanced functional expression in transgenic human T cells. *Clin Immunol* **119**: 135–145.
55. Szymczak, AL, Workman, CJ, Wang, Y, Vignali, KM, Dilioglou, S, Vanin, EF *et al.* (2004). Correction of multi-gene deficiency *in vivo* using a single 'self-cleaving' 2A peptide-based retroviral vector. *Nat Biotechnol* **22**: 589–594.
56. Munder, M, Schneider, H, Luckner, C, Giese, T, Langhans, CD, Fuentes, JM *et al.* (2006). Suppression of T-cell functions by human granulocyte arginase. *Blood* **108**: 1627–1634.
57. Zea, AH, Rodriguez, PC, Atkins, MB, Hernandez, C, Signoretti, S, Zabaleta, J *et al.* (2005). Arginase-producing myeloid suppressor cells in renal cell carcinoma patients: a mechanism of tumor evasion. *Cancer Res* **65**: 3044–3048.
58. Kuball, J, Dossett, ML, Wolfl, M, Ho, WY, Voss, RH, Fowler, C *et al.* (2006). Facilitating matched pairing and expression of TCR-chains introduced into human T-cells. *Blood* **109**: 2331–2338.
59. Zhang, T, He, X, Tsang, TC and Harris, DT (2004). Transgenic TCR expression: comparison of single chain with full-length receptor constructs for T-cell function. *Cancer Gene Ther* **11**: 487–496.
60. Yang, L, Qin, XF, Baltimore, D and Van Parijs, L (2002). Generation of functional antigen-specific T cells in defined genetic backgrounds by retrovirus-mediated expression of TCR cDNAs in hematopoietic precursor cells. *Proc Natl Acad Sci USA* **99**: 6204–6209.
61. Yang, L and Baltimore, D (2005). Long-term *in vivo* provision of antigen-specific T cell immunity by programming hematopoietic stem cells. *Proc Natl Acad Sci USA* **102**: 4518–4523.
62. Holst, J, Vignali, KM, Burton, AR and Vignali, DA (2006). Rapid analysis of T-cell selection *in vivo* using T cell-receptor retrogenic mice. *Nat Methods* **3**: 191–197.
63. Hacein-Bey-Abina, S, von Kalle, C, Schmidt, M, Le Deist, F and Wulffraat, N, McIntyre, E *et al.* (2003). A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med* **348**: 255–256.
64. Marshall, E (2003). Gene therapy. Second child in French trial is found to have leukemia. *Science* **299**: 320.
65. Check, E (2005). Gene therapy put on hold as third child develops cancer. *Nature* **433**: 561.
66. Pike-Overzet, K, de Ridder, D, Weerkamp, F, Baert, MR, Verstegen, MM, Brugman, MH *et al.* (2006). Gene therapy: is IL2RG oncogenic in T-cell development? *Nature* **443**: E5; discussion E6-E7.
67. Thrasher, AJ, Gaspar, HB, Baum, C, Modlich, U, Schambach, A, Candotti, F *et al.* (2006). Gene therapy: X-SCID transgene leukaemogenicity. *Nature* **443**: E5–E6; discussion E6–E7.
68. Woods, NB, Bottero, V, Schmidt, M, von Kalle, C and Verma, IM (2006). Gene therapy: therapeutic gene causing lymphoma. *Nature* **440**: 1123.
69. Recchia, A, Bonini, C, Magnani, Z, Urbinati, F, Sartori, D, Muraro, S *et al.* (2006). Retroviral vector integration deregulates gene expression but has no consequence on the biology and function of transplanted T cells. *Proc Natl Acad Sci USA* **103**: 1457–1462.
70. Zhao, Y, Parkhurst, MR, Zheng, Z, Cohen, CJ, Riley, JP, Gattinoni, L *et al.* (2007). Extrathymic generation of tumor-specific T cells from genetically engineered human hematopoietic stem cells via Notch signaling. *Cancer Res* **67**: 2425–2429.
71. Gattinoni, L, Klebanoff, CA, Palmer, DC, Wrzesinski, C, Kerstann, K, Yu, Z *et al.* (2005). Acquisition of full effector function *in vitro* paradoxically impairs the *in vivo* antitumor efficacy of adoptively transferred $CD8^+$ T cells. *J Clin Invest* **115**: 1616–1626.
72. Alves, NL, Arosa, FA and van Lier, RA (2005). IL-21 sustains CD28 expression on IL-15-activated human naive $CD8^+$ T cells. *J Immunol* **175**: 755–762.
73. Cavalieri, S, Cazzaniga, S, Geuna, M, Magnani, Z, Bordignon, C, Naldini, L *et al.* (2003). Human T lymphocytes transduced by lentiviral vectors in the absence of TCR activation maintain an intact immune competence. *Blood* **102**: 497–505.