Adoptive transfer of tumor- and minor antigen-specific T cell reactivity in mouse models

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Chapter 8

General discussion

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Passive transfer of T cell immunity has been proposed as an attractive form of cancer immunotherapy in cases where the endogenous T-cell repertoire is immune tolerant. Ideally, such T cells should be sensitive and specific, two criteria which (currently) not easily come together. Transfer of allogeneic T cells in the context of allo-SCT/DLI can be seen as a sensitive but not very specific form of immunotherapy, since it has been proven very successful for various malignancies[1], but is frequently complicated by Graft-versus-Host Disease (GvHD)[2]. Transfer of allogeneic T cell receptors into autologous T cells (a process referred to as TCR gene transfer, discussed in more detail in chapters 1 and 3) is anticipated to be more specific[3], but is in its current form only modestly efficient[4]. With this in mind, it seems warranted to develop strategies to increase the sensitivity of TCR gene modified T cells[5] and/or strategies that increase the specificity of allo-SCT/DLI[6]. Here I will discuss the progress made in pre-clinical (animal) models and provide some suggestions for future (pre-) clinical research.

Efficacy of TCR gene transfer

Proof of principle. Most tumors only express self-antigens to which the immune repertoire is tolerant. The aim of TCR gene transfer is to generate tumor specific T cells, which can be used for passive immunization in such situations. The ability of TCR gene modified T cells to function in an immune tolerant recipient and to target tumors expressing self-antigens seems therefore to be one of the critical issues to address[7]. Hereto we used transgenic mice expressing model antigens under tissue specific promotors (being ovalbumin in the pancreas and SV40 large T in the prostate), in which the high affinity T cell compartment specific for these model antigens is deleted in the thymus (REF[8] and de Witte, unpublished observations).

Central tolerance is a key mechanism to prevent autoimmune disease[9] and is mediated by expression of tissue specific antigens in a specialized subset of epithelial cells in the thymic medulla (mTECs[10]). The list of 'promiscuously' expressed antigens includes common targets for cancer immunotherapy such as the melanocyte differentiation antigens Mart1, GP100 and tyrosinase[11]. We therefore consider it useful to test T cell based tumor immunotherapies in murine models for central tolerance. Here we have shown that TCR modified T cells function in an immune tolerant recipient and can be used to target tumors expressing self antigens (chapters 4 and 5). Having provided proof of principle that TCR gene transfer can be used to circumvent immune tolerance, the next step was to determine which factors determine the *in vivo* efficacy. Parameters that may influence the outcome of adoptive cell therapy (ACT) can be manipulated at three levels, being the conditioning of the host environment, the design of the transgene and the composition of the T cell graft.

Conditioning of the host. The sole transfer of T cells carrying a TCR directed towards a self-antigen is insufficient to induce detectable immune responses (Chapter 6). The low levels of antigen and/or the encounter of antigen in absence of pro-inflammatory signals can possibly explain this. In ACT studies in which TCR transgenic cells[12;13] or tumor infiltrating lymphocytes (TILs)[14;15] are used to target melanoma, it has been shown that conditioning by either active vaccination or lymphodepletion results in profound immune responses and tumor regression. Here we performed a head-to-head comparison to determine which conditioning regimen is most suitable for TCR gene transfer and found that irradiation induced (sub-lethal) lymphodepletion was superior to active vaccination with recombinant viruses (chapter 6).

Based on data obtained in (pre-)clinical ACT or vaccination studies, modifications lymphodepletion-based TCR gene transfer protocols may result in further improvement. For instance, murine data suggest that myeloablative conditioning plus stem cell infusion results in an enhanced expansion and function of adoptively transferred TCR transgenic T cells as compared to non-myeloablative conditioning[13]. As an alternative to myeloablation, selective depletion of those cellular subsets that compete for homeostatic cytokines may be a more targeted approach to facilitate engraftment of antigen specific T cells (e.g. T cells and NK cells are described as cytokine sinks for homeostatic cytokines IL7 and IL15[16]). Finally, administration of immunostimulatory adjuvants (Table 1) may also considered to be used in combination with ACT[17].

Table 1: Adjuvant treatments proposed for combination immunotherapy

Adjuvant	Effect on immune system
Antagonistic anti-CTLA4 antibody[18]	CTLA4-B7 interaction suppresses T cell activation[19].
Agonistic anti-CD28 antibody[20-22]	CD28-B7 interaction is required for activation of naïve T cells.
Agonist antibodies targeting TNF-receptor superfamily members[23]	TNF-R superfamily members (e.g. CD27, OX40, and 4-1BB) promote survival of primed T cells[24].
Synthetic CpG -oligodeoxynucleotides (ODN)[17]	Stretch of unmethylated CpG dinucleotides activates APCs via TLR9 (identified in bacillus Calmette-Guerin DNA)[25].
IL-2 administration[17]	IL-2 was originally identified as T cell growth factor, but provides a range of pro-inflammatory signals[26].
Blocking TGFβ signaling via transfer of genes encoding a dominant negative TGFβ receptor[27;28] or via administration of small molecule inhibitors[29;30]	Pleiotropic cytokine with multiple immunosuppressive properties[31]. Tumors can secrete TGFβ[32].
Antagonistic PD-L1 antibody[33]	PD-L1 – PD-1 interaction suppresses T cell activation. Tumors can express PD-L1[34].
Administration 1 methyl trypthophan (1MT)[35]	Indoleamine 2,3-dioxygenase (IDO) catalyzes trypthophan degradation, resulting in an arrest in T cell proliferation[36]. Tumors can secrete IDO[37].
e.g. Antagonistic anti-CTLA4 antibody or depletion of $CD25^+$ cells[38].	T_{reg} cells is a T cell population that can functionally suppress an immune response by influencing the activity of another cell type[38].

In a recent set of experiments we tested some of these adjuvants in combination with irradiation induced sub-lethal lymphodepletion. Preliminary data suggest that administration of high dose IL-2 or blockade of TGFβ signaling through co-transfer of a dominant negative receptor[27] resulted in significantly higher T cell responses (de Witte et al, unpublished observations). However, in these experiments treatment was accompanied by severe adverse effects, indicating that under certain circumstances the specificity of TCR transduced T cells is not ensured (see below).

Modification of the TCR transgene (affinity and avidity). The majority of TCR genes which are currently available for clinical trials, are specific for melanocyte differentiation antigens, which have been isolated from tumor infiltrating lymphocytes found in melanoma patients[4;39-41]. Because these T cells have undergone negative selection, TCR affinity is relatively low. In addition, as discussed further in more detail, genetically introduced TCR genes are generally expressed at lower levels as compared to endogenous TCRs[4;42], which has a disadvantageous effect on T-cell avidity. Both the affinity (strength of binding of one TCR to one pMHC complex at one single site) and the avidity (the sum of all molecules participating in an interaction between two cells) will determine the sensitivity of a (redirected) T cell. We therefore consider it likely that the development of techniques to increase these two parameters will make an important contribution to the effectiveness of TCR modified T cells.

Both competition of introduced TCR molecules with endogenous TCR molecules for assembly with CD3 components, and formation of mixed dimers of endogenous and exogenous TCR chains play a role in the low surface expression of the transferred TCR[43]. Increasing the number of functional TCR heterodimers (and hence the avidity) has been shown *in vitro* to be possible by improving the translation through genetic optimization[42;44] and by modification of the TCR constant domain to facilitate pairing of transferred TCR chains[45-47]. But the true potential of these strategies can perhaps best be appreciated in *in vivo* studies, as shown in chapter 6. There we used TCR genes of which the unmodified variant was already relatively highly expressed. Gene optimization resulted in a rather modest increase in TCR surface expression. However, the gain in *in vivo* activity was unexpectedly striking, which underlines that not always all parameters contributing to *in vivo* effectiveness can be extrapolated from a culture dish.

Whereas these strategies to improve the avidity of TCR modified T cells are ready to be implemented in clinical studies, generation of high affinity TCRs is still in the developmental phase. One approach which holds great promise is adapted from the monoclonal antibody generation, in which mice are used as 'allogeneic donors'[48] (a more extended list of strategies is discussed in chapter 3). Due to genetic differences between mice and men (either by evolution or genetic engineering), a TAA derived from a human self-protein, can be foreign for the murine T cell repertoire. Upon immunization with such a TAA, murine T cells can recognize this antigen and mount an immune response with high affinity T cells. These TCRs can be isolated and used for human TCR gene transfer.

The proof of principle for this strategy has been given by Stanislawski et al, who showed that high affinity TCRs to the human oncoprotein MDM2 could be generated in mice[49]. The TCR isolated in this study was of murine origin and therefore potentially immunogenic in men, which makes clinical testing in this stage less attractive. As has been shown for high affinity monoclonal antibodies[48], immunogenicity may significantly be reduced by either *in vitro* replacement of murine parts of the TCR with its human analogues or by creating mice transgenic for (parts of) the unrearranged TCR locus. Based on the clear clinical value of murine derived human monoclonal antibodies[50], we expect that - once possible - transfer of 'artificial' high affinity TCRs will be preferred over 'natural' patient derived low affinity TCRs. Therefore we have chosen to perform pre-clinical murine studies with high affinity TCRs (chapters 4-6), which may not perfectly reflect the current clinical practice but hopefully will translate to the not too distant future.

Composition of the T cell graft. The fact that TCR genes can be transferred to a large pool of autologous T cells is considered as an advantage with regards to the speed in which high number of TAA-specific T cells can be obtained. When TCR modified T cells are used for adoptive transfer without further intervention, it generally implies that unmodified cells or T cells with low expression of the transgene will be co-transferred as well. In chapter 6 we show in lymphopenic recipients that adoptively transferred irrelevant cells hamper the function of TCR transduced T cells. This may be explained by the competition for factors that promote homeostatic proliferation[51], or by the development of regulatory T cells (T_{regs}), which have been shown to suppress immune responses[52;53]. In this light, the development of approaches such as MHC multimer technology[54], that would result in a more selective infusion of TCR-modified T cells may be desirable. The possibility of using monoclonal antibodies[55] or MHC tetramers[56;57] for ex vivo graft manipulation has been tested in mice and show a satisfactory preservation of T quality. However, considering the importance of transferring high numbers of retrovirally modified T cells[58], it is conceivable that T cell quantity post purification will be the most critical factor in determining the benefit of enrichment prior to transfer.

In addition to the frequency of TCR modified T cells within the graft, it seems plausible that also the type of T cell used for TCR transfer may influence the outcome of ACT. The acquirement of full effector function *in vitro* seems to impair the *in vivo* antitumor efficacy of adoptively transferred T cells[59], whereas T cells with a high capacity for immune reconstitution have been shown to contribute to long-term allo-responses[60]. The development of systems in which TCR modified T cells can be obtained *in vitro* from hematopoietic progenitor cells[61;62] may allow the generation TCR modified T cells which have not became effector cells *in vitro*. This activation status could potentially favour long term reconstitution with TAA-specific T cells.

Side-effects of adoptive T cell therapy.

GvHD, a term used in this thesis for autoimmune pathology caused by infused T cells in a TCR dependent fashion, is one of the most likely complications of ACT. ACT induced GvHD can be divided in 'on-target autoimmunity' and 'off-target autoimmunity', in which in the first case TAAs present on healthy tissue are targeted, whereas in the latter case T cell reactivity can be directed towards any self-antigen. The risk of GvHD post TCR gene transfer has until recently only be discussed in theory [REF[3] and chapter 3]. However, recent experiments performed by Bendle et al. suggest that with the ability to generate increasingly high TCR-gene modified T cell responses TCR gene transfer can become complicated by autoimmune pathology.

On-target autoimmunity. Because most TAAs are non-mutated self-antigens, TAA-specific T cells may also recognize the same antigen expressed on healthy tissue. It will depend on the type of tissue and the magnitude of the T cell attack whether on-target autoimmunity will be regarded as a serious adverse effect. TCRs directed towards antigens specifically expressed on non-vital tissues are in general considered to be 'safe candidates', of which TCRs recognizing melanocyte differentiation antigens are the most frequently studied examples. Concomitant autoimmune melanocyte destruction is commonly observed[12;14], which seems to be acceptable when it primarily results in vitiligo but may be considered a more serious complication when melanocyte differentiation antigens present in eye or brain are targeted as well[14;63]. Also ubiquitously expressed antigens such as p53[64], MDM2[49], and telomerase[65] are proposed to be attractive potential targets. These proteins play an essential role in tumor growth, and targeting such antigens may prohibit the selection of tumor-escape variants. The notion that these antigens are often overexpressed on tumor cells is hoped to provide a window of treatment, in which the cytolytic activity of adoptively transferred antigen-specific T cells can result in tumor regression, while leaving healthy tissues predominantly unharmed. However the likelihood of defining a sufficiently large window and the consequences of T cell attack of untransformed tissues remain important issues to be evaluated.

Off-target autoimmunity. As discussed in more detail in the introduction, recognition of self-peptides by endogenous T cells is for an important part prohibited by purging autoreactive thymocytes from the developing T cell repertoire in the thymus. Both exogenous T cells present in DLIs as endogenous T cells transferred with exogenous TCR genes, express TCRs which have not undergone negative selection in the thymus of the host, and therefore might contain TCR specificities that can recognize healthy tissue in the recipient. This type of GvHD is caused by TCRs that are not specific for TAAs, hence the term 'off-target' autoimmunity.

Off-target autoimmunity caused by DLI is directed towards mismatched Minor Histocompatibility Antigens (MiHAgs), which in theory could be derived from millions of polymorphisms[66]. However, in

murine models it has been shown that T cells responding to only a small collection of mismatched MiHAgs dominate the allo-response[67], which (at least partially) determines the target site of alloreactivity[68]. The same has been observed in clinical studies, in which the DLI responded with a relatively high frequency to the hematopoiesis-restricted MiHAgs HA-1 and HA-2[69]. These observations provide an incentive to manipulate the GvH-reaction on a T cell level, for instance by removing those immunodominant T cell specificities which are suspected to primarily contribute to GvHD pathology. We have assessed the feasibility of this approach in a well characterized murine transplantation model (B6→BALB.B), in which T cell responses against one of the characterized mismatched MiHAgs (H60) comprises around one-third of the total allo-response[70]. In chapter 2 we showed that a tetramer based technology can be used to effectively deplete T cells with a certain specificity from a polyclonal graft, and thereby alter the allo-response after DLI. But regardless the efficient removal of H60-specific T cells, GvHD development remained essentially unaltered. Two explanations may be given for this observation, either H60 is not essential for GvHD development or second, in absence of H60 specific T cells other T cell clones will contribute to the allo-response. As discussed in chapter 2, genetic studies with recombinant inbred strains demonstrate that H60 is neither required not sufficient for GvHD[71], suggesting that in the study presented in this thesis the first explanation can be applied. However, when not GvHD but recognition of splenocytes is chosen as a read-out for allo-reactivity, H60 specific T cells do dominate the allo-response up to almost 100%. In mice that received H60-depleteted T cells this allo-response is (at least partially) compensated by increased frequencies of alloreactive T cells with a yet unknown specificity, demonstrating that when an immunodominant T cell clone is depleted, other T cell specificities can take over (parts of) an immune response. Collectively, these data indicate that antigen-specific graft engineering is feasible, but that immunodominance does not necessarily translate into an essential role in GvHD.

As an alternative to the depletion of deleterious T cell specificities, enrichment of T cell specificities directed toward MiHAgs predominantly present on tumors and/or hematopoietic cells may also be considered. MiHAgs (such as HA-1 and HA-2) expressed on hematopoietic cells have been proposed as attractive candidates for patients with relapsed leukemia after allo-SCT, in which MiHAg differences between leukemic cells and the 'healthy' (donor derived) hematopoietic compartment may exist[72]. MHC tetramers may be used to purify MiHAg-specific T cells[56;57] or, in cases where T cell isolation is not feasible, TCR gene transfer may be considered as an alternative[73;74]. Unfortunately, the current list of identified human MiHAgs is still rather modest. As discussed in more detail in chapter 2, it seems that with an extension of this list possibilities to generate more specific allogeneic T cell grafts will increase[6].

Also TCR modified T cells can induce off-target auto-immunity, but the mechanisms are different when compared to GvHD caused by DLI. In theory a TCR modified T cell can become an autoreactive T cell in (at least) three different ways. Both animal and clinical studies are needed to show to what extend any of these scenarios will contribute to clinical GvHD. Here I will discuss all three

possibilities and propose some potential countermeasures. First, when 'TCR-donor' and 'TCR-recipient' are partially MHC-mismatched (an almost unavoidable situation for clinical application), redirected T cells will encounter self-peptide MHC complexes in the recipient that have not been present in the donor. The donor-derived introduced TCR has not undergone negative selection towards these mismatched self-peptide MHC complexes, which may therefore be seen and treated as 'foreign'. In chapter 4 we have used a murine model to assess the safety of TCR gene transfer in partially MHC-mismatched recipients and that data set provided no evidence of GvHD. We therefore concluded that TCR gene transfer can also be feasible in partially MHC-mismatched recipients. However, since the propensity of alloreactivity will be different for each individual TCR-pMHC combination, it is impossible to conclude from such studies that TCR gene transfer will be safe for any TCR in any recipient. Screening TCR modified T cells for reactivity with either a collection of cell lines each expressing a unique HLA allele[42] or with patient derived cells like hematopoietic cells may be used to perform a risk evaluation of for unwanted allo-reactivity prior to transfer. A second mechanism of how TCR gene transfer can result in auto-reactive T cells, is when introduced TCR chains form heterodimers with their endogenous counterparts. These so-called mixed dimers have unknown specificities, and can as a result potentially be autoreactive. By remodeling the TCR interface to facilitate preferred pairing of the introduced TCR molecules[45-47] or by precluding expression of the endogenous TCR, the formation of mixed dimers may be prohibited. Knock-down of endogenous TCR molecules may be achieved when TCR genes are introduced in T cell progenitor cells which have not yet recombined their endogenous TCR molecules[41;75]. Allelic exclusion is expected to prevent expression of endogenous TCR chains, although additional measurements such as inhibition of RAG molecules may be required to make this process sufficiently efficient. A final potential GvHD-mechanism in TCR gene transfer is when a previously 'ignorant' T cell becomes activated via the introduced TCR, which as a result can target self tissue of which it was previously unaware. Transferring TCR genes into pre-selected T cells with a known non-self specificity (e.g. virus specific T cells) have been suggested as an approach to prevent the 'bystander' activation of ignorant T cells[76].

To address the likelihood and consequences of TCR gene transfer mediated autoimmunity, mice have routinely been subject to histopathologic analysis. No signs of GvHD could be detected when viral vaccination was chosen as conditioning regimen (REF[77], chapter 4 and 5). The scale of the analysis (over a 100 animals, including a large cohort of partially MHC mismatched recipients and 3 different TCRs) made us interpret these data as encouraging, but with the remark that these data should not be taken as evidence that TCR gene transfer will never be complicated by GvHD (chapter 4). The correctness for this caution comes from a recent set of experiments, where sub-lethal irradiation is combined with immunestimulatory adjuvants such as high-dose IL-2 and blockade of TGFβ signaling. This combinatorial conditioning not only resulted in higher responses of TCR gene transduced cells, but also in a syndrome consistent with bone marrow failure (Bendle et al, unpublished observations). Although the exact etiology of the toxicity is still unknown (both with regards to role of the adjuvants

as with regards to the mechanism resulting in alloreactive TCR modified cells), these data underline the importance for pre-clinical safety evaluation of TCR gene transfer protocols.

Incorporating a safety switch. Apart from the question what type and level of autoimmune pathology can be expected, it is also interesting to discuss what degree of toxicity will be accepted. The latter issue is very difficult to address in murine studies. Factors such as the severity of the disease, the condition of the patient and the potential countermeasures available in case of side effects will each time be taken into consideration before (any) treatment or clinical study will be started. As is the case for allo-HSCT/DLI it is likely that both physicians and cancer patients are willing to accept some GvHD pathology of TCR modified T cells. However, if murine or clinical studies will learn that TCR gene transfer can be accompanied GvHD resulting in severe morbidity or even mortality which cannot be predicted beforehand, incorporation of a conditional safety switch could be of value in controlling toxicity[78]. Clinical useful safety switches should be effective, non-toxic and non-immunogenic. Here we have used the same murine model for auto-immune diabetes as in previous chapters 4 and 6, but for this set of experiments abrogation rather than induction of diabetes was used as a read-out. In this model we tested a recently developed caspase-9 based safety switch (iCasp9$_M$) [79], and found that this switch could successfully be used to halt an ongoing, otherwise fatal severe auto-immune attack of the pancreas (chapter 8). For these experiments we chose vaccination in stead of lymphodepletion as a conditioning regimen, because vaccination requires a relatively small ACT graft to induce severe autoimmune diabetes. Also after irradiation iCasp9$_M^+$ T cells could be eliminated, suggesting that this safety switch can be used as well to induce apoptosis of potentially autoreactive T cells when cell division is (presumably) driven by homeostatic proliferation. Whether the level of elimination is sufficient to prevent or revert lethal diabetes could not be addressed, since T cell numbers were to low to induced diabetes both in the experimental as in the control group. The difficulty in obtaining substantial T numbers is inflicted by the requirement for a certain threshold expression level of the suicide switch, which could only reached in 1-2% of genetically modified T cells. In that light it is conceivable that for successful clinical evaluation, developing strategies to generate sufficient cell numbers expressing a sufficient amounts of the safety switch may the largest hurdle to be taken.

Concluding remarks.

Before a physician wants to expose a patient to a potential novel therapy, he (or she) wishes to have a good indication about the risk-benefit ratio, and both allo-SCT/DLI and TCR gene transfer form no exception. Pre-clinical animal models can be very useful in making a valid prediction, facilitating a higher change of a successful move ‘from bench to bed’. The risk-benefit ratio of allo-SCT/DLI as treatment for malignancies such as CML is currently accepted, but certainly not considered satisfactory. Segregation of the GvHD from the GvL-effect seems to be the key challenge, which is thought to be feasible via *ex vivo* modification of the T cell graft. Data regarding MHC multimer-

based technologies suggest that T cell removal (chapter 2) or enrichment[56;57] is technically sufficiently promising to contemplate clinical testing, and for MiHAgs HA-1 and HA-2 enrichment may be a realistic option[80]. However, in general the knowledge on the identity of T-cell antigens that lead to GvHD or GvL is far from complete, and efforts to expand this knowledge seem to be crucial to take the full advantage of an allo-SCT.

In contrast to allo-SCT/DLI, TCR gene transfer is a far from established form of cancer immunotherapy. Preclinical animal studies (chapters 4 and 5) and a recent clinical phase I trial[4] have provided proof of principle, namely that TCR modified T cells can be used to target tumor antigens to which the endogenous T cells are immune tolerant. However, to further pursue clinical implementation, assessment of which factors determine the *in vivo* efficacy of TCR modified T cells and thorough evaluation of its safety is needed. Both immunological as well as tumor biological parameters will determine the clinical outcome of TCR gene transfer. This thesis has mainly focussed on the immunological part of TCR gene transfer. Because mice are considered to mirror human immunology remarkably well, and by keeping the relatively few differences in mind[81], it seems justified to use mice as a tool to determine how alterations in protocols for adoptive immunotherapies can yield more successful trials in men. Both the use of lymphodepletion as conditioning regimen as well as adaptations in the design of the transgene has greatly improved the clinical efficacy of TCR gene modified T cells in mice and are proposed to be implemented in clinical trials. Further improvements such as the development of platforms to generate high affinity human TCRs are expected to be made in the near future, and are anticipated to deliver a significant contribution to the quality of the TCR modified T cells.

In contrast to the similarity in T cell responses in mice and men, it is less obvious whether mouse models can be predictive of anti-tumor efficacy in clinical trials. To study tumor biology in mice one always has to revert to model systems, which can vary from transplantable tumor lines to transgenic and conditional spontaneous tumor models. But even the most sophisticated murine model cannot mimic all aspects of its human counterpart[82]. For example, in mice tumor growth is generally considerably faster, and genetic heterogeneity is lower. Therefore translation of experimental results to human situations should be done carefully and only with regards to those aspects of which resemblance is sufficiently met. This may be illustrated by the data presented in chapter 4, where we have tested TCR gene transfer in an SV40 large T-driven spontaneous tumor model and found that SV40-TCR transduced T cells can halt tumor development. Because central tolerance towards TAAs has been observed both in mice and humans (discussed in the section *'Proof of principle'*), we considered it justified to conclude that TCR gene transfer can be of value to target otherwise non-immunogenic tumor-associated antigens. Analysis of the remaining (pre-)malignant lesions revealed that – in contrast to some previous reports (REF[15] and chapter 6) – adoptive transfer of SV40-specific T cells did not result in the selection of antigen loss variants. This may very well be caused by the fact that the targeted protein is involved in cellular transformation, although the experiments did not

formally test this hypothesis. But since SV40-large T is of viral origin and under control of an artificial promoter, we regard this model not suitable for assessing the merit of choosing proteins essential for cell growth or transformation as targets for TCR gene transfer.

As for any new therapy, careful evaluation of potential toxicity is at least equally essential. Recent data suggest that also TCR gene transfer can be accompanied by GvHD, and it is therefore fair to advocate that assessing the safety of TCR gene transfer should be one of the important lines of translational research in the coming years, both in terms of risk-evaluation as in terms of developing strategies to control autoimmune pathology.

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