Adoptive T cell therapy as treatment for Epstein Barr Virus-associated malignancies : strategies to enhance potential and broaden application

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Chapter 8

Discussion

Cancer immunotherapy aims to destroy malignant cells by triggering an immunological response similar to the highly effective and specific elimination of virus-infected cells. One means of doing this is by adoptive transfer of tumor-specific T-cells. This strategy is particularly attractive as it allows for *ex vivo* generation, characterization and modification of a therapeutic product in the absence of tumor-related inhibitory factors. EBV-associated tumors provide an excellent model to develop and study this approach as the tumor associated viral antigens are immunogenic and the T-cell responses against these can be reactivated using established protocols. Homing, persistence and tumor-protective effects of adoptively transferred EBV-specific T-cells have previously been demonstrated for highly immunogenic EBV-positive tumors developing in immunocompromised hosts.[1-3] Here, we studied the feasibility and efficacy of this approach for EBV-positive NPC, a tumor that expresses subdominant viral antigens and can arise despite pre-existing EBV-immunity. Further, to enable a broader application of T-cell therapy to inherently less immunogenic tumors, we explored the transgenic expression of tumor-specific TCRs as a method to obtain T-cells specific for non-viral tumor-associated antigens. Finally, to address potential unwanted side effects of T-cells genetically modified to redirect their specificity or to enhance their function, we developed an inducible safety switch with desirable characteristics for use in T-cell therapy. Here, the results of these efforts are summarized and their implications for adoptive T-cell therapy are discussed.

Adoptive T-cell therapy as treatment for EBV type II latency tumors

Our phase I clinical trial of adoptive T-cell therapy as treatment for advanced NPC showed that CTL infusion is safe, although caution is required when treating patients with bulky tumors in close relation to viral organs, and provides the first indications of anti-tumor activity with clinical responses in 3 of 6 patients treated with refractory/relapsed disease (Chapter 3). This study addressed a number of important questions regarding the generation, characterization and efficacy of the autologous EBV-specific T-cells used for adoptive transfer. The first question was if CTL specific for the viral antigens expressed on this tumor could be reactivated and expanded from the peripheral blood of patients with advanced NPC as was previously demonstrated for healthy bone marrow donors.[2] To date, we successfully generated autologous EBV-specific CTL lines for 15 of 15 patients. These CTL lines expanded at similar rates as compared to CTL lines from healthy donors. This is in contrast to CTL lines from patients with relapsed EBV-positive Hodgkin's disease that required additional mitogenic stimulation to obtain sufficient expansion.[4,5] The normal growth rate despite the previous exposure to radiation and chemotherapy demonstrated the feasibility of this approach for this patient group.

The next question was if the CTL generated from patient PBMC had the desired phenotype and function. Secretion of cytokines such as IL-10 by the NPC tumor cells[6] may switch patient T-cells to a Th 2 phenotype, compromising their cytolytic activity. Further, impaired perforin expression and IFN-γ secretion in PBMC from NPC patients has been reported.[7] Nevertheless, all CTL lines, derived from autologous PBMC, lysed autologous LCL without reactivity towards autologous PHA blasts. Although the majority of T-cells in the CTL lines were CD8+, all contained a small number of CD4+ cells which has proved important for long term survival of infused T-cells in previous studies.[1,8] Thus, despite reported inherent defects of T-cells from NPC patients, autologous T-cells with the desirable cytolytic activity and phenotype could be reactivated and expanded *ex vivo*.

The availability of autologous APC poses a major block to the expansion of polyclonal antigen-specific T-cells. While non-specific methods of T-cell expansion, such as CD3/28 beads can expand T-cell clones without loss of specificity, this method is not effective for polyclonal T-cells.[9] Artificial APCs have been successfully used to expand T-cells in an antigen specific manner,[10,11] but a broad panel of such clinical-grade reagents is required to accommodate their use for antigen presentation through all HLA alleles. We chose to use LCL for reactivation and expansion of EBV-specific T-cells from PBMC, as these are readily generated from all patients using a minimal amount of blood. Moreover, they express costimulatory and cell adhesion molecules, which makes them excellent APC. However, a concern was whether LMP2-specific T-cells could be reactivated from the low number of precursors predicted to circulate in the peripheral blood of patients with LMP2-positive tumors,[12,13] using LCL that express immunodominant latent and early lytic cycle antigens.[14] By screening with a panel of peptides spanning the entire LMP2 sequence, we demonstrated that in the majority of CTL lines from patients with NPC and other EBV latency type II malignancies LMP2-specific T-cells were present (Chapter 2). As expected, the number of T-cells specific for LMP2 epitopes was low compared to the frequency of immunodominant antigen-derived epitopes, but nevertheless functional LMP2-specific T-cells could be reactivated and expanded using LCL.

This screening method further allowed us to characterize novel LMP2 epitopes recognized by these CTL. Hence, in addition to responses to previously described epitopes, 8 new epitopes were identified. The latter were restricted through HLA alleles for which no LMP2 epitopes were yet available. The majority of these and previously identified epitopes are conserved between the prototype B95-8 strain used to establish the LCL and viral strains from different geographical origin.[15] Thus, LCL-reactivated T-cells are expected to recognize LMP2-epitopes expressed on the tumor.

LMP2 peptides representing these epitopes and tetramers-derived from these provide valuable reagents to study the effects of immunotherapy. Monitoring the expansion and persistence of adoptively transferred T-cells is crucial for evaluating and improving T-cell therapy, and ultimately for understanding the interaction between the immune system and growing tumors. Using ELISPOT and tetramer assays we monitored the LMP2-specific T-cells populations, as identified in the infusion product, in the peripheral blood prior and following CTL infusion. In 4 of 8 patients with detectable LMP2-specificity in the infusion product a transient increase in the number of LMP2 specific T-cells was found. This indicates a limited expansion and limited persistence of the infused T-cells . However, it is unclear if the number of LMP2-specific T-cells in the peripheral blood reflects the anti-tumor activity of the infused T-cells at the tumor site. Further, despite the absence of a consistent and persistent increase in LMP2-specific immunity in the peripheral blood, clinical responses were observed in 3 of 6 patients with refractory disease. In future studies, the interpretation of the obtained results can be greatly facilitated by introducing a transgene in the infusion product that allows for discrimination from endogenous T-cells and tracking of adoptively transferred T-cells. In conclusion, this clinical study demonstrated feasibility and safety and the first hints of efficacy, which initiated investigation to further refine and enhance the potential of CTL therapy.

Strategies to improve T-cell therapy

A number of parameters could be responsible for the suboptimal efficacy of CTL therapy in the current setting. First, although the majority of the infused CTL lines were shown to contain LMP2-specific T-cells their number may be insufficient to eradiate all tumor cells. We therefore developed a protocol based on LMP2-expressing DCs for reactivation and LMP2-overexpressing LCLs for subsequent expansion of LMP2-specific T-cells. As both DCs and LCLs can be transduced with the chimeric Ad5Ad35 vector the production of only one clinical-grade vector is required. Using these LMP2-overexpressing APCs the specificity within the CTL lines was efficiently enriched for LMP2 specific T-cells (Chapter 4). This protocol is now being used in a clinical study of adoptive T-cell therapy for EBV-positive Hodgkin's disease.

An alternative strategy is the upregulated expression of immunodominant EBV antigens in the NPC tumor cells. A number of chemotherapeutic antigens, including cisplatinum and 5-fluorouracil, both of which are included in the standard treatment regimen for NPC, are shown to induce the switch from the latent to the lytic form of EBV infection in NPC tumor cells.[16] As a subpopulation of LCL expresses early lytic EBV antigens, LCL-reactivated CTL lines generally contain significant numbers of lytic antigen-specific T-cells as was shown for the CTL lines generated for the NPC patients (Chapter 3). Some of these chemotherapeutics also increase the expression of LMP1 in tumor cells, providing additional target antigen enhancement prior to CTL infusion.[17] Chemotherapy may therefore, in addition to its direct anti-tumor effect, sensitize the tumor for EBV-specific T-cell therapy.

A second immune evasion strategy used by tumor cells is the production of a variety of cytokines and chemokines that can negatively affect the maturation and function of immune cells. These include vascular endothelial growth factor, prostaglandin E_2, TGF-β and IL-10. For NPC in particular, enhanced levels of IL-10 at the tumor site and systemically[6] may inhibit antigen presentation and the induction of T helper type I responses.[18] Rendering T-cells resistant to these inhibitory factors may increase their efficacy. Transduction of EBV-specific T-cells with a dominant negative TGF-β receptor makes them insensitive to the anti-proliferative and anti-cytolytic effects of TGF-β.[19] Transgenic expression of IL-12 in CTL was shown to overcome the inhibitory effects of a T helper 2 environment while avoiding the systemic toxicity of recombinant IL-12.[20] These *in vitro* data suggests that harnessing EBV-specific CTL against one or more inhibitory factors present at the tumor site may enhance their efficacy and the value of this approach is currently being investigated in *in vivo* models.

The anti-tumor activity of adoptively transferred T-cells may further be inhibited by T-cell populations with a regulatory function. CD4+CD25+ T-cells are present in the peripheral blood and malignant effusions in patients with various types of cancer.[21,22] *In vitro* experiments have shown that these regulatory T-cells can inhibit T-cell responses in an antigen-specific or non-specific manner.[23,24] Such regulatory T-cells may contribute to the outgrowth of the potentially immunogenic tumor cells of NPC and may explain the lack of an efficient anti-tumor response in immunocompetent hosts. Further, it was demonstrated that regulatory T-cells could reverse the inhibition of tumor growth induced by adoptive transfer of tumor-specific T-cells.[25] This observation suggests that prior depletion of regulatory T-cell populations can enhance the efficacy of immunotherapeutic interventions. Indeed,

removal of CD4+CD25+ cells resulted in enhanced anti-tumor activity of a melanoma vaccine in mice.[26] Similarly, depletion of T-cells with an inhibitory effect prior to CTL infusion may result in improved efficacy. However, ideally, this approach would target a marker that is unique to regulatory T-cells. CD25 is a good candidate, although it is also expressed on activated T-cells and hence targeting this molecule may inadvertently deplete pre-existing tumor-specific T-cells. The desired effect may alternatively be obtained by general lymphodepletion if tumor-specific T-cells can be infused after lymphodepletion.

Finally, incomplete tumor responses post CTL therapy may be due in part to the failure of infused T lymphocytes to undergo adequate expansion. Only those T-cells that proliferate can entry into the memory pool and establish long-term anti-tumor protection.[27] However, the size of the T-cell compartment is maintained at a steady state by a number of potent homeostatic mechanisms involving cytokines or ligands, counting mechanisms, and the availability of space.[28] While these mechanisms are bypassed during immune responses, with increases in the size of spleen, lymph nodes and blood, there is a rapid return to a steady state when antigen stimulation subsides. Because of these homeostatic mechanisms, expansion of infused T-cells is likely to be limited in a steady state situation. In the context of a T-cell deficit however, mature T-cells proliferate to restore the steady state.[29] This may explain why, in the recipients of T-cell-depleted stem cell transplants, infused EBV-specific T-cells expand by 4 logs or more.[1,2] In contrast, when EBV-specific T-cells are given to NPC patients, in whom the T-cell compartment is already replete, expansion of adoptively transferred T lymphocytes is much lower or absent. Lymphoid depletion as a strategy to improve the efficacy of adoptively transferred has already shown evidence of success. When melanoma patients received cyclophosphamide and fludarabine prior to the adoptive transfer of large numbers of highly-activated melanoma-specific tumor infiltrating T-cells, peripheral repopulation and proliferation of the transferred cells was observed, as well as clinical responses (> 50% tumor reduction) in 18 of 35 patients treated.[30,31] Instead of chemotherapeutic agents, monoclonal antibodies specific for mature lymphocytes may provide a more specific means of obtaining the desired lymphodepletion. The mAb of choice should have low toxicity, a short half-life, high specificity and be available as a clinical grade reagent. Although the pan T-cell antibody anti-CD3 may have optimal specificity, its long half life and induction of tolerance reduce its attraction for this purpose.[32] mAbs specific for CD45, a pan lymphocyte marker, can profoundly deplete lymphocytes in peripheral blood and lymphoid organs, whilst sparing hematopoietic progenitor cells.[33,34] The pharmacokinetics of CD45 MAbs, with a half life of circa 8 hours are ideal for this purpose since they allow CTL infusion shortly after CD45 MAb administration and provide a window of several weeks for adoptively transferred CTL to proliferate prior to recovery of endogenous lymphocytes. We have implemented this method of lymphodepletion in our current study of CTL therapy for NPC.

Encouragingly, the clinical responses induced by adoptive transfer of melanoma-specific T-cells in lymphodepleted hosts were strongly associated with the level of persistence of the infused T-cells.[35] Lymphodepletion may have promoted the persistence of infused T-cells by enhancing the access to cytokines such as IL-7 and IL-15. IL-15 protects T-cells against activation-induced cell death and promotes homeostatic maintenance of memory CD8+ T-cells.[36] Further, in a lymphopenic environment the infused T-cells have a drive to proliferate which may allow them to transfer from effector memory to central memory cells and as such

providing long-term tumor protection.[37] Additional strategies to obtain long-lived function of adoptively transferred T-cells include their transduction with the required cytokines: the tumor-specific T-cells then locally provide the required cytokines thereby avoiding the adverse effects of systemic cytokine administration.[38] Further, vaccination strategies may improve persistence of infused T-cells. For example, DC vaccination induced cytokine production, enhanced proliferation, increased tumor infiltration and a more robust tumor response of adoptively transferred T-cells.[39] In the setting of EBV-associated malignancies irradiated LCL could be used for boosting of tumor-specific T-cells by vaccination. Clinical studies of these and other approaches are being initiated in our and other institutions to test their attributive effect to improved efficacy of T-cell therapy.

Strategies to broaden application of T-cell therapy

While EBV-related malignancies are elegant models to test general ideas about immunotherapy of cancer, unfortunately for most malignancies immunogenic and well-defined antigens are not yet available. Most tumor antigens for non-viral malignancies are either over-expressed differentiation antigens or aberrantly expressed fetal antigens. If adoptive immunotherapy for these forms of cancer is to be successful some new approaches must be undertaken. We must overcome the barrier of easily generating and expanding sufficient numbers of tumor-specific T-cells that are available for all patients regardless of HLA phenotype. Although methods usually relying on DCs as powerful antigen presenting cells are being developed these may still be impractical or not applicable for all antigens. For example, although HA-1 specific T-cells can be reactivated and expanded using peptide-pulsed DCs[40] this strategy is not successful in all cases. An attractive solution is TCR transfer: grafting T-cells with TCRs with the desired specificity. Although seemingly simple this approach has not been practical mainly for technical reasons including inefficient transduction of T-cells, poor expression or assembly of the transgenic TCR and the use of separate vectors for expression of the TCRα and β chains. We attempted to solve these problems using RD114 envelope-pseudotyped virus to obtain high transduction efficiency,[41] a retroviral vector that accommodates high transgene expression in T-cells (splicing SFG)[42] and a foot-and-mouth disease-derived 2A sequence to obtain equal expression of the TCR α and β chain from a single vector.[43] Using this expression system an HA-1 tetramer-positive population was detectable after a single transduction of EBV-specific CTL (Chapter 5). The HA-1 TCR transduced CTL had dual specificity for HA-1 and EBV-antigens and, importantly, cytolytic activity against hematopoietic cells natively expressing HA-1 was demonstrated.

We hypothesize that the main limitation to further enhance expression of the transgenic TCR is promiscuous cross pairing of transgenic TCRα and β chains with native TCRβ and α chains respectively. This leads to lower expression, lower function and potentially novel specificities. As other groups, we attempted to generate single-chain receptors,[44,45] but failed to detect any surface expression or function in this form. Currently, we are trying to introduce leucine zipper motifs that will force correct pairing of the transgenic TCRα and β chains.[46] The risk of TCRs with auto-immune reactivity as a results of cross-paring with native TCRs may be further reduced by transduction of antigen-specific CTL instead of polyclonal T-cells with unselected specificity.[47]

Once we have developed a strategy for readily achievable, high level functional transgenic TCR expression, this approach should be transferable to any TCR. However, as high avidity CTL are required for optimal anti-tumor activity,[48,49] the main challenge will be to obtain high avidity CTL clones specific for tumor antigens. HA-1 provides an ideal target in that the allo-nature of this response likely increases the presence of high affinity receptors. The majority of tumor associated antigens, however, are self antigens, resulting in a peripheral T-cell repertoire that is devoid of high avidity antigen-specific T-cells, due to self tolerance.[50] A number of strategies have been developed to circumvent tolerance to self-antigens to obtain high affinity tumor-specific TCRs. These include the induction of allo-restricted antigen-specific T-cells[51,52] and vaccination of HLA-transgenic mice with peptides representing self antigen epitopes[50,53]. Alternatively, low affinity TCRs isolated from a tolerized environment can be engineered to enhance their affinity.[54,55] Further, as CD4+ T-cells play an important role in the induction and persistence of tumor immunity, it is desirable to extend this approach to HLA class II-restricted TCRs. So far only a limited number of HLA class II-restricted tumor antigen-derived epitopes have been identified. Grafting CD4+ T-cells with MHC class I-restricted TCRs provides an alternative means to obtain tumor-specific CD4+ cells. Willemsen et al demonstrated this approach is feasible when the CD8α is co-expressed with the transgenic MHC class I TCR.[56] Finally, rapid screening techniques (as proposed in Chapter 5) need to be developed to obtain a large array of high affinity tumor-antigen specific TCRs restricted through different HLA alleles to allow application of this strategy to a broad patient group.

Chimeric TCRs are very attractive since they graft a surface specificity. Unlike native TCR they are not HLA restricted, nor are they susceptible to downregulation of HLA molecules and defects in antigen processing. Moreover, T-cell mediated effector functions are more likely to result in tumor cell lysis than humoral immune response alone. Cytokine secretion upon T-cell activation by tumor antigen will result in the recruitment of additional components of the immune system, amplifying the anti-tumor immune response. The main problem with the chimeric TCR approach is the number of truly tumor-specific target antigens available. In the absence of these, markers of non-essential tissue such as CD19 and CD20 (B cells) or prostate surface proteins may be used as alternatives.

The clinical benefits obtained so far with chimeric TCRs based on a CD3-ζ have been limited, at least in part because of incomplete T-cell activation by the tumor cells, which do not provide the required co-stimulatory signals. Indeed, incorporation of a CD28 signaling domain clearly improved efficacy in animal models,[57,58] but remains limited to tumor growth inhibition. The recently proposed concept that different co-stimulatory molecules are required to provide proliferative and survival signals suggested that incorporation of addition costimulatory signals in artificial TCRs is necessary for optimal function.[59] Incorporation of OX40 in our model chimeric receptor allowed for extensive and prolonged expansion of redirected T-cells upon stimulation with tumor cells even in the absence of exogenous IL-2 (Chapter 6). This increased expansion may be the result of a stronger proliferative signal but more likely results from protection against apoptosis as OX40 has been shown to be instrumental in maintaining high expression of anti-apoptotic molecules.[60] The lack of increased activity of T-cells transduced with a TCR containing OX40-ζ in the absence of CD28, is in line with the observation that the physiological expression of both OX40 and OX40L requires CD28-B7 interactions.[60] Thus, the combined incorporation of CD3-ζ, CD28 and OX40

in the endodomain of the tumor-specific TCR ensures the presence of essential signals for target cell lysis, clonal activation and proliferation as well as early and sustained survival signals resulting in a potent anti-tumor response *in vitro*. It remains to be determined if this triple domain can further enhance the anti-tumor activity obtained with CD28.CD3-ζ based TCRs . Animal studies have already shown the first indication that OX40 engagement can enhance anti-tumor immunity.[61,62] Incorporation of OX40 in the endodomain of a tumor-specific TCR may provide a similar beneficial effect while circumventing the risk of the induction of auto-immunity when using an OX40 activating antibody.[63]

Safety using genetically modified T-cells

Genetic modification of T-cells used for adoptive transfer is likely to be the next incremental step in the development of adoptive T-cell therapy. Strategies of rendering T-cells resistant to tumor evasion mechanisms or to provide them with cytokines that enhance their survival have shown promising results *in vitro*.[19,20] In addition, grafting T-cells with the desired specificity will greatly facilitate the generation of tumor-specific T-cells to a broader range of antigens. However, these genetically modified T-cells may have unwanted toxicity caused by the transgene or by targeting antigens that are not solely expressed on tumor cells.[35,64,65] These concerns can be addressed by incorporation of a safety switch that allows for elimination of transduced cells in the event of toxicity. We developed an inducible caspase 9 that in contrast to other safety mechanisms has low immunogenic potential and selectively and virtually completely eliminates transduced cells (Chapter 7). In contrast to inducible death molecules that act as initiators of the apoptosis pathway, inducible caspase remains functional in the presence of most inhibitors of apoptosis. This is important to ensure function in T-cells that are relatively apoptosis-resistant including memory T-cells and malignantly transformed T-cells. The optimal function of inducible caspase 9 requires selection for T-cells with bright transgene expression. This can be achieved by co-expression of non-immunogenic marker genes such as CD20, truncated CD34 or truncated NGFR.[66-68] The development of retroviral vectors that allow for reliable co-expression of multiple gene makes the expression of a transgene, a suicide gene and a marker gene from a single vector a feasible possibility/goal.[69]

In the majority of studies depending on gene transfer to T-cells, oncoretroviral vectors that rely on integration in the host genome for transgene expression were used. Murine retroviruses have been associated with the induction of leukemia in two children receiving corrective therapy for X-linked severe combined immunodeficiency.[70] In this study patients received CD34+ bone marrow stem cells transduced with the common gamma chain – a critical proliferative signaling protein. It is difficult to extrapolate this potential danger to studies of transduced peripheral blood T-cells especially when bearing in mind that over 100 patients have received retrovirally transduced T-cells without subsequent leukemogenesis. Nevertheless, vector designs that reduce the risk of insertional mutagenesis are warranted. Self-inactivating (SIN) retroviral vectors in which the LTRs become inactivated upon integration reduce the risk of vector driven overexpression of genes in close proximity to the unemployed 3' LTR. Flanking the transgenic promoter-enhancer in the retroviral cassette with a chromatin insulator provides and additional safety mechanism.[71] The use of SIN vectors was so far inhibited by the difficulty of obtaining high titer virus. This limitation may now be overcome as RD114-pseudotyped transient transfection produced vector affords us high titer virus that facilitated efficient transduction of T-cells (Chapter 5-7).

Future of adoptive T-cell therapy

From the clinical studies of adoptive T-cell therapy including the study described in this thesis, valuable expertise has been gained with the generation, characterization and monitoring of tumor-specific T-cells. With improved knowledge of the interaction between the immune system and malignant cells insights are emerging how to enhance efficacy. Improved vector design, improved transduction protocols and a suicide switch allow for the clinical implementation of T-cells genetically modified to obtain the desired specificity and enhance potency. Well designed clinical trials are now required to evaluate the effect of modifications on expansion, persistence and function of adoptively transferred T-cells.

Crucial in this is the monitoring of grafting and homing of the infused T-cells. Monitoring the number of tumor-specific T-cells using ELISPOT or tetramer assays does not discriminate between those T-cells that were adoptively transferred and a population arising de novo in the host. Similarly, the TCR-Vβ spectratype of the infusion product is a surrogate indicator.[72] Genetic modification provides a unique marker that permits infused T-cells to be distinguished from a newly arising population of identical specificity and phenotype.[73] Slightly different vectors can be used to mark different cell population allowing differential tracking.[74] Further, as a single transduced cell and its progeny have a unique site of integration of the retroviral vector this "signature" can be used to follow individual T-cell clones over time. Highly valuable information regarding expansion, persistence and homing of infused T-cells has been obtained using gene marked virus and tumor-specific T-cells in adoptive T-cell therapy studies.[1,3,5,75,76] During the clinical study described in this thesis gene marking was not available due to safety concerns following the two cases of leukemia in children receiving retrovirally modified stem cells.[70] Also for the immediate future at least, it is unlikely that retroviral transduction of adoptively transferred cells purely for marking purposes will be pursued. However, studies involving genetically improved T-cells also provide gene marking. Here, the potential benefit of the modification would outweigh the risks of insertional mutagenesis, in particular when used to treat cancer patients with relapsed/refractory disease.

Although monitoring the fate of adoptively transferred T-cells provides essential information, only a combination of improved anti-tumor immunity and clinical response can demonstrate the effect of immunotherapy. This requires sensitive tumor markers and imaging techniques in particular when treating patients with minimal residual disease. For NPC, the concentration of cell-free EBV-DNA as detected in plasma is emerging as a reliable marker for diagnosis and response to treatment.[77] However, the sensitivity of this method, in other words what size tumor results in a detectable level of EBV-DNA in plasma, still needs to be determined. Further, in our clinical study, evaluation of tumor responses proved difficult in a number of cases as MRI and CT scan did allow for discrimination between radiation-induced fibrosis and residual NPC.[78] We therefore implemented the more sensitive PET-scan as method of choice in our current clinical studies. Combined imaging of the adoptively transferred T-cells and the targeted tumor would provide highly valuable insight in the efficacy of adoptive T-cell therapy strategies. MRI imaging of tumor-specific T-cells labeled with physiologically inert nanoparticles has been shown a sensitive real-life imaging technique to study T-cell migration and homing in mice.[79] Transgenic expression of a reporter that can be visualized by PET scan (e.g. sodium–iodine-symporter) may become available as a non-invasive imaging technique for long-term monitoring of the infused cells.[80]

Key to the rapid implementation of new insights in cancer immunotherapy is successful interaction with industry and regulatory agents.[81] Reluctance by industry to make their products (e.g. cytokines) available for clinical study forces the use of less optimal reagents. Agreements to share liability and financial risk between industry and government could address this issue. Regulatory rules set down by government agencies for phase I clinical studies represent a tremendous burden to the clinical researcher. Standards applicable to the pharmaceutical industry are often mandated. Extremely expensive testing and re-testing of clinical products drains grant money, as does the requirement for extensive infrastructures for quality assurance and data monitoring. While such standards are reasonable for drugs developed for use by a large number of patients for non-life-threatening disorders, the regulatory burden for even the simplest study acts a powerful deterrent to investigators. To allow for rapid execution of novel clinical studies, simplification of requirements should be implemented. Government agencies should create centralized facilities for generating vectors and cell products, since high throughput of production and testing can greatly reduce the costs of staffing and facilities and concentrate expertise. Specialized committees that include members working in the immunotherapy field may be best able to address issues relevant to biological agents. Such changes would foster an effective relationship between regulatory agencies and clinical investigators with the mutual goal of moving the field forward in safe and efficient manner.

In conclusion, this thesis describes incremental advances made in overcoming barriers to the successful use of adoptive T-cell therapy. Our studies and others have demonstrated the feasibility of generating sufficient autologous tumor-specific T-cells from patients with advanced malignancy for clinical use. Improved reactivation and expansion methods are now becoming available for selection of T-cell specific for less immunogenic tumor antigens.[82-84] Our protocol to generate LMP2-specific CTL has already been implemented in an ongoing clinical study. For non-immunogenic tumor antigens, or antigens for which tolerance exits, native TCR transfer using modern retroviral vector techniques, may soon allow any TCR specificity to be grafted onto autologous T-cells in a clinically feasible manner. Harnessing T-cells with costimulatory molecules or other immune modifiers is a promising strategy to overcome immunosuppressive strategies employed by tumor cells to prevent their eradication, the value of which in now being evaluated in animal models. Inducible caspase 9 provides a non-immunogenic highly effective suicide gene that will allow many of these new approaches to be implemented in clinical protocols. Together, engineering work on T-cell selection and genetic modification, such as presented in this thesis, may well allow us to develop adoptive T-cell therapy into an effective non-toxic treatment modality for cancer.

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