Engineering T cell immunity by TCR gene transfer

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SCOPE OF THE THESIS

The immune system has evolved effective mechanisms to protect our body against infectious disease. T cells play an essential role in this protection. The development of antigen-specific T cell responses is achieved in three distinct steps:

I) Recruitment: T cells with appropriate TCR specificities for the antigen are selected from the total pool of T cells.
II) Expansion: recruited T cells expand to higher numbers.
III) Differentiation: T cell subsets differentiate into appropriate subsets.

Following this process, the resulting T cell population(s) eliminate the pathogen and establish a subsequent T cell memory. While being markedly efficient in most situations, T cell responses of sufficient strength are sometimes not established. Examples are persisting pathogen infections (HIV, HBV, HCV) as well as cancer. Tumors in particular pose a challenge in terms of recognition as a "threat" to the immune system since they are not "foreign" but rather "self" which have acquired malignant potential as a result of genetic alterations. It can be argued that tumors are not immunogenic due to their self-origin. However, indirect support for the notion that tumors are immunogenic and that the immune system plays a role in tumor control comes from case reports of spontaneous tumor regressions of various cancer types[1-5]. Furthermore, the discovery of various tumor antigens[6-12] and the reports of frequent T cell responses against such tumor antigens[13, 14] argue that antigen-specific T cell responses against tumors can develop. Therefore, anti-tumor T cell responses are not generally absent in cancer patients, but do not mediate immunity against the tumor. This raises the question of whether T cell immunity can be engineered in those cancer patients?

Many T cell based immunotherapies such as vaccination[15, 16], immunmodulation by blockade of T cell checkpoints with monoclonal Antibodies (mAb)[17-20] and adoptive cell therapy utilizing autologous tumor-infiltrating lymphocytes (TIL)[21-24] aim to mobilize the endogenous T cell repertoire against the tumor by inducing T cell responses against tumor antigens. Especially the latter two approaches have shown remarkable success in the treatment of cancer patients with advanced malignancies, most notably metastatic melanoma. For melanoma there is a strong indication for the existence of T cell reactivity against tumors[6-14] opening the opportunity to further enhance such T cell reactivity with immunotherapeutic interventions. However, such evidence of anti-tumor T cell reactivity is more rare for most other tumors. While the molecular basis for such differences is unknown, this may account at least in part for the observation that different malignancies have varying response rates to immunotherapeutic interventions[17, 19]. Furthermore, it may contribute to the fact that some malignancies are not eligible for some treatments, e.g. TIL therapy is restricted to melanoma and renal cell carcinoma. Therefore, many treated patients do not ultimately develop curative endogenous anti-tumor T cell immunity after such interventions (of note, even a high percentage of patients with metastatic melanoma will not be responsive). Thus, it seems possible that the endogenous T cell compartment is compromised in many cancer patients with regards to, first, the number and/or affinity of tumor-reactive TCRs present, and second, the effector function of tumor-reactive T cells.

TCR gene transfer offers the possibility to create a tumor reactive T cell compartment, thereby engineering T cell immunity against tumor in cases where it is otherwise absent. This approach refers to the redirection of T cells towards a defined antigen by introduction of new TCRαβ genes. It was pioneered with the discovery that T cell specificity can be transferred between T cells by TCRαβ genes[25]. The manipulation of the TCR compartment with TCR gene transfer is possible in three ways:

I) Recruitment: one (or more) TCRs with selected antigen-specificity and affinity can be introduced into autologous T cells.
II) Expansion: TCR-modified T cells can be expanded *ex vivo* and infused in high numbers.
III) Differentiation: TCR gene transfer can be performed into T cell subsets with different functional properties that may be additionally manipulated by genetic engineering (e.g. introduction or deletion of genes), thereby creating T cell activities of choice.

The scope of this thesis is to explore strategies and tools to optimize TCR gene transfer as an engineering strategy for T cell immunity against cancer. In particular, we assess the safety of TCR-modified T cells, evaluate strategies to support the function of TCR-modified T cells *in vivo* and develop technologies for the isolation of tumor-reactive TCRαβ genes and the general analysis of disease-associated TCR repertoires.

In **Chapter 2** we discuss lines of development for TCR gene transfer with regards to safety and enhanced efficacy. We argue that the safe and effective use of TCR-modified T cells critically depends on both the selection of suitable target antigens and the use of appropriate engineering strategies for the introduction of TCR genes into T cells. The expression of target-antigens in normal tissues has led to harmful reactivity of TCR-modified T cells against such tissues in clinical studies[26-28]. As outlined in detail in **Chapter 2**, the class of Cancer/Germline (C/G)-antigens seems to contain promising candidates, such as NY-ESO-1[29], in order to avoid such toxicities. In addition, the targeting of patient-specific neo-antigens arising from tumor mutations might offer new possibilities with regards to safety and possibly treatment efficacy[12, 30, 31].

Apart from safety concerns regarding the reactivity of the introduced TCR, it has been argued that adverse effects in TCR gene transfer may arise from the formation of TCRαβ heterodimers in TCR-modified T cells, comprised of an introduced and endogenous TCR chain (mixed TCR-dimers)[32]. In **Chapter 3** we demonstrate that the formation of such mixed TCR-dimers – which have an unpredictable specificity and can also be formed in human T cells[33] – can lead to fatal TCR gene transfer induced Graft-versus-Host Disease (Ti-GVHD) in mice. In order to avoid the formation of mixed TCR-dimers, we test different engineering strategies to enhance pairing of introduced TCRαβ genes and demonstrate that they can limit or even prevent the occurrence of Ti-GVHD.

Many human malignancies suppress T cell functions in their microenvironment by engaging inhibitory signaling pathways in T cells, such as PD-1[34, 35] or the TGF-β receptor[36-39]. Thus, interference with such inhibitory pathways in TCR-modified T cells is of interest in order to enhance their anti-tumor reactivity. It has been described

that the modification of T cells with siRNA targeting T cell checkpoints such as PD-1[40] or key regulators of T cell effector function like Cbl-b[41] enhances anti-tumor T cell reactivity. In **Chapter 4** we engineer T cells to express both a tumor-reactive TCR and a dominant-negative TGF-β receptor II (dnTGF-βRII), rendering them insensitive to suppression by TGF-β. In an autochthonous mouse model of prostate carcinoma we demonstrate that these engineered T cells, expressing a tumor-reactive TCR and dnTGF-βRII, eradicate large, invasive prostate carcinomas, leading to complete, sustained tumor regression and prolonged survival.

The isolation of TCRs specific for antigens that can be targeted with TCR gene transfer requires tools to detect and isolate antigen-specific T cells and efficient strategies to identify TCRαβ gene sequences from these T cells. In **Chapter 5** we describe the development of MHC-class I multimer technology for various HLA-alleles allowing detection of antigen-specific T cell responses by flow cytometry[13, 14, 42]. Using a novel high-throughput TCR gene capture approach in **Chapter 6**, we analyze TCR repertoires from such antigen-specific T cell populations and isolate TCRαβ genes to assemble a library of C/G-antigen specific TCRs. We further demonstrate that TCR gene capture can also be used for both the monitoring of the diversity of disease-associated T cell subsets (such as tumor-reactive $CD8^+$ T cells among TILs) and the identification of dominant TCRs in such populations. In **Chapter 7** we present an alternative strategy allowing the identification of TCRαβ sequences on a single-cell level. This technology holds great promise for the in-depth analysis of TCR repertoires, e.g. the TCR composition of intratumoral T cell populations. Finally, the prospects for TCR gene transfer as a clinical treatment for cancer, in particular in the context of the findings described in this thesis, are discussed in **Chapter 8**.

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