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A PERSPECTIVE FOR TCR GENE TRANSFER: FROM ‘OFF-THE-SHELF’ TO ‘PERSONALIZED MEDICINE’?
From the initial demonstration that the introduction of new TCR genes can confer an additional antigen-specificity to T cells\(^1\), TCR gene transfer has quickly progressed into a clinically feasible treatment within the last decade\(^2\)-\(^4\). Engineered T cells can be highly potent after infusion into patients as shown in the latest clinical trial with TCR modified T cells targeting the Cancer/Germline (C/G)-antigen NY-ESO-1\(^4\) and clinical trials in which CD19-specific Chimeric Antigen Receptor (CAR)-modified T cells have been infused into patients with chronic lymphocytic leukemia\(^5\)-\(^6\). The above TCR trial led to an objective clinical response rate of 53\%, although the duration of responses was unsatisfactory. Furthermore, the number of severe adverse events observed in recent trials with either TCR or CAR-modified T cells provides further evidence for the potency of such engineered T cells (J.H. vd Berg, Manuscript in preparation)\(^2\),\(^3\),\(^7\)-\(^10\). In several cases, it is apparent that the cognate epitope recognized by the introduced TCR or CAR was presented not exclusively on tumor cells but also on cells from other tissues. Thus, the selection of antigens with a truly tumor-restricted expression pattern appears to be a major challenge. This selection is further complicated by the fact that for optimal treatment efficacy additional factors have to be taken into account. Common considerations for the selection of safe and effective targets of TCR gene transfer are (Chapter 2)\(^11\)-\(^13\): first, low/absent expression in vital non-tumor tissues (safety) and, second, high, homogenous and sustained expression in tumor lesions (efficacy). However, with the exception of some hematological diseases, thus far it has proven difficult to identify target antigens that meet both of these criteria. The first clinical studies with TCR-modified T cells initially targeted melanocyte differentiation antigens (i.e. Melan-A, gp100), which are expressed in multiple normal tissues. More recently, C/G-antigens have been targeted (Chapter 2) which may be sufficiently tumor-restricted in some, but not all cases\(^4\),\(^9\),\(^14\). Moreover, they often show a heterogeneous expression pattern within tumor lesions which raises concerns with regards to full eradication of lesions. As a further concern, it remains challenging to prove tumor-restricted expression patterns of target antigens with current technologies since they may not uncover potential expression in rare, but possibly vital, cell types. Therefore, the safety profile of chosen antigens can ultimately only be defined in clinical studies at the moment.

The sole class of non-viral antigens for which tumor-restricted expression can be safely assumed are antigens arising from tumor-specific mutations\(^15\)-\(^18\). There is some evidence for the T cell recognition of such antigens on tumor cells\(^15\),\(^16\),\(^19\) suggesting that they might not only be a preferable choice with regards to safety but may also lead to tumor regression.

The common vision for TCR gene transfer is the idea of an “off-the-shelf” treatment. The goal is to assemble libraries of TCRs against tumor antigens, which can then be applied according to the requirements of the individual patient (i.e. the HLA-haplotype and the expression patterns of target antigens in tumor lesions of the patient). The broadness of the available TCR libraries with regards to HLA-restriction and targeted antigens will critically determine the number of patients benefiting. A high coverage will require TCRs against many antigens (expressed in different cancer types and disease stages).
and TCRs restricted to different HLA-types. However, the vast majority of tumor-specific mutations are unrelated to cellular transformation and thus likely to be patient-specific. Therefore, when considering the class of tumor-specific mutations as the most preferable class of target antigens, the idea of an “off-the-shelf” TCR gene transfer treatment is not feasible. Instead this raises the question of whether such patient-specific antigens may be targeted by TCR gene transfer of autologous tumor-reactive TCRs – in essence providing a “personalized autologous medicine”.

There are possible advantages in the utilization of a patient’s endogenous TCR repertoire to engineer T cell immunity. Both the blockade of inhibitory receptors of T cell function (PD-1, CTLA-4) with monoclonal antibodies as well as TIL therapy have shown marked success in the treatment of metastatic melanoma. These approaches aim to mobilize the endogenous T cell repertoire of the patient against the tumor. Although the nature of anti-tumor responses induced by these treatments is only partially understood, they emphasize that endogenous TCRs can mediate tumor regression in some cases and that a broad anti-tumor reactive TCR repertoire might be available for TCR gene transfer. In particular for tumor-specific mutations, it can be expected that a tumor-reactive TCR repertoire is present in a very high number of patients, since T cells against such antigens are not deleted in the thymus.

The isolation and transfer of high numbers of TCRs on a patient-specific basis in a clinically relevant time frame would have appeared to be futuristic a few years ago. However, technical advances make such an approach a realistic goal now. As demonstrated in Chapter 6, the high-throughput isolation of patient-specific TCRs is possible. Single-cell based approaches as published and reported in Chapter 7 will further extend the possibilities to identify tumor-reactive TCRs in individual patients. The current bottleneck for TCR gene transfer of tumor-reactive TCR on a patient-specific basis is the production of clinical-grade retrovirus which is costly with regards to time and finances. However, non-viral gene transfer systems such as transposon-based approaches might offer alternatives.

When considering which autologous tumor-reactive TCRs to use for TCR gene transfer, the preferable option with regards to safety is the selective transfer of TCRs against tumor-specific mutations. It is technically feasible to detect T cells that recognize such antigens on a patient-specific basis. However, to date, such a procedure is still time-consuming and may miss potentially beneficial TCRs. For example, such screens are commonly based on exome-sequencing and thereby may miss antigens from alternative transcription/translation events (of note, such ‘unconventional’ antigens as well are likely to be highly tumor-restricted). In addition, they usually rely on prediction of MHC-class I binding of mutated peptides, which is not equally reliable for all MHC-alleles.

Alternatively, one may consider the identification of high numbers of autologous tumor-reactive TCRs (e.g. demonstrated in Chapter 6) and their use for TCR gene transfer – even without knowledge of the antigen-specificities of transferred TCRs. In this way a broad, tumor-reactive TCR repertoire can be utilized for “autologous TCR gene transfer”. The resulting TCR repertoire will likely contain TCRs against tumor-specific mutations but also against other tumor-antigens. There is some
indication that such an ‘unselective’ transfer can be safe. First, any TCR transferred on a patient-specific basis will have undergone prior thymic selection in the same individual. Second, the experience with TIL therapy may suggest that the transfer of autologous TCRs, at least derived from intratumoral T cells, could be safe. However, the potential risk in ‘transplantation’ of tumor-reactive TCRs without selection on tumor-specific mutations lies in the accidental engineering of strong T cell responses against potential harmful targets (e.g. some MAGE antigens). Such TCRs might be transferred from exhausted, minimally functional T cells into T cells with very high effector capacity. Therefore, it seems advisable to implement the use of a “safety-switch” in such autologous TCR-modified T cells in order to better control their in vivo function.

In conclusion, it is tempting to speculate that “autologous TCR gene transfer” might overcome current hurdles in TCR gene transfer, especially with regards to the need of selecting defined, safe antigens. However, in order evaluate the prospects of “autologous TCR gene transfer” it will be essential to determine whether tumor-reactive TCRs that are suitable to engineer T cell responses of sufficient strength to eradicate tumor lesions are generally present in cancer patients.

To this end, one can analyze the TCR repertoire in patients receiving immunotherapeutic treatments (e.g. anti-CTLA-4, anti-PD-1, TIL therapy) for cancer to uncover whether differences with regards to the frequency, diversity or affinity of tumor-reactive TCRs exist. It seems plausible that marked differences in these aspects may distinguish responding and non-responding patients. The technologies described in Chapter 6 and 7 can be employed to determine TCR sequences in TIL in a high-throughput fashion. Identified TCRs can then be retrovirally introduced in PBL to allow assessment of autologous tumor recognition. The isolation of TCR genes will enable one to test the avidity of TCR modified T cells for the autologous tumor, uncovering whether differences in TCR “quality” rather than “frequency” or “diversity” play a role. Such an analysis could initially be focused on intratumoral CD8+ T cells and then be expanded to other intratumoral and peripheral T cell subsets. If no significant differences between TCR repertoires of responding and non-responding patients exist, this would argue that the endogenous TCR repertoire commonly has a tumoricidal potential. Since the lack of anti-tumor responses in some of the patients is then related to other factors, e.g. impaired T cell function due to phenotype or immune suppression (see below), autologous TCR gene transfer would seem promising since T cell function could be tailored by engineering approaches. Although a residual tumor-reactive TCR repertoire is likely to be present in every patient (in particular against antigens not subjected to thymic selection), differences in the frequency, diversity or affinity of tumor-reactive TCRs may be uncovered between patients. In case such ‘ineffective’ TCR repertoires are uncovered, it may be conceivable to generate (additional) TCRs against tumor-specific mutations of this patient to obtain a potent tumor-reactive TCR repertoire. Such TCRs can be obtained with allo-CTL systems, display systems, or possibly even from the autologous naïve T cell repertoire and then be isolated with TCR gene capture (Chapter 6) or single TCR identification methods (Chapter 7).
For “autologous TCR gene transfer” it will be possible to use two further advantages of TCR gene transfer: first, tumor-reactive TCRs from exhausted cells can be transferred to a “fit” T cell population and second, TCR-modified T cells can be harnessed to withstand tumor-induced immune suppression. To assess the potential benefits of both possibilities, it seems advisable to test whether both exhaustion and immune suppression are factors hampering the success of immunotherapies thus far, i.e. whether they differ between responding and non-responding patients receiving such treatments.

In order to examine the contribution of cell “fitness”, one can test whether tumor-reactive T cells in patients not responding to immunotherapeutic interventions are commonly less functional than their counterparts in responding patients. Indirect support for this hypothesis comes from the observation that clinical response to TIL therapy correlates with expression levels of CD27 and telomere length of TIL cells, suggesting that less differentiated and less exhausted T cells exhibit better anti-tumor function. Experimentally, this question can be addressed by determining the frequency of tumor-reactive TCRs (as outlined above) in different phenotypic T cell subsets. If tumor-reactive TCRs are primarily found in terminally differentiated and exhausted T cells, it seems likely that transfer of such autologous TCRs into less differentiated T cells with a higher capacity to eliminate tumor cells will enhance anti-tumor reactivity in the patient. Of note, such an approach might be tested in xenograft animal models in order to compare the anti-tumor efficacy of a TIL product and TCR modified T cells expressing TCRs derived from TIL of the same patient. In parallel, it will be essential to test whether tumors in non-responding patients are particularly immunosuppressive. Tumor specimens from both patient cohorts have to be analyzed for expression of ligands of inhibitory receptors for T cell function (e.g. PD-L1), the secretion of inhibitory cytokines (e.g. IL-10, TGF-β) and the presence of immunosuppressive cell types (e.g. regulatory T cells and Myeloid-derived Suppressor cells). A difference between the two cohorts with regards to the extent of immune suppression in the tumor microenvironment would argue that strategies are warranted to overcome such immune suppression. Engineering approaches, for example the transfer of a tumor-reactive TCR into T cells harnessed to withstand such immune suppression as described in Chapter 4 and other studies, might offer sophisticated solutions towards this aim.

Concluding remarks
Although TCR gene transfer has shown some potential in the treatment of cancer both safety and efficacy have to be improved. Thus far, the clinical experience of TCR gene transfer proves that the selection of targets suitable to treat large groups of patients with the same TCR is far from a routine procedure. In the light of the realization that even tumors of the same histological origin can differ greatly in their antigen expression pattern, the vision for TCR gene transfer might have to change towards a highly personalized treatment. A better understanding of the aspects which determine the effectiveness of individual TCR repertoires to mount effective anti-tumor T cell responses will be critical to engineer curative T cell responses against cancer.
REFERENCES

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