The next step toward GMP-grade production of engineered immune cells

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ABSTRACT
Removing less potent T cell subsets as well as poorly- or non-engineered cells can optimize effectiveness of engineered T cell therapy against cancer. We have recently described a novel, GMP-ready method for the purification of engineered immune cells that might further boost the clinical success of cancer immunotherapy.

Abbreviations: allo-SCT, allogeneic Stem Cell Transplantation; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; GMP, Good Manufacturing Practices; GvHD, Graft versus Host Disease; RNAi, Ribonucleic Acid interference; TALEN, Transcription Activator-Like Effector Nucleases; TCR, T Cell Receptor.

Adoptive transfer of genetically engineered T cells is a promising strategy in the fight against cancer. An increasing number of clinical trials show the high-potential of cancer immunotherapy using immune cells engineered to express tumor specific immune receptors, which most recently attracted interest from patients and “big pharma”. This is reflected by the impressive number of clinical trials currently recruiting patients for treatment with genetically modified T cells.¹ To further exploit this potent application of cancer immunotherapy, various possibilities may be considered.

In addition to defining the best immune receptor,¹ optimizing the composition of the engineered T cell graft is likely to contribute to the success of clinical outcome. Currently, the engineered T cell graft contains a very heterogeneous population of T cells that are engineered, caused (A) by the vast variety of CD3⁺ subsets in peripheral blood mononuclear cells, as well as (B) the fact that current protocols only redirect a fraction of immune cells. Thus, usually most clinical trials administer a very diverse product including many different immune cell subsets as well as engineered, poorly- and non-engineered immune cells.

The presence of multiple T cell subsets in an infused cell product can lead to dampening of the immune response by e.g., regulatory T cells, or in the context of an allogeneic stem cell transplantation (allo-SCT) induce graft vs. host disease (GvHD). In order to overcome such obstacles and to increase long-term memory, transfer of selected immune subsets has been proposed.² For example, in an elegant primate model Berger et al. demonstrated that central memory T cells, as defined by a CD62L⁺ phenotype, show an increased capacity to persist after adoptive transfer.³ However, in the context of an allo-SCT the very same subsets might be harmful. When the CD62L⁺ T cell population was depleted from the graft in mice, GvHD was significantly reduced.⁴ Therefore the right choice of subset does not only depend on the desired immunological phenotype of engineered immune cells, such as central memory T cells, but also on the context of clinical application. Cells appropriate for use in an autologous setting might be harmful when used for allo-SCT. In the context of allo-SCT, downregulation of endogenous receptors might be an additional important engineering step.⁵ Regardless of the desired subset, processing cell fractions in a good-manufacturing-practices (GMP) certified environment is usually cumbersome and expensive due to the fact that sequential isolation steps using multiple GMP-grade antibodies are necessary.

A second important step toward a more defined product is selecting immune cells with maximal receptor expression in order to reduce unwanted bystander activity by poorly- or non-engineered immune cells. At present, efforts to increase the purity of the engineered immune cells mainly utilize positive selection, which can result in unwanted activation of T cell subsets. Furthermore, this method is often based on the expression of an additional transgene like truncated CD19 or proteins like epidermal growth factor receptor which are normally absent in the hematological cell lineage.⁶ These strategies do not only interfere with the expression of the introduced immune receptor, which can be detrimental when affinity of used receptors is low (J. Kuball, unpublished observation), but more importantly also lead to immunogenicity, altered homing or the rejection of engineered immune cells.

Therefore, we propose in a recent issue of Clinical Cancer Research, a novel GMP-ready strategy to remove poorly- and non-engineered T cells from a cellular product based -in contrast to recent efforts- on negative selection.⁷ We demonstrate
that interference with endogenous αβTCRs combined with GMP-grade anti-αβTCR beads can provide highly purified untouched engineered immune cells without the additional need for selection markers. We used a tumor specific γδTCR 7,8 to naturally interfere with endogenous αβTCRs, a readily translatable strategy. A T cell editing technique such as RNAi, TALENs, Zinc Finger Nucleases or CRISPR/Cas9, to knock out the endogenous TCR chains, provides an alternative approach for the introduction of a tumor specific receptor lacking natural interference with endogenous αβTCRs. However, clinical translation of these techniques might still need some years. GMP-grade anti-αβTCR beads recently became available and are typically used in the context of haematopoietic stem cell transplantations by others 9 and us. 10

Furthermore, clinical devices for apheresis and magnetic cell sorting are well established in daily routine, therefore the combination of such techniques opens a new avenue toward the broader application of engineered immune cells in men with more purified products. This has the potential to significantly reduce “off-target” effects. In addition, we observed increased antitumor responses, both in vitro and in vivo, of the αβTCR depleted cells as compared to a bulk engineered cellular product. Even though our approach resulted in a gradual re-expression of the endogenous αβTCR over time, allo-reactivity remained absent and tumor control preserved. Our method can be applied to virtual any engineered immune product in which competition with endogenous αβTCRs takes place.

Choosing the most potent subset of T cells and increasing the purity of an engineered cellular product (Fig. 1) are two complementary strategies that can bring cancer immunotherapy to the next level. Key is to exploit readily available GMP-grade methods to reduce costs and time to clinical application, in order to democratize implementation.

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No potential conflicts of interest were disclosed.

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**Figure 1.** Choosing the most potent subset of T cells and increasing the purity of an engineered cellular product. The most potent subset of T cells will be selected from leukapheresis material, followed by the gene transfer of a tumor specific immune receptor, e.g., γδTCR and subsequent downregulation or additional knock-out of the endogenous αβTCR. Poorly- or non-transduced immune cells will be depleted from the cellular product before infusion in the patient.