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Engineering T cells for adoptive therapy: outsmarting the tumor

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Adoptive transfer of T cells gene-engineered with antigen-specific receptors, whether it be chimeric antigen receptors (CARs) or T cell receptors (TCRs), has proven its feasibility and therapeutic potential in the treatment of tumors. Despite clinical successes, the majority of patients experiences no or non-sustainable clearance of solid tumors, which is attributed to local T cell evasive mechanisms. A rapidly expanding understanding of molecular and cellular events that contribute to a reduction in numbers and/or activation of intra-tumor T cells has facilitated the development of gene-engineering strategies, enabling T cells to counter immune tolerance. Here, we present an overview of gene-engineering approaches and considerations to improve tumor-selectivity and effectiveness of adoptively transferred T cells.

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Engineering T cells to treat tumors: a short introduction

The concept to combat malignant disease by utilizing the patient's own immune system has established itself as an effective alternative and/or addition to treatments such as surgery, chemotherapy or radiotherapy. A promising and currently employed immune treatment is the adoptive transfer of T cells (AT) engineered with chimeric antigen receptors (CARs) or T cell receptors (TCRs). To this end, patient-derived T cells are equipped with receptors recognizing a given tumor antigen, redirecting them to selectively destroy cells expressing this antigen. While the use of CARs has shown impressive results in B cell leukemia's (ALL) with objective and complete responses (OR/CR) up to 94%, their current use in the treatment of solid tumors is failing to corroborate the same success (reviewed in [1]). TCR-engineered T cells have demonstrated clinical benefit in patients with multiple myeloma (OR: 80%; CR: 2%), metastatic melanoma (OR: 55%; CR: 6%) and metastatic synovial sarcoma (OR: 61%; CR: 20%) (reviewed in [2]). These clinical results notwithstanding, AT can be accompanied by therapy-related toxicities, generally related to recognition of target antigens or highly similar target antigens outside tumor tissue and overt T cell activation [1,2]. In addition, in particular when treating solid tumors, AT is generally marked by a large fraction of patients with no clinical response and, in case patients do respond, non-sustainability of responses. This suboptimal success rate coincides with limited accumulation and activation of T cells within tumors and poor persistence of these cells in the periphery [3,4]. In order to further enhance therapeutic efficacy, gene engineered T cells need to address two major challenges. First, receptors need to be selected that mediate effective and safe T cell responses; and second, local immune suppressive mechanisms need to be antagonized to ensure sufficient numbers and function of therapeutic T cells at the tumor site.

In this perspective, we will shortly touch upon selection of safe receptors to enable T cells to see tumor-specific antigens. Subsequently, we will zoom in on specific mechanisms employed by solid tumors to restrict accumulation and activation of intra-tumoral T cells, and outline gene-engineering approaches to overcome such T cell evasive mechanisms.

T cell engineering: safe antigen-specific receptors

When addressing therapeutic safety by choosing a sufficiently immunogenic target antigen, it is critical to verify that its expression is restricted to tumor cells, thus avoiding possible destruction of healthy tissues. Once an antigen is selected, antigen-binding moieties (as a source for CARs) or TCRs can be obtained through various immunization or molecular platforms. In case of TCRs, receptors need to undergo stringent efficacy and specificity assessment due to their intrinsic capacity of recognizing multiple, highly similar epitopes sharing an identical recognition motif. More detailed information on the selection and evaluation of tumor antigens and corresponding TCRs can be found in [5•]. Another strategy to address safety of AT entails incorporation of suicide genes into therapeutic T cells. This approach, although potentially valid in certain settings (reviewed in [6,7]) requires caution as it may not be compatible to the fast

kinetics of serious adverse events observed in clinical trials (reviewed in [2]).

High numbers of active, intra-tumoral T cells make all the difference

When shifting to therapeutic efficacy, there are several lines of evidence pointing to the importance of high numbers and activity of intra-tumoral CD8 T cells. First, tissues that harbor highly effective CD8 T cells, such as allo-rejected kidney transplants, show a distinctive signature of genes related to T cell trafficking and T cell effector functions [8]. Second, it has been well-recognized that a low number of immune effector cells, in particular CD8 T cells, inside tumors are associated with worse prognosis for various tumor types [9,10]. Moreover, absence of local T cell immunity, when captured from the expression of multiple genes that cover trafficking and activity of effector T cells, predicts no or low responsiveness to immune therapies in a pan-cancer setting [11••,12•]. Third, the predictive value of local T cell immunity may go beyond the value of markers of mere tumor antigenicity or mutational load [13–15].

How do tumors prevent accumulation and activation of CD8 T cells?

The tumor micro-environment poses barriers towards trafficking and intra-tumoral activation of T cells. The trafficking of T cells towards tissue normally starts with extravasation, which is often hampered by limited expression of T cell-specific adhesion molecules, co-stimulatory ligands or chemoattractants by endothelial cells in the setting of tumor tissue. Shut-down of T cell-specific chemoattractants may be linked to epigenetic silencing [16] or disruptive post-translational modifications [17,18]. Notably, tumor cell-derived molecules such as VEGF can induce expression of FAS-ligand (FASL), which can mediate killing of FAS-positive CD8 effector T cells [19]. Following extravasation, T cells normally migrate through supportive tissue, a process that is again hindered in the setting of tumor tissue, with prominent roles for cancer-associated fibroblasts and extracellular matrix (ECM) components, such as type I collagen [20,21].

Even in case of successful trafficking towards and within the tumor site, T cells will generally be confronted with areas of hypoxia. Although hypoxia provides a stimulus for neo-vascularization and breaking CD8 T cell tolerance to restore tissue homeostasis, sustained hypoxia is accompanied by immature vasculature, CD8 T cell evasion and tumor progression [22]. Amongst other mechanisms, hypoxia is reported to decrease CD8 T cell numbers via enhanced production of TGFβ1 by tumor cells as well as enhanced numbers of T regulatory cells (Tregs) and myeloid derived suppressor cells (MDSCs) [22–24]. In addition, tumor cells may reduce expression of co-stimulatory ligands, such as B7-1, B7-2 or B7-H2, enhance expression of co-inhibitory ligands, such as PD-L1 and PD-L2 [25], shield themselves with immune-suppressive chemoattractants, such as CXCL12 [26], or demonstrate enhanced necrotic cell death. A tumor micro-environment that is nutrient-poor and does not favor T cell co-stimulation may push intra-tumoral T cells into metabolic exhaustion [27••]. Collectively, deficiency in numbers and/or activation of immune effector cells, in particular Th1 cells, provides insufficient intra-tumoral inflammation and enables tumors to escape from CD8 T cells. Figure 1a depicts a selection of mechanisms that tumors employ to become immune tolerant.

Gene-engineering to enhance accumulation and activation of intra-tumoral CD8 T cells

To reinvigorate T cell responses, advanced gene-engineering strategies have been developed. These strategies are diverse in nature and depend on technical advancements (nicely reviewed in [6,7]). Here, we zoom into several gene-engineering strategies that enhance the trafficking and/or intra-tumoral activation of T cells, and which are illustrated in Figure 1b.

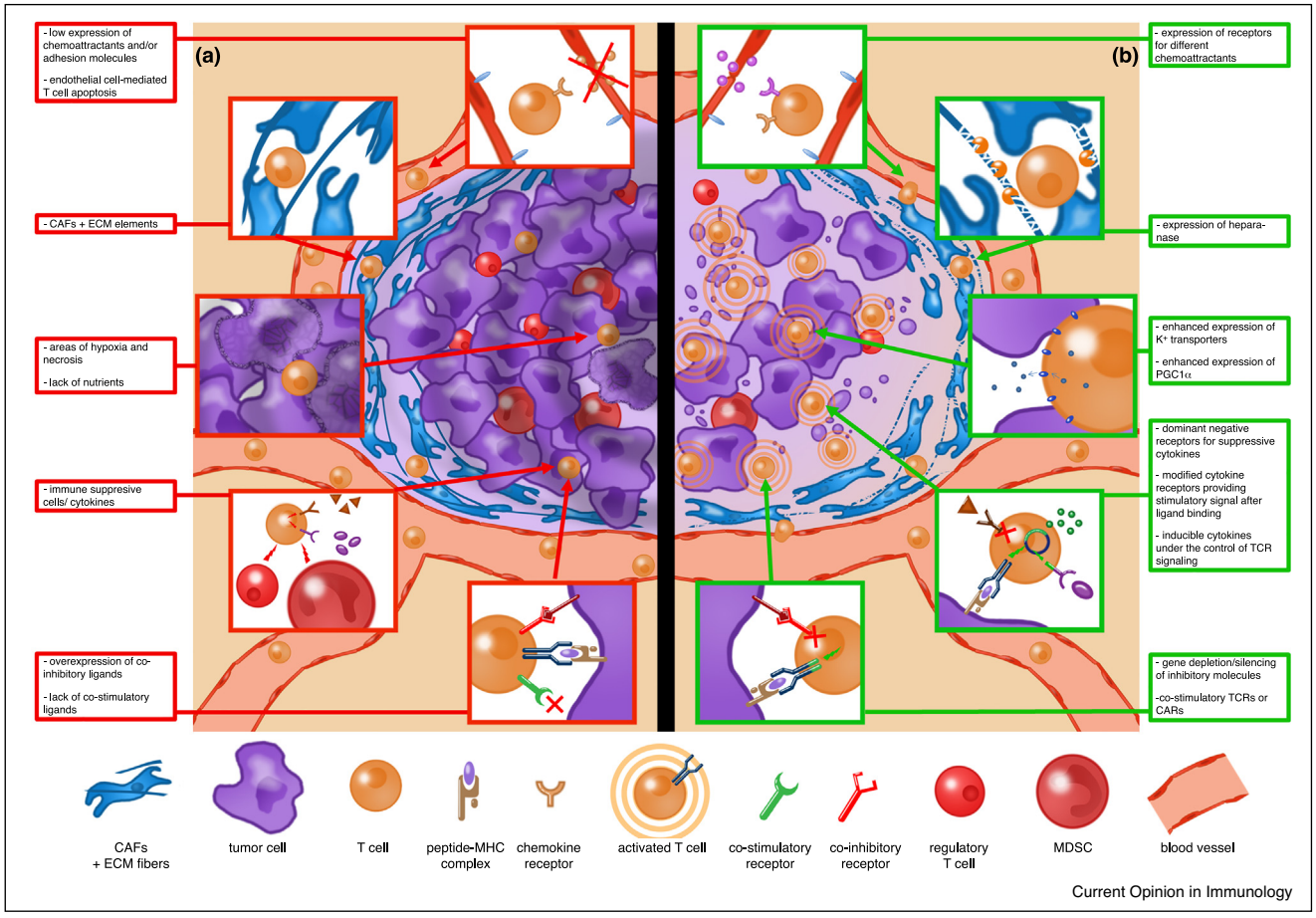
Gene-engineering addressing T cell trafficking

In order to enhance numbers of T cells, gene-engineering strategies have been used to improve T cell extravasation and migration towards and within tumors. One strategy relies on gene-transducing T cells with chemokine receptors, such as CXCR2, CCR2b and CX3CR1, where T cell recruitment and responsiveness towards various tumors in preclinical models improved [28–30]. Another strategy that enhances T cell extravasation and inhibition of tumor growth encompasses the targeting of the vasculature of established tumors via T cells gene-engineered with a CAR directed against VEGFR2 [31]. T cell migration through supportive tissue was shown to benefit from enforced expression of heparanase by T cells, facilitating the degradation of extracellular matrix (ECM) components, thereby contributing to tumor T cell infiltration and anti-tumor activity [32].

Gene-engineering addressing T cell activation

In order to enhance intra-tumoral activation of administered T cells, various approaches have been reported, including (but not limited to): enhancement of T cell co-stimulation; metabolic reprogramming of T cells; driving T cells into an inflammatory phenotype; and resistance of T cells towards tumor cell death. Addressing T cell co-stimulation, one straightforward approach would entail TALEN-mediated PD-1 gene inactivation, effectively blocking this negative feedback loop in therapeutic T cells [33•]. Along the same line, depletion of the negative regulators of T cell activation CBLB or adenosine 2A receptors using small interfering RNAs, resulted in enhanced anti-tumor efficacy of T cells [34,35]. To affect only those T cells that recognize antigen within tumors, CARs and TCRs have been developed that incorporate co-signaling molecules to provide T cell activation

Figure 1



Gene-engineering of T cells to treat immune tolerant tumors. **(a)** Mechanisms of T cell evasion employed by tumor tissues; **(b)** Gene-engineering approaches to make T cells cope with evasive mechanisms. (CAF = cancer associated fibroblast; ECM = extracellular matrix; MDSC = myeloid derived suppressor cells; MHC = major histocompatibility complex.)

without the need for co-stimulatory ligands. CARs equipped with signaling modules derived from CD28 or CD137 have already demonstrated clinical benefit in hematological malignancies [1], whereas CARs equipped with modules derived from CD278 demonstrated development of IFNγ$^+$/IL17$^+$ T cells with enhanced anti-tumor reactivity [36]. An alternative strategy involves co-transduction of T cells with a CD28-containing CAR and a CD137 ligand, resulting in enhanced therapeutic efficacy towards CD19-positive tumors [37]. With respect to TCRs, Govers and colleagues showed that incorporation of CD28 enhanced T cell responses directed against melanoma [38••]. Notably, when using TCRs that incorporate CD278 these anti-tumor responses became more pronounced and were accompanied by increased intra-tumoral accumulation and prolonged peripheral persistence of CD8 T cells (*Kunert, manuscript in preparation*). Intriguingly, T cells equipped with such co-stimulatory antigen-specific receptors are less prone to intra-tumoral metabolic exhaustion, showing enhanced mitochondrial biogenesis and oxidative phosphorylation [39••]. Notably, shifting metabolic pathways in T cells by enforced expression of PGC1α or OPA1, involved in mitochondrial biogenesis and fusion, respectively, resulted in enhanced anti-tumor responses and T cell persistence [27••,40•]. Besides metabolic constraints, intra-tumoral T cells also face suppression through release of potassium ions by dying, necrotic tumor cells, often present in developing tumors. Genetic introduction of the potassium channel $K_v1.3$ in T cells, and their adoptive transfer into melanoma-bearing mice, resulted in improved rejection of tumors [41].

T cell responsiveness towards immune-suppressive cytokines, such as TGFβ1, may be targeted through the introduction of DNRII into T cells, a dominant-negative receptor for this cytokine [42]. Another example includes transduction of therapeutic T cells with a modified IL-4 receptor, equipped with an IL-7 signaling domain, thus conveying tumor-generated and immune-suppressive IL-

4 into an activation signal [43]. As systemic administration of immune modulatory agents, such as inflammatory cytokines, is often limited due to overt impact on immunity, approaches that enable release at the tumor site are of particular interest. To this end, CAR or TCR T cells have been developed that harbor constructs from which IL12 or IL18 are expressed under the control of nuclear factor of activated T cells (NFAT). These were shown to mediate improved anti-tumor effects upon administration to mice [44,45•]. T cells with inducible IL12 were observed to yield toxicities both in mice and patients with melanoma [45•,46]. In contrast, T cells with inducible IL18 showed clear anti-tumor effects which were accompanied by enriched numbers of intra-tumoral CD8 T cells when treating melanoma-bearing mice [45•], a polarization towards Th1 cells and presence of inflammatory immune cells when treating pancreatic cancer or lung cancer-bearing mice [47•] without detectable side effects. Interestingly, in the latter study, IL18, combined with a co-stimulatory CD28-containing CAR, resulted in potent T cell skewing towards T-bet^{hi}, FoxO1^{lo} cells, possibly enabling T cells to resist metabolic exhaustion effector differentiation. For an overview of gene-engineering strategies to boost T cell trafficking and intra-tumoral activation, please refer to Table 1.

Non-gene engineering strategies

Although this perspective focuses primarily on approaches to counter CD8 T cell evasion by gene-engineering, it needs mentioning that a myriad of non-engineering approaches is currently also under investigation. Examples include: antibodies against checkpoints; depletion of MDSC [48], or inhibitors of IDO1 to prevent tryptophan shortage [49], CXCR4 to prevent cancer cell shielding [26] or PTPN2 to override blockade of IFN response pathways [50••]. Interestingly, exposing gene-engineered T cells prior to AT to inhibitors of FAS or AKT signaling pathways has been shown to preserve the pool of naïve and early memory T cells and enhance *in vivo* persistence and anti-tumor response [51].

As our understanding of the mechanisms that govern immune escape grow, new possible targets for gene-engineering and none-gene-engineering approaches emerge. For example, antigen presentation and/or IFN response pathways are considered critical for effective T cell responses as evidenced by mutations in β2-microglobulin and JAK1/2 that associate with resistance to checkpoint blockade and potentially other immune therapies [52,53]. Similarly, oncogenic signaling pathways, such as β-catenin and type I IFN signaling, have been recognized for their contributions to CD8 T cell evasion [54,55].

Future perspective of gene-engineering: a new avenue of combination treatment

As detailed above, engineering T cells with antigen-specific CARs or TCRs enables T cells to target tumor cells with high specificity and sufficiently high avidity towards a given antigen. While this measure equips T cells with the potential to 'see' tumor cells, selection of additional gene-engineering approaches is considered an effective next step to ensure sufficient numbers and activation of intra-tumoral T cells. Technical advancements, such as the synthetic Notch (synNotch) platform that dynamically enables conditional expression of receptors or molecules [56], are expected to further facilitate the therapeutic implementation of gene-engineering of antigen-specific T cells. With an increased understanding of how tumor type, patient characteristics and choice of therapy affect disease progression and immune evasion, it is necessary to not only generate patient-tailored and disease-tailored gene-engineered T cells. Rather, it is also important to refine methods to detect immune suppressive mechanisms, such as immune genomic tools to interrogate Next Generation Sequencing (NGS) data and multiplex analysis of cellular and molecular markers in tissues and blood. Information on presence of tumor-selective target antigens, in combination with dominant immune escape mechanisms, is expected to guide optimal combination therapies [10].

Table 1

Gene-engineering strategies to overcome selected shortcomings of T cell immunity[a]

Category of T cell evasion	Gene-engineering strategies to counter T cell evasion	Refs.
T cell trafficking	Expression of chemoattractant receptor	[28–30]
	Expression of endothelial growth factor receptor	[31]
	Secretion of ECM-degrading enzyme	[32]
T cell activation	Depletion/silencing of inhibitory receptor	[33•,34,35]
	Expression of co-stimulatory ligand	[37]
	Expression of co-stimulatory CAR/TCR	[1,36,38••]
	Expression of molecules that enhance mitochondrial activity	[27••,40•]
	Expression of ionic transport channel	[41]
	Expression of modified receptors for suppressive cytokine	[42,43]
	Inducible secretion of inflammatory cytokine	[45•,47•]

[a] Gene-engineering strategies to overcome a selected shortcoming may have beneficial effects towards other shortcomings as well since T cell properties, such as co-stimulation, metabolism and inflammation, may share common cellular and molecular pathways (see text for details).

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