

Cellular Therapy with Engineered T Cells, Efficacy, and Side Effects: Gene Editing/Gene Therapy

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60.1 Introduction

The cellular basis of cancer immune surveillance, already hypothesized in ancient times, was only proven with the advent of HCT. Indeed, the discovery of the nature of GVHD and its antileukemic effects (Weiden et al. 1979) was followed by the first successful attempts of adoptive immunotherapy using donor leukocytes (Kolb et al. 1990). To address the significant GVHD risk associated with allogeneic T cells, several

approaches of T-cell manipulation were developed and tested (Table 60.1). Some of these strategies rely on the genetic manipulation of T cells. First, suicide gene therapy approaches were established to promote GVL and immune reconstitution while controlling GVHD. More recently, strategies based on the genetic transfer of tumor-specific T-cell receptors (TCRs) or chimeric antigen receptors (CARs) were developed to improve antitumor efficiency of T cells. This chapter provides an overview of this vastly evolving area.

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Table 60.1 T-cell-based cellular therapy approaches to increase GVL/GVI while taming GVHD

Strategy	Mechanism of action	References
Infusions of pathogen (i.e., CMV, EBV)-specific T cells	Isolation and infusion of T cells specific for opportunistic pathogens to control posttransplant infectious morbidity and mortality	Riddell et al. (1992), Rooney et al. (1995), Koehne et al. (2003)
Infusions of T cells depleted of alloreactive specificities	In vitro activation of host-reactive T cells followed by their depletion, infusion of remaining cells with the aim of promoting immune reconstitution with a reduced GVHD risk	André-Schmutz et al. (2002), Hartwig et al. (2008), Mielke et al. (2008)
Infusions of regulatory T cells	Isolation and/or expansion T-cell subsets with regulatory properties to promote immune reconstitution with a reduced GVHD risk	Groux et al. (1997), Chen et al. (2003), Trenado et al. (2004), Brunstein et al. (2011), Di Ianni et al. (2011), Bacchetta et al. (2014)
Infusion of T cells depleted of regulatory T cells	Infusion of T cells depleted of regulatory T cells to increase the antileukemic activity of DLI	Maury et al. (2010)
Infusions of leukemia-specific T cells	Isolation and infusion of T cells specific for leukemia-associated antigens to boost the GVL potency of DLI	Warren et al. (2010), Bornhäuser et al. (2011), Chapuis et al. (2013), Comoli et al. (2017)
Infusion of alpha-/beta-depleted T cells	Infusion of a graft in vitro depleted of conventional alpha/beta T cells, thus enriched of gamma-delta T cells, endowed with antitumor activity and a low GVHD potential	Lang et al. (2015), Airolidi et al. (2015), Maschan et al. (2016)
Infusion naïve-depleted T cells	Infusion of donor T-cell subsets in vitro depleted of naïve cells, with the aim of promoting immune reconstitution with a reduced GVHD risk	Bleakley (2015)
Infusions of CIK	Infusions of in vitro activated donor CIK cells to promote GVL and reduce the risk of GVHD	Introna (2007), Introna et al. (2010)
Suicide gene therapy	Donor lymphocytes are genetically engineered to express a suicide gene and then infused after HCT to promote GVT and immune reconstitution while selectively controlling GVHD with the prodrug-mediated activation of the suicide gene	Bonini et al. (1997), Ciceri et al. (2009), Fehse et al. (2004), Di Stasi et al. (2011), Zhan et al. (2013), Oliveira et al. (2015)
CAR/TCR T cells	Lymphocytes are genetically engineered to express a chimeric antigen receptor (CAR) or a T-cell receptor (TCR) that confers to T cells specificity for an antigen expressed by cancer cells	Kochenderfer et al. (2010), Porter et al. (2011), Brentjens et al. (2013), Morgan et al. (2006), Robbins et al. (2011)

CIK cytokine-induced killer

60.2 Suicide Gene Therapy

The transfer of a suicide gene into donor lymphocytes was designed and tested at preclinical and clinical level in the 1990s, with the aim of transferring the entire donor T-cell repertoire, inclusive of cancer and infectious specificities, to transplanted patients while enabling the selective elimination of the transferred lymphocytes in case of GVHD (Bonini et al. 1997). The first suicide gene, and to date the most extensively tested in clinical trials, is thymidine kinase of herpes simplex virus (HSV-TK). In dividing cells, HSV-TK expression confers selective sen-

sitivity to the antiviral drug ganciclovir. Upon retroviral gene transfer, HSV-TK is stably expressed by donor T lymphocytes not interfering with their functionality. However, when exposed to ganciclovir, highly proliferating HSV-TK-expressing T cells (TK cells) will die in a dose-dependent manner. Thus, if ganciclovir is administered during GVHD to patients treated with TK cells, activated and thus proliferating alloreactive TK cells will be eliminated. The HSV-TK/ganciclovir suicide system proved highly effective in controlling GVHD in several transplant settings, including haploidentical HCT (haplo-HCT). After T-cell-depleted haplo-

HCT, the infusion of TK cells promoted broad and rapid immune reconstitution, which, being associated with GVHD control, led to abrogation of late transplant-related mortality (Ciceri et al. 2009). Overall, clinical results obtained with TK cells led to their conditional approval by EMA in 2016, thus representing the first genetically engineered medicinal product approved for cancer patients in Europe. Although when infused after haplo-HCT TK cells could be detected for more than 14 years (Oliveira et al. 2015), their persistence might be limited when cells are infused to immunocompetent patients, due to the viral origin of HSV-TK and to its subsequent immunogenicity in humans. Alternative suicide genes were designed and tested in clinical trials. iCasp9, in particular, is an innovative suicide gene based on human components and thus with a reduced risk of immunogenicity, which was recently proposed and successfully tested in clinical trials (Di Stasi et al. 2011; Zhou et al. 2014). Overall, more than half of the patients who had received suicide gene-expressing donor T cells experienced a clinical benefit in terms of immune reconstitution and GVL. Of notice, all cases of GVHD were completely controlled by the suicide gene/prodrug systems.

60.3 CAR-T Cells

60.3.1 CAR-T-Cell Clinical Efficacy

CARs are designer molecules comprised of several components: an extracellular antigen-binding domain, usually the variable light and heavy chains of a monoclonal antibody (scFv), a spacer and transmembrane region that anchors the receptor on the T-cell surface and provides the reach and flexibility necessary to bind to the target epitope, and an intracellular signaling module, most commonly CD3 zeta and one or more costimulatory domains that mediate T-cell activation after antigen binding, resulting in their profound proliferation and eventually selective tumor cell killing.

The first clinical development was the use of CARs specific for the B-lineage marker CD19.

Several groups demonstrated that CD19 CAR-T cells are able to induce durable complete remissions in patients with chemotherapy- and radiotherapy-refractory B-cell ALL, NHL, and CLL (Maude et al. 2014; Park et al. 2018; Turtle et al. 2017).

However, in a fraction of patients, resistance mechanisms to CD19 CAR-T-cell therapy have become apparent, including the development of leukemia cell variants that lost their CD19 antigen expression, particularly in ALL. Several mechanisms may contribute to the development of this phenotype including lymphoid-to-myeloid transdifferentiation, selection of preexisting CD19-low/CD19-negative leukemia clones, and emergence of clones that lost the specific epitope targeted by the CD19 CAR due to alternative splicing (Gardner et al. 2016; Sotillo et al. 2015; Ruella and June 2016). In ALL, CD19-low/CD19-negative leukemia cells may still express CD20, CD22, and/or CD123 that are being pursued as rescue antigens. A recent study highlighted the potential to re-induce remissions in patients that had relapsed with CD19-low/CD19-negative leukemia and subsequently received CD22 CAR-T cells (Fry et al. 2018). Unfortunately, CD22 itself is prone to internalization and downregulation, and indeed a significant proportion of patients experienced successive CD22-low/CD22-negative leukemia relapse. At present, combinatorial targeting of CD19 with either CD20, CD22, or CD123 is being explored, either through bi-specific CAR constructs with two scFvs *in cis* or through co-expression of two CAR constructs in the same T cells (Zah et al. 2016).

Since 2017, outcomes of CAR-T-cell clinical trials (Table 60.2) led to the approval by FDA and EMA of six CAR-T-cell therapeutic products, including four specific for CD19, approved for B-cell lymphoma and acute lymphoblastic leukemia (<https://doi.org/10.1056/NEJMoa1707447>, <https://doi.org/10.1056/NEJMoa1804980>, [https://doi.org/10.1016/S0140-6736\(20\)31366-0](https://doi.org/10.1016/S0140-6736(20)31366-0), <https://doi.org/10.1056/NEJMoa1914347>), and two directed to BCMA and used for multiple myeloma ([https://doi.org/10.1016/s0140-6736\(21\)00933-8](https://doi.org/10.1016/s0140-6736(21)00933-8), <https://doi.org/10.1056/NEJMoa2024850>). All

Table 60.2 First clinical trials with CAR-T cells

Clinical application	Antigen	No. of patients	Clinical response	Toxicity	No. of T cells infused/kg	CAR design	Gene transfer vector	Safety technology	References
ALL	CD19	30	90% CR, 79% MRD	B-cell aplasia CRS Neurotoxicity	$0.76\text{--}20 \times 10^6/\text{kg}$ BW	FMC63 scFv CD8 alpha spacer 4-1BB costim	<i>Lentivirus</i>	None	Maude et al. (2014)
ALL	CD19	16	88% CR, 75% MRD	B-cell aplasia CRS Neurotoxicity	$3 \times 10^6/\text{kg}$ BW	SJ25C1 scFv CD28 ECD spacer CD28 costim	γ -Retrovirus	None	Davila et al. (2014)
ALL	CD19	29	93% CR, 86% MRD	B-cell aplasia CRS Neurotoxicity	$2 \times 10^5\text{--}2 \times 10^7/\text{kg}$ BW	FMC63 scFv IgG4 Hinge spacer 4-1BB costim	<i>Lentivirus</i>	EGFRt depletion marker	Turtle et al. (2016)
NHL/CLL	CD19	15	53% CR, 26% PR	B-cell aplasia CRS Neurotoxicity	$1\text{--}5 \times 10^6/\text{kg}$ BW	FMC63 scFv CD28 ECD spacer CD28 costim	γ -Retrovirus	None	Kochenderfer et al. (2015)
NHL/CLL	CD19	32	50% CR, 72% ORR	B-cell aplasia CRS Neurotoxicity	$2 \times 10^5\text{--}2 \times 10^7/\text{kg}$ BW	FMC63 scFv IgG4 Hinge spacer 4-1BB costim	Lentivirus	EGFRt depletion marker	Turtle et al. (2016)
CLL	CD19	20	21% CR, 53% PR	B-cell aplasia CRS Neurotoxicity	$2 \times 10^5\text{--}2 \times 10^7/\text{kg}$ BW	FMC63 scFv IgG4 Hinge spacer 4-1BB costim	Lentivirus	EGFRt depletion marker	Turtle et al. (2017)
MM	CD19	10	1CR, 2PR	B-cell aplasia CRS (mild)	$1\text{--}5 \times 10^7$ (total)	FMC63 scFv CD8 alpha spacer 4-1BB costim	Lentivirus	None	Garfall et al. (2015)
MM	BCMA	12	1 CR, 1PR, 2 VGPR	Hematologic (cytopenia) CRS Neurotoxicity	$0.3\text{--}3 \times 10^6/\text{kg}$ BW	C11D5.3 scFv CD28 ECD spacer CD28 costim	γ -Retrovirus	None	Ali et al. (2016)

ALL acute lymphoblastic leukemia, NHL non-Hodgkin lymphoma, CLL chronic lymphocytic leukemia, MM multiple myeloma, BCMA B-cell maturation antigen, CR complete remission, MRD minimal residual disease, PR partial remission, VGPR very good partial remission, CRS cytokine release syndrome, kg kilogram, BW body weight, scFv single-chain variable fragment, costim costimulatory domain, IgG immunoglobulin G, ECD extracellular domain, EGFRt, epidermal growth factor receptor

approved products are manufactured by retroviral (γ -retroviral or lentiviral) gene transfer and made headlines due to their considerable market price and the complex logistics behind this treatment. This involves harvesting the patient's T cells at a leukapheresis center, shipping to a centralized manufacturing facility to perform CAR gene transfer and T-cell expansion, and return shipment of the cryopreserved cell product. There is a recent increase in the use of exportable, GMP-certified manufacturing devices that are anticipated to provide on-site, point-of-care CAR-T-cell manufacture to reduce costs and wait time. Currently, approved cellular products are tested as earlier lines of treatment, and an intense effort is ongoing toward the identification of new CAR targets for further hematological malignancies, e.g., SLAMF7 (Gogishvili et al. 2017), CD44v6 (Casucci et al. 2013), CD38 (Mihara et al. 2009), CD33, CD70, CD123, CLL-1, and FLT3 (<https://doi.org/10.1111/ejh.14047>). Clinical progress of CAR-T cells for solid tumors has been lacking behind, since it meets—besides identification of suitable target antigens—several additional challenges, including but not limited to difficult accessibility, immunosuppressive tumor microenvironment, and high degrees of heterogeneity. Novel CAR constructs and treatment concepts are underway to address these difficulties (<https://doi.org/10.1038/s41586-023-05707-3>).

60.3.2 Side Effects and Their Management

Results from pioneering clinical studies investigating CAR-T cells in patients with hematological cancers highlight the frequent occurrence of severe adverse reactions, which in some cases were fatal. The most obvious toxicity by CAR-T cells is the elimination of lineage cells expressing the target antigen of choice. For example, profound and, in some cases, long-lasting B-cell aplasia was observed after the infusion of CD19 CAR-T cells in patients with ALL, NHL, and CLL (Maude et al. 2014; Park et al. 2018; Turtle et al. 2017). By analogy, BCMA CAR-T cells

are expected to induce plasma cell ablation in MM patients. The depletion of antibody-producing cells, or their precursors, in turn causes hypogammaglobulinemia, requiring constant supplementation with immunoglobulins. Besides these expected on-target/off-tumor effects, a new class of on-target/on-tumor adverse reactions is represented by the cytokine release syndrome (CRS) and by neurotoxicity. CRS is initiated by CAR-T-cell recognition of tumor cells, igniting the release of massive amounts of inflammatory cytokines, possibly by recruiting cells of the innate immunity. A master cytokine of the CRS is IL-6, as demonstrated by prompt and often complete response to the anti-IL-6 receptor monoclonal antibody tocilizumab. CRS symptoms range from high fever, headache, and myalgia to life-threatening cardiocirculatory and renal insufficiency. Clinical data reported so far utilize three slightly different systems for severity grading, which makes it difficult to draw meaningful comparisons in CRS liability between CAR-T-cell trials (Table 60.3). Nonetheless, there is a generalized consensus on the fact that severe CRS is more frequent in ALL compared to NHL and that high tumor burden is an important risk factor. Different from CRS, the pathophysiology of neurotoxicity by CAR-T cells remains an uncharted territory and decisively worthy of further research, given its highly dismal prognosis, as demonstrated by several cases of lethal cerebral edema (Berger et al. 2023). Initially thought to be caused by tumor recognition by CAR-T cells within the brain, neurotoxicity is now recognized to be independent from leukemic localization to the CNS. Moreover, unresponsiveness to tocilizumab suggests that excessive IL-6 signaling may not be sufficient to explain neurotoxicity and that additional pharmacological measures should be investigated. Finally, in a substantial proportion of patient, profound and in some cases very long-lasting leukopenia associated with high rates of infectious complications was reported after CD19 CAR-T therapy and referred to as CAR-HEMATOTOX (Rejeski et al. 2021).

Table 60.3 CRS severity scoring systems

	Penn scale	CTCAE	Lee et al. (2014)
Grade 1	Mild reaction treated with antipyretics and/or antiemetics	Mild reaction, no treatment needed	Non-life-threatening reaction responsive to symptomatic treatment
Grade 2	Moderate reaction requiring hospitalization and i.v. therapy (no fluid resuscitation)	Moderate reaction responsive to symptomatic treatment within 24 h	Moderate reaction requiring oxygen <40%, fluid resuscitation or low-dose pressors. Any G2 organ toxicity
Grade 3	Severe reaction requiring high-flow oxygen or noninvasive lung ventilation, fluid resuscitation, or low-dose pressors	Prolonged reaction nonresponsive to symptomatic treatment	Severe reaction requiring oxygen >40%, high-dose pressors. Any G3 organ toxicity
Grade 4	Life-threatening reaction requiring high-dose pressors and/or mechanical ventilation	Life-threatening reaction, pressor, or ventilator requirement	Life-threatening reaction requiring mechanical ventilation. Any G4 organ toxicity

60.4 TCR Gene Transfer, TCR Gene Editing, and Future Perspectives

In contrast to CARs that only bind surface molecules, TCRs recognize small pieces (peptides) derived from any cellular protein and presented by MHC molecules. Since the vast majority of tumor-specific/tumor-associated antigens are expressed intracellularly, they will only be addressable by TCRs, but not CARs. Moreover, therapeutically relevant cancer driver mutations in most cases happen in intracellular proteins (e.g., signal transducers).

At the same time, the advantage of TCRs represents a major hurdle for broad clinical application: Any transgenic TCR only functions in the context of one specific HLA complex. Thus, in order to offer TCR-T-cell therapy to virtually all candidate patients, for each antigen, a whole set of active TCRs will have to be established for different HLA molecules.

The first TCR gene therapies were applied to melanoma patients (MART-1 antigen), but meanwhile many cancers have been addressed. Based on their almost complete absence in adult tissues, cancer/testis antigens (e.g., NY-ESO1) represent particularly promising targets. Many studies showed significant antitumor activity, but on-target and off-target activities were associated with severe side effects, including mortality (Morris and Stauss 2016).

Genome editing has been proposed to improve efficacy and decrease side effects of TCR gene

therapy. Editing might be used to knock out the endogenous TCR to increasing expression of the transgenic one and decreasing the mispairing risk between endogenous and transgenic TCR chains (potentially leading to autoreactive T cells) (Provasi et al. 2012), and is required for the development of allogeneic T-cell products (<https://doi.org/10.1126/scitranslmed.aaj2013>). The recent development of CRISPR/Cas9 and its application to T cells have significantly increased the efficiency of gene disruption and of homology-directed repair (HDR) while also permitting simultaneous multiple gene editing (<https://doi.org/10.1126/scitranslmed.abg8027>). TCR-edited T cells were proven safe in a pilot study on cancer patients (<https://doi.org/10.1126/science.aba7365>). Moreover, targeted integration in the TCR locus can improve long-term expression of transgenic TCRs (<https://doi.org/10.1038/s41551-019-0409-0>) or CARs (Eyquem et al. 2017). Finally, replacing nuclease activity with alternative enzymes, such as deaminases for nucleotide conversion, is further improving our toolbox for T-cell engineering (<https://doi.org/10.1056/NEJMoa2300709>).

In conclusion, T-cell therapies have become a promising novel anticancer weapon. Their broad application will require (1) identification of additional targets, (2) availability of TCRs against established targets for many HLA molecules, (3) implementation of innovative gene transfer and genome-editing biotechnological tools, and (4) improved methods for large-scale GMP production.

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