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REVIEW ARTICLE

Safety and Tolerability of Adoptive Cell Therapy in Cancer

Benita Wolf[1] · Stefan Zimmermann[1] · Caroline Arber[1] · Melita Irving[1] · Lionel Trueb[1] · George Coukos[1]

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Abstract
Adoptive T cell therapy (ACT) is a safe and effective personalized cancer immunotherapy that can comprise naturally occurring ex vivo expanded cells (e.g., tumor-infiltrating lymphocytes [TIL]) or T cells genetically engineered to confer antigen specificity (T-cell receptor [TCR] or chimeric antigen receptor [CAR] engineered T cells) to mediate cancer rejection. In recent years, some ACTs have produced unprecedented breakthrough responses: TIL therapy has moved from melanoma to solid tumor applications, TCR-engineered cells are developed for hematologic and solid tumors, and CAR-engineered T cells have received Food and Drug Administration (FDA) approval for the treatment of patients with certain B-cell malignancies. Although results are encouraging, to date, only a small percentage of patients with advanced malignancies can benefit from ACT. Besides ACT availability and accessibility, treatment-related toxicities represent a major hurdle in the widespread implementation of this therapeutic modality. The large variety of observed toxicities is caused by the infused cell product or as side effects of accompanying medication and chemotherapy. Toxicities can occur immediately or can be delayed. In order to render those highly promising therapeutic approaches safe enough for a wider pool of patients outside of clinical trials, an international consensus for toxicity management needs to be established.

Key Points

Our work provides an overview of important toxicities of adoptive cellular immunotherapy.

We discuss the concepts of tumor infiltrating lymphocyte (TIL), T-cell receptor (TCR), and chimeric antigen receptor (CAR) adoptive T-cell therapy (ACT).

We discuss toxicity pathomechanisms and review up-to-date treatment strategies.

Part of a theme issue on "Safety of Novel Anticancer Therapies: Future Perspectives". Guest Editors: Rashmi R Shah, Giuseppe Curigliano.

✉ George Coukos
George.Coukos@chuv.ch

[1] Department of Oncology, Lausanne University Hospital, and Ludwig Institute for Cancer Research, University of Lausanne, 1066 Lausanne, Switzerland

1 Introduction

T cells harbor great potential to treat cancer [1]. So-called adoptive cell immunotherapy (ACT) uses T cells isolated from patients' tumors (tumor-infiltrating lymphocytes or TILs) or genetically engineered with T-cell receptors (TCRs) or chimeric antigen receptors (CARs) [2].

TIL therapy employs naturally occurring T cells and has been established over decades with very promising results largely in melanoma [3, 4], but also in ovarian cancer [5–7] and colorectal cancer [8, 9]. In contrast to TILs, gene transfer-based T-cell therapy strategies have been developed to confer new target specificity to peripheral blood T cells (Fig. 1), and new generations of CARs provide increased functionality to overcome tumor-specific immune tolerance. TCRs recognize peptides derived from tumor-associated antigens (TAAs) presented in the context of human leukocyte antigen (MHC)-restricted antigen peptides [2, 10, 11]. CARs are antibody recognition domains linked to TCR and other costimulatory signaling molecules (Fig. 2b) [12–14]. Both have been shown effective in refractory tumors [10, 15].

For all three ACT approaches discussed in this review, T cells are expanded ex vivo and re-infused in large numbers into a lymphodepleted cancer patient (Fig. 1)

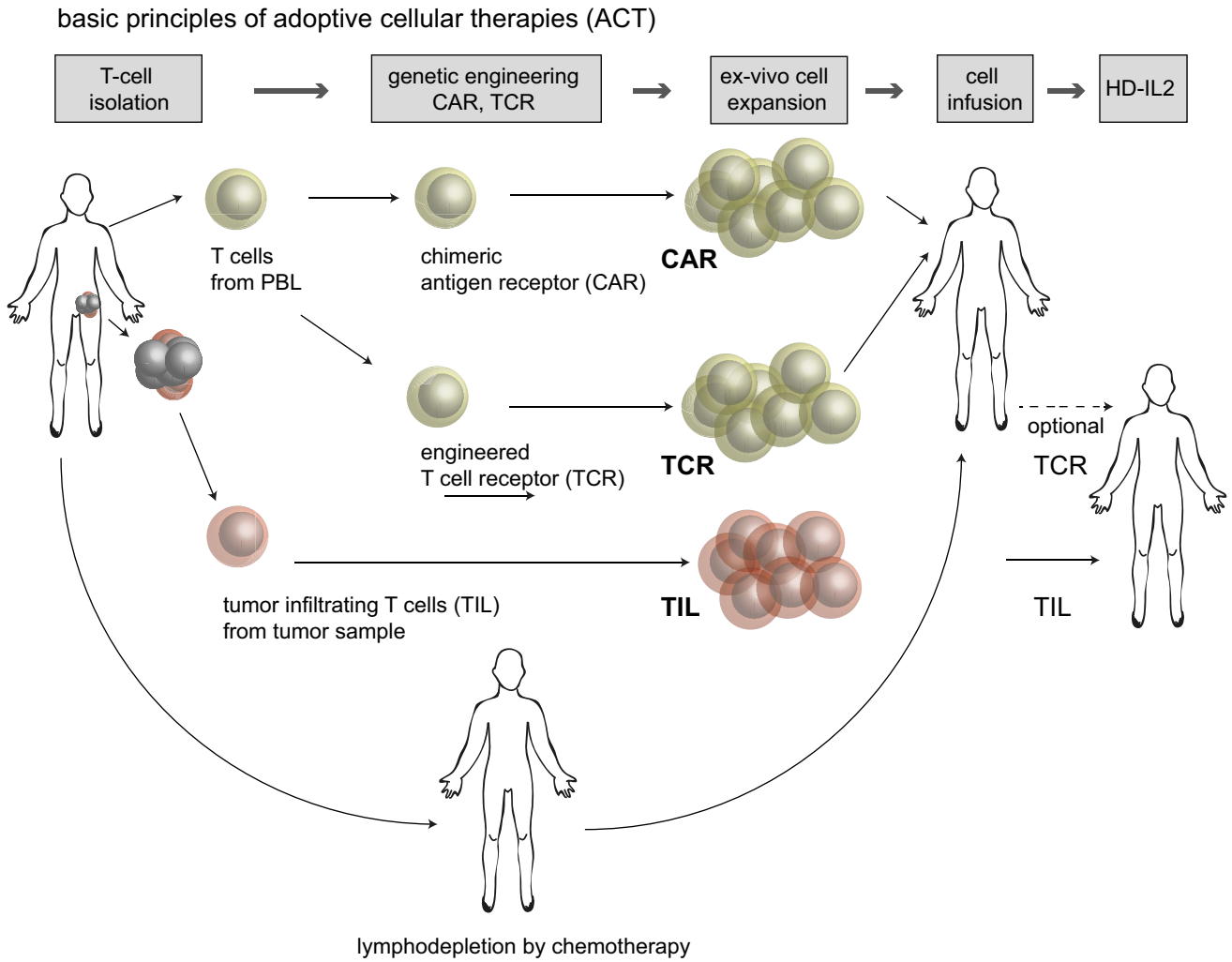

Fig. 1 Basic principles of adoptive cellular immunotherapy. Please note that high-dose IL-2 is administered in TIL ACT and administration is optional in TCR T-cell ACT. Lymphodepletive chemotherapy consisting of cyclophosphamide and fludarabine is administered in TIL and TCR-T ACT and optional in CAR-T ACT. *ACT* Adoptive T-cell therapy, *CAR* chimeric antigen receptor, *HD-IL-2* high-dose interleukin 2, *PBL* peripheral blood lymphocyte, *TCR* T-cell receptor, *TIL* tumor-infiltrating lymphocytes

[2], although TCR-ACT has been effective without lymphodepletion as well [16]. Lymphodepleting chemotherapy before TIL infusion reduces regulatory T cells (Treg) and resident tumor microenvironment cells competing for T-cell homeostatic cytokines, increases the levels of Toll-like receptor ligands [17, 18], and favors the proliferation of the infused T cells through homeostatic expansion. This translates into a markedly improved T-cell survival and response rate and duration in melanoma [19, 20].

After treatment with TIL and in most TCR-ACT clinical trials, patients receive high-dose interleukin 2 (IL-2) in order to bolster T-cell division expansion within the host (Fig. 1).

TIL and engineered T cells (TCR and CAR) are currently applied mainly within clinical trials at highly specialized centers. The landscape is nonetheless rapidly evolving. In August 2017, the FDA approved the first anti-CD19 CAR T-cell product, tisagenlecleucel (Kymriah, Novartis, Basel, Switzerland), for the treatment of pediatric and young adult patients with relapsed and/or refractory B-cell precursor acute lymphoblastic leukemia [21]. In October 2017, a second anti-CD19 CAR, axicabtagene ciloleucel (Yescarta, Kite Pharma, Santa Monica, CA, USA), was approved by the FDA for the treatment of adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy. Both products received approval in Europe in 2018. Despite great promise and rapid development of ACT, treatment-related toxicities remain an important issue. Preventing or managing unwanted toxicity has therefore emerged as a key component in the successful clinical application of these technologies. This article will review the treatment principles and toxicities of the three most prominent classes of ACT: TILs, TCR-engineered T cells, and CAR T cells.

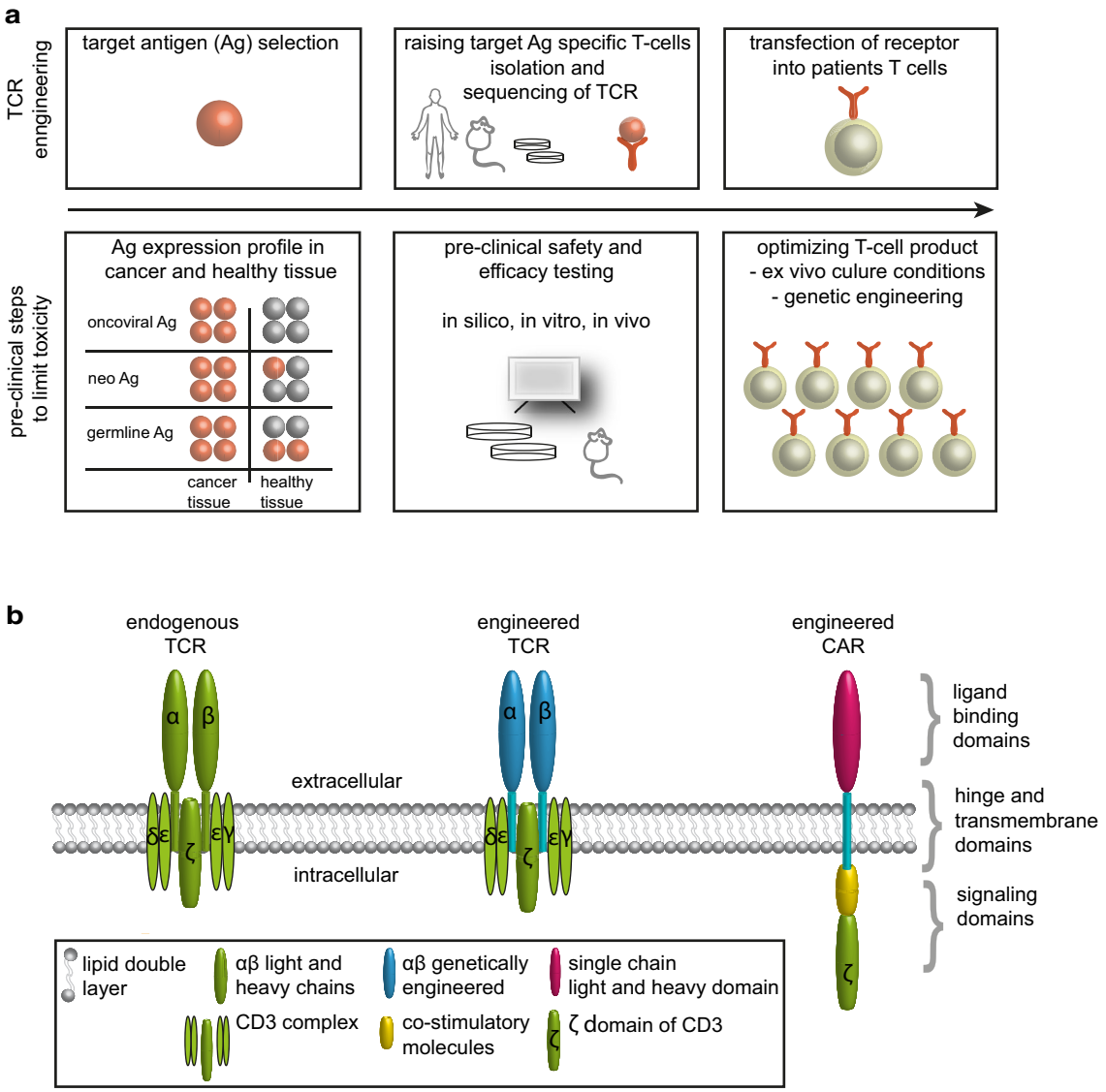

Fig. 2 TCR and CAR ACT. **a** Schematic overview over the process of T-cell receptor engineering for TCR ACT. The upper row of boxes describes the steps in the engineering process; the boxes below describe safety measures that are applied in parallel in order to limit clinical toxicity. Adapted from Kapanen et al. 2015 [130]. **b** Schematic of components of endogenous TCR, genetically engineered TCR and CAR. Adapted from June et al. 2015 [13]. *ACT* adoptive cellular therapy, *CAR* chimeric antigen receptor, *TCR* T-cell receptor

2 Tumor-Infiltrating Lymphocytes (TILs)

2.1 Treatment Principle

TIL therapy consists of the administration of autologous ex vivo expanded T cells that naturally infiltrate tumors. Figure 1 illustrates the general sequence of TIL ACT: following surgical resection of a suitable lesion, TILs are isolated, cultured and expanded ex vivo in the presence of IL-2 to generate a T-cell product of predominantly T-effector-memory cells [22]. Before TIL reinfusion, patients undergo lymphodepleting chemotherapy. High doses of recombinant IL-2 are administered after TIL reinfusion to facilitate TIL expansion and engraftment in vivo. Response rates appear higher in patients treated with minimally cultured 'young' TIL that retain a higher proliferative potential and a higher lytic activity [23].

Most lymphodepleting regimens consist of fludarabine and cyclophosphamide; as an example, the NIH Surgery Branch regimen combines 5 days of fludarabine 25 mg/m^2 (D-7 to D-3) and 2 days of cyclophosphamide 60 mg/kg (D-7, D-6; Fig. 1) [9]. After completion of TIL infusion, patients are treated with IL-2, usually administered as bolus infusions; the largest dataset originates from high-dose (HD) IL-2 (typically boluses of ≥ 600,000 IU/kg), although low-dose regimens (≤ 600,000 IU/kg) have been described

[4, 19, 24–27]. Most patients treated receive between two and ten doses of HD IL-2, with a median of approximately six doses [4, 25–30]. Usually, an interval of 8 h is chosen between doses.

2.2 Clinical Results and Applications

While most TIL clinical trials from 1988 to date have been for metastatic melanoma [4, 25–30], they have also been reported for renal cell carcinoma, breast cancer, and colorectal cancer [8, 9]. Overall response rates in melanoma patients, mostly highly pretreated, ranged from 27.5 to 57%, and complete response rates from 6.4 to 22% [24–26, 28–31]. Median overall survival was either not reached at the time of publication or rather variable and ranged from 8.5 months [31] to 16.4 months [28] in treated and evaluable patients. Two-year overall survival was reported as 40% in two studies [25, 31]. It should be mentioned that most studies have not reported results as an intent-to-treat analysis, with 20–40% [19, 24–26, 28] of patients being taken off study due to rapid disease progression before TIL reinfusion, or unsuccessful TIL expansion [28]. Furthermore, few studies have comprehensively described toxicity, the majority reporting only on grade 3 or higher adverse events.

2.3 Toxicities

TIL therapy requires an inpatient hospital stay with a median duration of 20 days [26, 28]; patients are discharged upon hematologic and other systems recovery. Non-myeloablative lymphodepleting chemotherapy causes both hematological and non-hematological toxicities. Transient cytopenia including neutropenia, lymphopenia as well as prolonged depression of CD4 + T cells are observed in virtually all patients [4, 9, 24–32]. Neutropenic fever occurs in 37–51% of patients [24, 26]. Granulocyte-colony stimulating factor (G-CSF) as well as blood product support is routinely required, with a median of five red blood cell transfusions and 30 units of platelets [4, 9, 24–32] (Table 1). Side effects are managed according to standards of good clinical practice [33]. In the absence of prophylaxis, a minority of patients experience opportunistic infections, including *Pneumocystis jirovecii* pneumonia (e.g., 6% in [26]) or *Herpes zoster* reactivation (e.g., 9% in [26]), thus mandating routine prophylaxis for a minimum duration of 6 months post-chemotherapy. Non-hematological high-grade toxicities include diarrhea (e.g., 12% in [28]), hyperbilirubinemia (e.g., 14% in [28]) and fludarabine-induced neurotoxicity [25, 26, 28]. The overall mortality from this regimen is < 1% [25, 27].

High-grade toxicity attributed to the TIL infusion product itself is exceedingly uncommon. Immediate infusion reactions to TILs are rare and mainly low grade [2, 9, 26, 27, 32, 34–39], and they are difficult to discriminate from early reactions to low residual levels of IL-2 remaining in the TIL product after ex vivo culture. Allergic reactions include acute release of cytokines with fever, skin reaction and dyspnea or delayed symptom onset. Management is symptomatic and use of corticosteroids discouraged. In case of a side effect related to TIL infusion, every effort should be taken not to stop the cell infusion, if the clinical symptoms allow it [2, 40, 41] (Table 1). Autoimmune melanocyte destruction, manifesting as vitiligo or uveitis, may occur in approximately 35% and 15% of patients, respectively [26].

High-dose IL-2 is associated with transient and typical dose-limiting toxicity. Both efficacy and toxicity are dose- and schedule-dependent (reviewed in [42–45]). Although high-dose IL-2 is associated with significant morbidity, the incidence and severity of toxicities have decreased overall as more patients have been treated worldwide and clinicians have gained experience in the prevention and management of side effects [4, 26, 32, 45, 46] (Table 1). The implementation of lymphodepleting chemotherapy greatly limits the immediate IL-2 toxicity relative to an immunocompetent host, as it eliminates resident lymphocytes as a source of cytokines contributing to IL-2 side effects [19].

Generally, toxicities associated with high-dose IL-2 therapy are transient and can be managed using standard interventions [43]. Infusion of IL-2 requires an adequate hospitalization setting offering hemodynamic and respiratory monitoring as well as personnel to conduct frequent physical examination, blood tests, and radiological imaging when required, according to protocol. IL-2 toxicity can manifest in multiple organ systems, most significantly the heart, lungs, kidneys, and central nervous system. The most common manifestation is capillary leak syndrome, resulting in a hypovolemic state and extravascular fluid accumulation. Most patients become tachycardic and hypotensive 4–6 h after IL-2 administration, mimicking a sepsis-like pathophysiology. Usual management includes cautious crystalloid fluid boluses (max 1000–1500 mL/day) and careful avoidance of fluid overload, which can precipitate pulmonary edema due to capillary leak. Systolic arterial blood pressure can usually be stabilized to a new baseline of approximately 80–90 mmHg. Heart rate must generally return below 100 beats/min before administering the next IL-2 dose [43]. Cardiac arrhythmias happen rarely. In case of transient atrial flutter or fibrillation, IL-2 continuation is possible if rhythm returns to normal sinus rate. In case of ventricular arrhythmia, definitive discontinuation of IL-2 is mandatory. Capillary leak syndrome can contribute significantly to the development of oliguria, cardiac ischemia, dyspnea from pulmonary congestion (3–47%, [25, 28]) requiring intubation in 6–9% of patients [24, 26] and mental status changes (confusion). Treatment is mainly supportive. In addition, thyroid dysfunction is a relatively common sequel of IL-2 therapy, with 9% of patients presenting hypothyroidism

Table 1 List of toxicities encountered in ACT by immune-depletive, non-myeloablative chemotherapy by fludarabine and cyclophosphamide, high doses of IL-2 and infused cell product as well as their management according to existing guidelines

ACT	Component	Toxicity	Management
TIL/TCR/CAR	Fludarabine	Myelosuppression, thrombopenia, anemia, neutropenia	Standard clinical practice, empiric broad-spectrum antibiotics and growth-factor support in case of fever
		Gastrointestinal side effects, anorexia, nausea vomiting	Standard clinical practice
		Malaise, fever, chills, fatigue, and weakness	Standard clinical practice
		Neurological side effects: paresthesia and visual disturbances	Standard clinical practice
	Cyclophosphamide	Myelosuppression, anemia, thrombopenia, neutropenia	Standard clinical practice
		Gastrointestinal side effects, anorexia, nausea, vomiting	Standard clinical practice
		Reversible alopecia	Standard clinical practice
		Urological side effects, cystitis, hematuria and hemorrhagic cystitis	Standard clinical practice
	TIL infusion	Acute cytokine release by the infused cells (see also cytokine release syndrome under CAR T cells)	G1 continue TIL infusion
		Fever, skin reactions, dyspnea	G2 stop TIL infusion until symptoms are G1
		Autoimmune complications from attacking 'self-antigens', also expressed by some normal tissues, and off-target toxicities	G3 symptoms stop infusion
		Vitiligo	
		Uveitis	
	IL-2	Capillary leak syndrome with hypotension, tachycardia, edema, weight gain, and hypoalbuminemia, failure of vital organs due to low perfusion, gastrointestinal bleeding or kidney infarction, change of mental state	[42–46], avoid steroids as long as possible
		Respiratory insufficiency with pulmonary edema or pleural effusion	Supportive care e.g., nasal oxygen, diuretics, intubation
		Renal failure due to hypotension	Supportive care, e.g., hydric support, preferring ringer lactate
		Arrhythmias (supraventricular and ventricular), angina pectoris, myocardial infarction	Supportive care, treatment of myocardial infarction according to guidelines
		Hepatic dysfunction	Supportive care
		Hematologic abnormalities, including anemia, leukopenia, and thrombocytopenia, are common during IL-2 therapy, but are rarely severe or dose limiting	Supportive care
		Constitutional symptoms, chills, fever, malaise, and fatigue	Supportive care e.g., pethidine in case of G2/G3 chills
		Gastrointestinal symptoms, nausea, vomiting or diarrhea, bleeding	Supportive care
		Neurological adverse events: confusion, dizziness, disorientation and somnolence. Altered sleeping patterns, agitation and paranoia. Rare: leukoencephalopathy	Supportive care

Table 1 (continued)

ACT	Component	Toxicity	Management
		Endocrine and metabolic disorders. Thyroid dysfunction, mainly hypothyroidism, subclinical presentation, hyperthyroidism is less frequent, potassium, calcium, magnesium, and phosphorus levels commonly decrease	Supportive care
		Dermatological effects, erythema, pruritus on the face and neck and progress to the trunk and extremities within 3 days	Supportive care
CAR/TCR	Cytokine release syndrome (can be observed in all ACT, but most frequently seen in CAR ACT)	General, depending on grade, according to Lee et al. [105] and Neelapu [99]	
		Fever	Acetaminophen, ibuprofen, and a cooling blanket
		Hypotension	Fluid bolus, if refractory anti-IL6 (tocilizumab or siltuximab), if refractory vasopressor, TNF antibody etanercept
		Hypoxemia	Nasal oxygen, intravenous tocilizumab (8 mg/kg)
		Neurotoxicity	Intravenous tocilizumab (8 mg/kg)
CAR	CAR	CAR T-cell-related encephalopathy syndrome (CRES), toxic encephalopathic state with symptoms of confusion and delirium, and occasionally seizures and cerebral edema	Intensive care support, tocilizumab, corticosteroids
		Fulminant hemophagocytic lymphohistiocytosis (HLH) (also known as macrophage-activation syndrome [MAS]) severe immune activation, lymphohistiocytic tissue infiltration, and immune-mediated multiorgan failure	Intensive care support, dexamethasone, etoposide, alemtuzumab

G1–4 grading of symptom severity according to CTCAE (Common Terminology Criteria for Adverse Events)

ACT adoptive cell therapy, *CAR* chimeric antigen receptor, *IL-2* interleukin 2, *TCR* T-cell receptor, *TIL* tumor-infiltrating lymphocytes, TNF tumor necrosis factor

requiring hormone replacement, and 7% of patients presenting hyperthyroidism [47]. In rare severe cases, vasopressors, intubation or continuous hemofiltration may be indicated. Safe administration of high-dose IL-2 depends on the experience of the caring team, adherence to standards of IL-2 administration and patient assessment guidelines, and that patient-eligibility criteria are respected. Further, it is important to carefully assess vital parameters prior to each high-dose IL-2 administration and strictly recognize and avoid contraindications as determined by the clinical study protocol [42, 43, 45, 46] (Table 1). Toxicities accompanying TIL therapy are mostly low grade, transient and manageable by standard supportive care but patients should only be treated in specialized centers.

3 T-Cell Receptor (TCR)-Transduced T Cells

3.1 Treatment Principle

Antigen specificity of T cells is endowed by their TCR, which binds a cognate ligand consisting of a peptide presented in the major histocompatibility complex (MHC), the so-called pMHC complex. TCR-ACT consists of autologous T lymphocytes engineered ex vivo to express an exogenous cancer-specific TCR (as described in Figs. 1 and 2a); this redirects autologous peripheral T cells to recognize a specific cancer antigen processed and presented in the context of the patient's MHC. The use of peripheral T cells obviates the need to harvest and expand natural lymphocyte clones. A crucial determinant of both efficacy and safety is the affinity of the chosen TCR for its target pMHC. TCRs in the upper end of natural affinity are associated with higher efficacy but affinity thresholds have been reported beyond which T-cell activity levels drop, and cross-reactivity becomes an important risk [48]. The ideal pMHC target of a candidate TCR comprises a peptide from a tumor antigen that is exclusively expressed by cancer cells (expression in non-essential normal tissues may be tolerable), that is essential for cancer cell survival to reduce the risk of tumor escape through down-regulation, and that is presented on frequent MHC molecules (reviewed in [49]). TCRs selected for gene modification are usually obtained from naturally occurring tumor-reactive T cell clones, although TCRs have also been isolated from mice transgenic for human HLAs that have been vaccinated with the targeted human antigen. In addition, the affinity of natural TCRs can be optimized by structure-based rational design [50] as well as by phage display screening technology [51, 52] (Fig. 2a). Although successful in enhancing the performance of the transduced T cells against cancer, non-natural TCRs may also carry a higher risk of 'off-tumor, on-target' toxicity (recognition of the pMHC expressed at low levels in normal tissues), or 'off-tumor, off-target' toxicity (cross-reactivity with a different pMHC expressed in normal tissues). Notably, the mispairing of introduced TCR subunits with endogenous TCR subunits can generate autoreactive T cells [53, 54], but this can be minimized by optimal transgene design or gene editing [55].

For TCR T-cell ACT, peripheral T lymphocytes are activated and gene-modified to express the TCR, and then expanded in culture. As described in the TIL section, patients are usually pretreated with lymphodepleting chemotherapy, and high-dose IL-2 may be administered after cell transfer. In sharp contrast to autologous TIL therapy, the genetic engineering of the T cells to express specific TCRs may lead to a high rate of toxicity mediated by the cell product itself due to autoreactivity, as discussed above. Rigorous pre-clinical testing is performed in order to negatively select autoreactive TCRs [49].

3.2 Clinical Results and Applications

Several TCR-ACT clinical trials have been conducted (Table 2) in patients with melanoma [10, 56–58], colorectal cancer [59], esophageal cancer [16, 60, 61], other carcinomas [60, 62], advanced multiple myeloma [63], acute myeloid leukemia, and myelodysplastic syndrome [64]. While the targeting of tissue differentiation antigens such as MART-1 has had limited success, TCR against cancer-germline antigens such as melanoma-associated antigen (MAGE)-A3 [58, 60, 61], and New York esophageal squamous cell carcinoma (NY-ESO)-1 [63, 65] have demonstrated high response rates between 23 and 80% with rare durable and complete responses.

3.3 Toxicities

Toxicities resulting from lymphodepleting chemotherapy and high-dose IL-2 were described in the TIL section. The infused T cells can cause acute cytokine release syndrome (CRS), as well as tissue-directed autoimmune reactions [66]. Cytokine release is triggered by the engagement of infused T cells with the targeted tumor cells (Table 1 and CAR section), and its severity depends on the number and fitness of infused T cells, their avidity for the tumor antigen and the tumor bulk. The resulting clinical picture is a systemic inflammatory response syndrome, characterized by fever, tachycardia, hypotension, vasodilation, and capillary leak [66]. Severe forms of CRS can progress to shock and fatal multi-organ failure. Management is similar to that for responding to side effects of high-dose IL-2, described above (Table 1) and which has been reviewed extensively in the literature [42, 43, 46]. Mild forms of CRS can be treated with non-steroidal anti-inflammatory drugs and anti-pyretic drugs.

Table 2 Selected clinical trials using TCR ACT, encountered toxicities and their management

Study	Trial ID	Disease	MCH	Antigen	Receptor origin	Receptor generation	T-cell origin	Patients (*n*)	Treatment response	TCR treatment-related toxicities	Mechanism of toxicity	Management	IL-2 yes/no
Morgan et al. [10]	Phase I, approved by the NIH IRB, NCI IRB, NIH RAC, and FDA-CBER	Melanoma	Class 1	MART-1	Human	In vivo (receptor DNA sequence obtained from TIL from responding patient)	Autol T cells	15	PR 2/15	No reported toxicities	NA	NA	No
Johnson et al. [57]	Phase I, NCI-07-C-0174, and NCI-07-C-0175	Melanoma	Class 1	MART-1: 27–35 epitope	Human	In vivo (receptor DNA sequence obtained from TIL from responding patient)	Autol PBL	20	PR 6/20	Up to G2 skin, G2 eye, and G3 ear toxicity, 14/20 skin tox, 11/20 eye tox, 10/20 ear tox	On-target toxicity, expression of target antigen in uvea, ear, skin	Corticosteroids	Yes HD
Johnson et al. [57]	Phase I, NCI-07-C-0174, and NCI-07-C-0175	Melanoma	Class 1	gp100: 154–162 peptide	Mouse	In vivo (RACE TCR isolation, selection for highest anti-tumor affinity independent of CD4 or CD8 molecule)	Autol T cells	16	CR 1/16, PR 2/16	Up to G2 skin, G2 eye, and G3 ear 15/16 skin tox, 4/16 eye tox, 5/16 ear tox	On-target toxicity, expression of target antigen in uvea, ear, skin	Corticosteroids	Yes HD
Davis et al. [62]	Phase I, approved by the NIH IRB, NCI IRB, NIH RAC, and FDA-CBER	Various epithelial cancers	Class 1	p53	Mouse	In vivo (p53 264–272 peptide sequence was synthesized)	Autol T cells	14	PR 1/9	No reported toxicities	NA	NA	Yes HD

Table 2 (continued)

Study	Trial ID	Disease	MCH	Antigen	Receptor origin	Receptor generation	T-cell origin	Patients (*n*)	Treatment response	TCR treatment-related toxicities	Mechanism of toxicity	Management	IL-2 yes/no
Robbins et al. [65]	Phase I, approved by the NIH IRB, NCI IRB, NIH RAC, and FDA-CBER	Melanoma and synovial sarcoma	Class 1	NY-ESO-1	Mouse	In vitro affinity enhanced TCR (by amino acid substitution)	Autol T cells	17 Total (11 melanoma, 6 synovial carcinoma)	Melanoma CR 2/11, PR 3/11; synovial sarcoma PR 4/6	No reported toxicities	NA	NA	Yes HD
Parkhurst et al. [59]	Phase I, NCT00923806	Colorectal	Class 1	CEA	Mouse	In vivo (CEA sequence from COS7 CEA expressing cells)	Autol T cells	3	PR 1/3, 3/3 with decreased serum CEA protein levels	Severe transient dose-limiting inflammatory colitis 3/3 up to G3	On-target toxicity, expression of target antigen in colon	Corticosteroids	Yes HD
Morgan et al. [61]	Phase I, NCT01273181	Melanoma, synovial sarcoma, and esophageal cancer	Class 1	MAGE-A3	Mouse	In vivo (MAGE-A3 sequences cloned from tumor RNA)	Autol T cells	9 Total (7 melanoma, 1 esophageal, 1 synovial)	Melanoma CR1/7, PR 4/7; synovial carcinoma 0/1; and esophageal carcinoma NR 0/1	3 patients developed mental disturbances and 2 died from necrotizing leukoencephalopathy	On-target toxicity with unexpected MART-A3 expression in the brain	High-dose steroids, levetiracetam, and carbidopa-levodopa	Yes HD
Linette et al. [58]	Phase I, NCT01350401 and NCT01352286	Melanoma and myeloma	Class 1	MAGE-A3	Human	Affinity enhanced	Autol T cells	2		2/2 patients died from cardiogenic shock	Cross-reactivity with presented epitope of an unrelated protein (titin) expressed in the heart	High-dose steroids, levetiracetam, and carbidopa-levodopa	Not described

Table 2 (continued)

Study	Trial ID	Disease	MCH	Antigen	Receptor origin	Receptor generation	T-cell origin	Patients (*n*)	Treatment response	TCR treatment-related toxicities	Mechanism of toxicity	Management	IL-2 yes/no
Chodon et al. [56]	Phase I, NCT00910650	Melanoma	Class 1	MART-1	Human	No information	Autol T cells	14	Short-term regression 9/14	2/14 G3 respiratory distress	Not investigated, most likely not TCR related	High-dose corticosteroids, intubation	Yes, HD
Kageyama et al. [16]	Phase I, UMIN Clinical Trials Registry ID: UMIN000002395	Esophageal cancer	Class 1	MAGE-A4	Human	Human MAGE-A4 specific CTL clone	Autol T cells	10	3/10 patients who had minimal tumor lesions at baseline survived for > 27 months	No reported toxicities	NA	NA	No
Rapoport et al. [63]	Phase I, NCT01352286	Multiple myeloma	Class 1	NY-ESO-1/LAGE1	Human	Affinity enhanced	Autol T cells	20	CR 14/20, dPR 2/20, PR 2/20, SD 1/20, and PD 1/20	G3 or lower AEs included 3/20 with G3 colon aGVHD, 2/20 with G2 skin aGVHD	Not related to TCR, lymphocyte infiltrate in colon lower than normal tissue	Not described	No

Table 2 (continued)

Study	Trial ID	Disease	MCH	Antigen	Receptor origin	Receptor generation	T-cell origin	Patients (*n*)	Treatment response	TCR treatment-related toxicities	Mechanism of toxicity	Management	IL-2 yes/no
Tawara et al. [64]	http://www.umin.ac.jp, #UMIN000011519	AML and high-risk MDS	Class 1	WT1	Human	Isolated from CTL clone using RACE, including siRNA for endogenous TCR	Autol PBL	8	4/8 PR or SD	0/8 G3 toxicity, 7/8 skin reaction (G1), 1/8 none, 1/8 facial edema (G1), 1/8 dermatitis (G1), 1/8 fever (G1), 1/8 phlebitis (G2), 1/8 arrhythmia (G1), 1/8 stomatitis (G1)	Not related to TCR	Not reported	No
Lu et al. [60]	Phase I, NCT02111850	Cervical cancer, esophageal, urothelial, osteosarcoma	Class 2	MAGE-A3	Human	MAGE-A3-specific TCRs were isolated from a regulatory T-cell clone (6F9) and an effector clone (R12C9)	Autol PBL	17	4/17 with response, 1 CR, 2 PR at highest dose level ($n=9$)	2/17 transient transaminase elevations G3/G4	Not investigated, could be TCR related	Not reported	Yes, HD

AEs adverse events, *aGVHD* acute graft-versus-host disease, *AML* acute myeloid leukemia, *Autol.* autologous, *CBER* Center for Biologics Evaluation and Research, *CEA* carcinoembryonic antigen, *CR* complete response according to RECIST, *CTL* cytotoxic T lymphocyte, *dPR* deep partial response, *FDA* Food and Drug Administration, *gp100* glycoprotein 100, *HD* high dose, *IRB* institutional review board, *LAGE1* cancer/testis antigen 2, *MAGE-A3* melanoma-associated antigen 3, *MART-1* melanoma-associated antigen recognized by T cells, *MDS* myelodysplastic syndrome, *MHC* major histocompatibility complex, *NA* not applicable, *NCI* National Cancer Institute, *NIH* National Institute of Health, *NR* non-responder, *NY-ESO-1* New York esophageal squamous cell carcinoma 1, *PBL* peripheral blood lymphocytes, *PD* progressive disease, *PR* partial response according to RECIST, *RAC* Recombinant DNA Advisory Committee, *RACE* rapid amplification of cDNA ends, *SD* stable disease according to RECIST, *TCR* T-cell receptor, *TIL* tumor-infiltrating lymphocytes, *WT1* Wilms tumor protein

The nature of autoimmune toxicity is largely dependent on the target antigen of the TCR. For example, severe adverse events have been reported when TCR-ACT was directed against lineage antigens; that is, antigens overexpressed in tumors but also expressed at low levels by the normal tissue of origin. For example, high-grade on-target colitis was reported upon administration of TCR-transduced T cells targeting carcinoembryonic antigen (CEA), expressed highly in gastrointestinal cancers but also at low levels in the normal intestine [59], while on-target skin reactions were observed with TCRs against melanoma-specific antigens MART-1 and gp100 [56, 57], also expressed by normal melanocytes.

Careful selection of the target and the TCR mitigates the risk of excess toxicity during clinical development (Fig. 2a). Commonly targeted and potentially safe antigens for TCR ACT include oncoviral antigens, cancer germline (testis) antigens such as NY-ESO-1, and tumor neo-antigens [67]. Oncoviral antigens are highly immunogenic, but only present in 10–15% of all malignancies; TIL specific to Epstein-Barr virus (EBV) epitopes resulted in high response rates with durable responses in patients with EBV-associated nasopharyngeal carcinoma [36], and anti-human papillomavirus (HPV)-specific TIL administered in metastatic cervical cancer evoked durable complete responses [38].

Cancer germline antigens are normally expressed in gonads and the thymus but some exhibit cancer-specific expression and are shared among many tumor types [65, 68]. MAGE-A3 and NY-ESO1 have been targeted in metastatic melanoma, metastatic synovial sarcoma, or multiple myeloma [61, 65]. Since the affinity of the wild-type TCRs to these targets is usually weak, affinity-enhanced TCRs have been generated to increase anti-tumor activity, bearing the risk of losing strict specificity and generating cross-reactivity with other self-antigens. Thus far, anti-NY-ESO1 TCR T cells have demonstrated a clinical benefit without toxicity [63, 65]. However, treatment of melanoma patients with an HLA-A*0201 restricted TCR directed against the germline antigen MAGE-A3 produced lethal neurotoxicity; deep characterization of the molecular basis for the toxicity revealed that the TCR also recognized HLA-A*0201 epitopes in MAGE-A9 and A12, and that MAGE-A12 was expressed in the human brain (in addition to possibly MAGE-A9) [61]. Furthermore, lethal off-target cardiotoxicity was observed in patients receiving ACT with an HLA-A*01 restricted TCR against MAGE-A3 due to unexpected cross-reactivity of the TCR with a titin epitope in the HLA-A*01 background, exclusively expressed in the heart in beating cardiomyocytes [58, 69].

In order to limit on-target toxicity for oncoviral and germline antigens, their absence from panels of healthy tissue is tested in silico, using online databases (Human Protein Atlas, CGA database), and in vitro using polymerase chain reaction (PCR) cDNA libraries and immunohistochemistry (IHC) in tissue panels [49, 70]. TCRs are tested against random epitopes and allogeneic MHC molecules using, for example, lymphoblastoid B-cell lines with various MHC allotypes [71, 72]. Further testing for self-avidity and efficient cellular processing and presentation is recommended [49] as well as screening against a combinatorial peptide library and additional cell subsets to detect off-target toxicity due to cross-reactivity [73]. Various techniques to reduce the risk of mispairing [74], including siRNA-induced silencing of endogenous TCR [75] have been described.

Neo-antigens resulting from somatic DNA alterations in cancer cells are by definition tumor-specific and are potentially recognized by a high-affinity T-cell repertoire, and as such represent attractive targets for immunotherapy both for their safety and efficacy [76, 77]. Neo-antigens are mostly patient-specific (i.e., with very few being shared among patients); their utilization, however, requires high-throughput methods for neo-epitope and TCR identification [76, 78, 79]. The rapid development of whole genome sequencing approaches might help to find neo-antigen targets for ACT from circulating tumor DNA (reviewed in [80]). Very recent developments in molecular-genetic methodology like CRISPR/CAS9 genetic engineering could be useful for supporting the development of personalized TCR-ACT, and there is currently a first trial recruiting at the National Institutes of Health (NIH) using individual TCRs (ClinicalTrials.gov Identifier: NCT03412877).

Management of toxicities depends on the organ system involved as well as the type of toxicity. In reported clinical trials, side effects resulting from on-target toxicity as reported after the MAGE-3 TCR study were managed using symptomatic therapy (e.g., for seizure control) and immunosuppression using corticosteroids [61]. Efforts to limit toxicity by inducible T-cell suicide are discussed in chapter 5 below.

4 CAR T Cells

4.1 Treatment Principle

A CAR combines an extracellular antigen-binding domain, which typically comprises a single-chain variable fragment (scFv) from a monoclonal antibody, or a natural ligand [81] that confers recognition of a tumor-associated antigen, with an intracellular domain carrying signaling motifs capable of T-cell activation and costimulation [12]. Currently, the most common method of ex vivo genetic engineering of T cells is via lentiviral and gamma-retroviral vector-based transduction methods [82–85]. These allow for stable integration of the desired transgene(s). Alternative non-viral delivery technologies include electroporation for transient expression

[86], and transposon/transposase delivery systems that allow larger gene cargo [87, 88].

In contrast to TCRs, CARs can recognize any molecule present on the surface of target tumor cells in a non-MHC restricted manner. MHC-independent antigen recognition enables CAR-modified T cells to treat any patient whose tumor expresses the target antigen, and thus, unlike TCR-ACT, CAR-ACT permits the treatment of tumors that have acquired defects in antigen processing and MHC presentation [89]. While antigen recognition by CARs occurs by engagement of larger epitopes, imparting less risk of cross-reactivity [90], solid tumors remain an important challenge for CAR therapy as there exist few bona fide tumor antigens, thus running the risk of on-target/off-tumor toxicities (Fig. 2b).

4.2 Clinical Results and Applications

Administration of CAR-modified T cells that target the B-cell lineage differentiation antigen CD19 (CAR19) has led to impressive clinical responses in patients with acute B-cell leukemia, chronic lymphocytic leukemia, diffuse large B-cell lymphoma, and other non-Hodgkin lymphomas (NHLs) [15, 91–97], which led to their regulatory approval. CAR19 has therefore entered the mainstream and is a valuable therapeutic option for patients with hematologic malignancies.

4.3 Toxicities

Toxicities arising from CAR therapy include toxicity from lymphodepleting chemotherapy, as described in the TIL section, CRS and CAR T-cell-related encephalopathy syndrome (CRES), and auto-immune events. CRS is the most commonly observed toxicity. While in the majority of cases CRS presents as a mild, flu-like disorder with fever, malaise, headache, tachycardia, and myalgias, in a proportion of patients it can rapidly evolve into a sepsis-like symptomatology, with vascular leak, hypotension, rash, pulmonary edema, systemic coagulopathy, and multi-organ failure [98]. The severity of CRS correlates with tumor burden [21]. Most toxicities are grade 1–2 and manageable [99]. Some predictive biomarkers for the occurrence of CRS like the dose of infused CAR T cells, disease burden or preexisting endothelial activation have been established but warrant further clinical trials for their validation [100].

Since algorithms for accurate and consistent grading and management of the toxicities were lacking, a CARTOX (CAR T-cell therapy-associated toxicity) working group has been formed and guidelines for diagnostic, grading, and treatment of toxicities have been published in 2018 [99]. This review also includes a list of lethal events observed to date in CAR T-cell trials. The same working group presented CAR treatment guidelines for pediatric patients [101]. The magnitude and timing of the toxicities associated with CAR T-cell therapy vary considerably across different CAR T-cell constructs and across different diseases (acute lymphocytic leukemia [ALL] versus NHL) [102]. For example, in the pivotal multicenter ZUMA-1 trial of a CAR19 bearing the CD28/CD3ζ (28/ζ) endodomain in 101 patients with refractory aggressive B-cell NHL, the rates of grade ≥ 3 CRS and neurological toxicities were 13% and 28%, respectively [103]. Conversely, in an interim analysis of the JULIET trial of a CAR19 bearing the 4-1BB/CD3ζ (BB/ζ) endodomain in 51 patients with relapsed or refractory diffuse large B-cell lymphoma, these rates were 26% and 13% [104].

Symptoms of CRS can be graded according to Lee et al. [105]. Rarely, CRS can develop into a fulminant hemophagocytic lymphohistiocytosis (HLH), characterized by hepatosplenomegaly, hepatotoxicity, jaundice, and diffuse lymphoadenopathy, or macrophage activation syndrome (MAS) with high fever, hepatosplenomegaly, hepatotoxicity, jaundice, coagulopathy, hypofibrinogenemia, cytopenia, hypertriglyceridemia, and extreme hyperferritinemia. Plasma levels of IL-6, IL-10, and interferon-gamma (IFNγ) have been found to be very high during CRS [106], and they also correlate closely with the expansion and persistence of CAR T cells [107]. Although IFNγ is likely produced directly by CAR T cells, IL-6 is contributed largely by activated macrophages, which must persist despite chemotherapy according to recent preclinical studies [108, 109]. Given the potential key role of macrophages in CRS induced by CAR T cells, it has been recommended that candidate patients be screened for hereditary mutations predisposing to HLH, including *PRF1*, *MUNC13-4*, *STXBP2*, and *STX11* [98].

Intensive monitoring and prompt management of toxicities are essential to minimize the morbidity and mortality associated with this potentially curative therapeutic approach (Table 2). Table 1 shows CRS treatment options according to Neelapu et al. [99]. Corticosteroids have been part of the management. The potential to attenuate the clinical efficacy of CAR T cells is a concern, although short-term steroid treatment did not appear to limit the efficacy of CAR T-cells [91, 106]. Blockade of IL-6 receptor (IL-6R) with the commercially available, FDA-approved antibody tocilizumab, along with anti-tumor necrosis factor alpha (TNFα) antibody etanercept, produced prompt resolution of the symptomatology without affecting the expansion or efficacy of CAR T cells [106]. Effective IL-6 blockade can also be achieved through siltuximab, a commercially available IL-6 blocking antibody [98]. IL-6 blockade is recommended to be administered early in case of CRS [99, 110]. Recent preclinical research shows that IL-1 is also required to trigger CRS [108, 109], indicating that IL-1 blockade might be useful in the management of CAR therapy toxicity.

The second most worrisome CAR-specific side effect is acute onset neurotoxicity (CRES), which can occur in association or independently of CRS. CRES is described as a biphasic phenomenon with a first phase that can occur together with CRS and is responsive to tocilizumab treatment, followed by a second phase that is not responsive to IL-6R blockade [99]. Early signs of CRES include decreased attention, coordination problems, agitation or delirium with preserved alertness, headache, and language deficits. In the majority of cases, symptoms resolve within 4 weeks, in more severe cases, cerebral edema, seizures, focal deficits, and diminished consciousness including coma can occur. In 133 patients receiving an anti-CD19 CAR bearing the BBz endodomain, neurologic adverse events (AEs—any) were recorded in 40%, presenting a median of 4 days after CAR T-cell infusion [111]. The highest grade neurotoxicity evolved within a median of 1 day from the onset of neurotoxicity, while the duration of reversible neurologic AEs was < 4 weeks (median 5 days) in all but one patient. There were four deaths due to CRES: two from acute cerebral edema, one from disseminated intravascular coagulation and multifocal brainstem hemorrhage, and one from cortical laminar necrosis and coma. These largely occurred during the dose-escalation phase of the study, in patients who received a dose of CAR T cells subsequently determined to be above the maximally tolerated dose. In > 90% of patients, neurologic AE presented in the presence of CRS, and patients without CRS only presented grade transient 1 neurotoxicity. In addition to CRS, the severity of neurotoxicity correlated with CAR T-cell expansion in vivo, higher disease burden, higher dose of CAR T cells, and a fludarabine-containing chemotherapy preparative regimen. Severe CRS was a major risk factor for grade ≥ 3 CRES, and plasma IL-6 levels > 500 pg/mL within 6 days of CAR T-cell infusion were associated with grade ≥ 4 neurotoxicity in 100% of patients [111].

The pathophysiology mechanisms of CRES are under investigation. A careful review of clinical, laboratory, and autopsy data from the above patients suggested that brain endothelial cell activation is an early event in CRES, which leads to breakdown of the blood–brain endothelial barrier and entry of inflammatory cytokines and CAR T cells in the brain, leading in severe cases to local severe inflammation, cerebral edema, hemorrhage, and infarctions [111]. Mouse models have revealed that CRES is largely driven by activation of endogenous macrophages, recruited and activated by CAR T cells. Such monocytes produce IL-1 and nitric oxide, which drive the neurotoxicity, and monocyte depletion in the mice prevented CRES. Tocilizumab could prevent systemic CRS but not the delayed-onset lethal CRES, while the IL-1 receptor antagonist anakinra could effectively reverse CRES in mice without affecting the anti-leukemia efficacy of CAR T cells [108, 109].

The CARTOX working group developed algorithms for grading and management of CRES [99]. Treatment is symptom dependent. Anti-IL-6R therapy can be considered to relieve systemic toxicity of CRS. However, based on the recent mouse evidence, the use of IL-1 antagonist anakinra should be evaluated in the clinic. In higher grade CRES, administration of corticosteroids should also be considered [99].

Severe immune-mediated adverse events, which can be on-target [80] or off-target (as explained in more detail in the TCR section) following CAR T-cell infusion, have been appreciated. In order to limit on-target toxicity, careful selection of the target antigen is key, as discussed already in the TCR section of this article. Therapy with CAR T cells against carbonic anhydrase-9 (CAIX), for example, delivered to 12 patients with CAIX-expressing metastatic renal cell carcinoma had to be stopped because of G2–G4 liver toxicity due to CAIX expression in the bile duct epithelium [112].

Several attempts have been made to limit toxicity from CAR-ACT through engineering solutions [113]. For example, the so-called split-signaling CARs entail the co-transfection of T cells with two distinct CARs, one (zeta-CAR) that provides the main antigen binding ectodomain and a CD3ζ endodomain and a second (costimulatory-CAR) that recognizes a second antigen on target tumor cells with a different ectodomain linked to a costimulatory endodomain. Engagement of the zeta-CAR drives suboptimal activation of T cells upon antigen recognition, while engagement of the costimulatory-CAR boosts T-cell activation upon recognition of the second antigen. This combinatorial strategy therefore requires the simultaneous expression of the two antigens to fully activate CAR T cells, which occurs on the tumor, and avoids the CAR T-cell activation against normal tissues, which may express only one of the two antigens [114].

5 Management of Adoptively Transferred T cells to Reduce Autoimmune Toxicity

Agents suppressing effector T cells could be useful in the management of acute TCR-ACT autoimmune toxicities. Corticosteroids are most readily used, such as pulse corticosteroids (methylprednisolone) followed by a taper. Clinicians must also familiarize themselves with drugs used in acute allotransplant rejection as further means to control acute autoimmunity, including rabbit anti-thymocyte globulin (rATG-thymoglobulin), mycophenolate, tacrolimus, and/or anti-CD52 antibody alemtuzumab [115].

Additional safety strategy approaches include suicide genes that can eliminate CAR-T or TCR T cells on command [116]. For example, T cells transfected with the herpes

simplex virus thymidine kinase (HSV-TK) can be subsequently eliminated by the use of the prodrug ganciclovir, which induces apoptosis specifically in HSV-tk transfected CAR T cells. This has been successfully tested in clinical trials in order to avoid graft versus host disease after allogenic hematopoietic stem cell transplantation [117–119]. Another strategy employs an inducible caspase 9 suicide gene, integrated in the delivered transgene [120–122]. This particular suicide gene can be selectively activated by a chemical inducer of dimerization (CID) small molecule, which has been shown to increase safety in an allogenic stem cell transplantation setting [120] and is about to be tested in CAR T cells in several phase I/II clinical trials (e.g., NCT03639844).

Beside suicide gene engineering, T-cell death can be achieved using antibody-dependent cell-mediated cytotoxicity (ADCC). A pre-clinically validated suicide strategy is retroviral delivery of the CD20 molecule into T cells, which allows targeting transduced T cells in vivo with anti-CD20 monoclonal antibody [123]. An alternative approach has combined epitopes from CD34 and CD20, enabling CD34 selection, cell tracking, as well as deletion after anti-CD20 monoclonal antibody administration [124]. Another approach has introduced a 10-amino acid tag of c-myc protein into the TCR sequence allowing elimination with anti-myc tag monoclonal antibody administration [125]. Finally, another approach has used truncated human epidermal growth factor receptor (EGFR) polypeptide/anti-EGFR monoclonal antibody [126]. The above methods rely on elimination of transduced T cells through ADCC, which can be slow, especially following high-dose chemotherapy, and are unable to control a rapidly expanding T cell population in the lymphodepleted host.

6 Conclusions

ACT immunotherapy shows great promise for treating and eradicating advanced metastatic cancers, but clinicians must familiarize themselves with its potential side effects. Except for CAR19, which is approved for B-cell malignancies in the US and Europe, all ACT is administered within clinical trials in specialized centers. Adverse events may be immediate or delayed, and although usually mild, they can be severe and persist for the duration of the genetically modified T-cell lifespan [127]. Unique to T-cell therapies is the potential for extraordinary long-term persistence of transferred T cells for up to 10 years or longer [128, 129]. This persistence extends the promise for long-term surveillance of residual tumor cells and possible elimination and definitive cure of tumors, but also increases the timeline of potential toxicities far beyond those of chemotherapy or antibody-based therapies.

The rapidly growing knowledge regarding the interaction between the immune system and tumors, together with rapid advances in technology, will support the development of TIL, TCR, and CAR-T ACT to move toward the goal of treating cancer with high degree of safety, high efficacy, and low cost. The CARTOX working group treatment algorithms for toxicity management in adults and pediatric patients provide guidelines for building the medical practice of CAR19 T-cell therapy and offer a solid framework for establishing standardized and safe practices in the development of adoptive cell therapy with further CARs, TCRs, and TILs.

Compliance with Ethical Standards

Institutional Review Board approval was not required because this is a review of the literature.

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