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Benita Wolf, Stefan Zimmermann, Caroline Arber, Melita Irving ...+2 more authors

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Safety and Tolerability of Adoptive Cell Therapy in Cancer

Benita Wolf, Stefan Zimmermann, Caroline Arber, Melita Irving, Lionel Trueb and
George Coukos

Department of Oncology, Lausanne University Hospital, and Ludwig Institute for
Cancer Research, University of Lausanne, Lausanne CH-1066, Switzerland.

Corresponding author: George.Coukos@chuv.ch

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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 ACT 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 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 wide 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 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 over important toxicities of adoptive cellular immunotherapy.

We introduce the concepts of TIL, TCR and CAR ACT.

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

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 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 [10, 2, 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 [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 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.

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. Fig.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 25mg/m² fludarabine (D-7 to D-3) and 2 days of 60mg/kg cyclophosphamide (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 $\geq 600,000$ IU/kg), although low-dose regimens ($\leq 600,000$ IU/kg) have been described [24, 25, 19, 26, 27, 4]. Most patients treated receive between two and ten doses of high-dose IL-2, with a median of approximately six doses [28, 25-27, 29, 30, 4]. Usually, an interval of 8 hours is chosen between doses.

2.2 Clinical results and applications

While most TIL clinical trials from 1988 to date have been for metastatic melanoma [28, 25-27, 29, 30, 4], 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% [28, 24-26, 31, 29, 30]. Median overall survival was either not reached at the time of publication or rather variable and ranged from 8.5 month [31] to 16.4 month [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 % [28, 24, 25, 19, 26] 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 [28, 26]; 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 [28, 24-27, 31, 29, 30, 32, 4, 9]. Neutropenic fever occurs in 37 - 51% of patients [24, 26]. 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 [28, 24-27, 31, 29, 30, 32, 4, 9] (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 [28, 25, 26]. The overall mortality from this regimen is less than 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 [34, 35, 26, 27, 36, 37, 32, 2, 38, 9, 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 [40, 41, 2] (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 [26, 32, 4, 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 hours after IL-2 administration, mimicking a sepsis-like pathophysiology. Usual management includes cautious crystalloid fluid boluses

(max 1000-1500 ml/day), 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 b/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%, [28, 25]) 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 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 [43, 45, 42, 46] (Table1). 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. 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 Fig.1 and Fig.2a); this redirects autologous peripheral T cells to recognize a specific cancer antigen processed and presented on 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. TCR 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 downregulation, 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 [56-58, 10], colorectal cancer [59], esophageal cancer [16, 60, 61], other carcinomas [62, 60], 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 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 (Table1) and which has been reviewed extensively in the literature [43, 42, 46]. Mild forms of CRS can be treated with non-steroidal anti-inflammatory drugs and anti-pyretic drugs.

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, i.e. 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 EBV epitopes resulted in high response rates with durable responses in patients with EBV-associated

nasopharyngeal carcinoma [36], while anti-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 [68, 65]. 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 a 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 recognized also HLA-A*0201 epitopes in MAGE-A9 and A12, and that MAGE-A12 was expressed in 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 [69, 58].

In order to limit on-target toxicity for oncoviral and germ-line antigens, their absence from panels of healthy tissue is tested *in silico*, using online databases (Human Protein Atlas, CGA database) and *in vitro* using PCR cDNA libraries and IHC in tissue panels [70, 49]). 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. [78, 76, 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 prove useful for supporting the development of personalized TCR-ACT and there is currently a first trial recruiting at 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 (for example 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 which 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 bone 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 [91-96, 15, 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, cytokine release syndrome (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 mild, as a 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 multicentre ZUMA-1 trial of a CAR19 bearing the CD28/CD3 ζ (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/CD3 ζ (BB/ ζ) endodomain in 51 patients with relapsed or refractory DLBCL, these rates were 26% and 13% [104].

Symptoms of CRS can be graded according to Lee [105]. Rarely CRS can develop into a fulminant haemophagocytic lymphohistiocytosis (HLH), characterized by hepatosplenomegaly, hepatotoxicity, jaundice and diffuse lymphadenopathy, or macrophage activation syndrome (MAS), with high fever, hepatosplenomegaly, hepatotoxicity, jaundice, coagulopathy, hypofibrinogenemia, cytopenia, hypertriglyceridemia and extreme hyperferritinemia. Plasma levels of interleukin-6 (IL-6), IL-10 and interferon-gamma (IFN γ) 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 IFN γ likely is 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 commercially available, FDA approved antibody tocilizumab, along with anti-TNF α antibody etanercept, produced prompt resolution of the symptomatology without affecting the expansion or efficacy of CAR T cells [106]. Effective IL-6 blockade can be

achieved also through siltuximab, a commercially available IL-6 blocking antibody [98]. IL-6 blockade is recommended to be administered early in case of CRS [110, 99]. Recent preclinical research shows that IL-1 is also required to trigger CRS [109, 108], 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 termed CAR T cell-related encephalopathy syndrome (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 four 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 AEs (any) were recorded in 40%, presenting a median of four days after CAR-T cell infusion [111]. The highest grade neurotoxicity evolved within a median of one day from the onset of neurotoxicity, while the duration of reversible neurologic AEs was less than four weeks (median 5 days) in all but one patient. There were 4 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 over 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 \geq grade 3 CRES, and plasma IL-6 levels greater than 500 pg/mL within 6 days of CAR T-cell infusion were associated with \geq 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 mouse 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 be also considered [99].

Severe immune-mediated adverse events, which can be on-target [80] or off-target (as explained in more detail 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 due to 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 CD3 ζ 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 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 which 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 allogeneic 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 allogeneic 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 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 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.

7. Compliance with ethical standards

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

7.2. Conflict of interest:

The authors of this manuscript declare relationships with the following companies:

Prof. George Coukos, Dr. Lionel Trueb, Dr. Stefan Zimmermann, Dr. Benita Wolf, Dr. Melita Irving and Prof. Carline Arber are co-investigators in lovance and Kite Pharma sponsored clinical studies at CHUV, Lausanne. Prof. Carline Arber receives royalties of Cell Medica. Prof. Carline Arber has 1 patent issued in the field of engineered T-cell therapies licensed to Cell Medica in 2016. Prof. Carline Arber has two provisional patent applications filed in the field of engineered T cell therapies. Prof. Carline Arber received consulting fees from Kite/ Gilead.

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Figure and Table legends

Figure 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. PBL: peripheral blood lymphocyte. CAR: chimeric antigen receptor, TIL: tumor infiltrating lymphocytes, TCR: T-cell receptor, HD-IL-2: high dose interleukin 2.

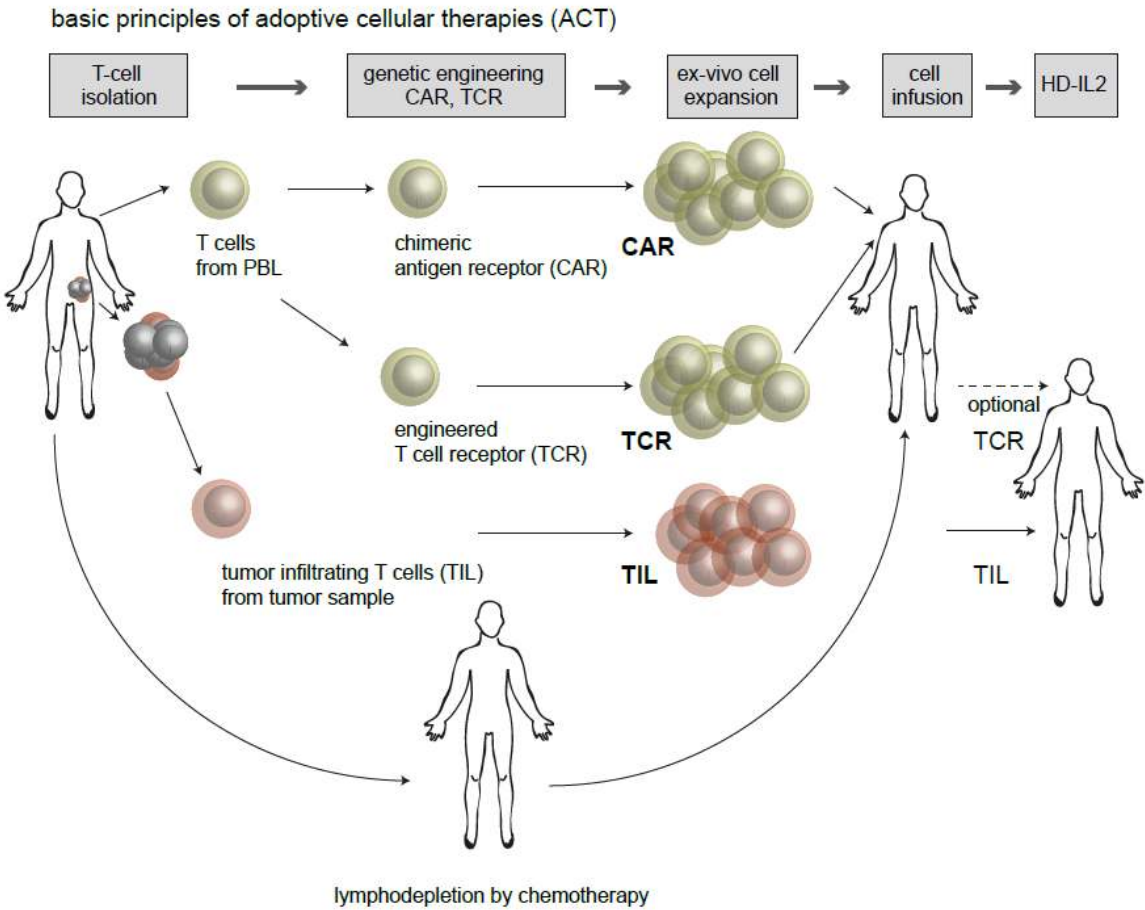


Figure 2: TCR and CAR ACT.

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. TCR: T-cell receptor, ACT: adoptive cellular therapy. Adapted from Kapanen et al. 2015 [130].

b: Schematic of components of endogenous TCR, genetically engineered TCR and CAR, TCR: T-cell receptor, CAR: chimeric antigen receptor. Adapted from June et al. 2015 [13]

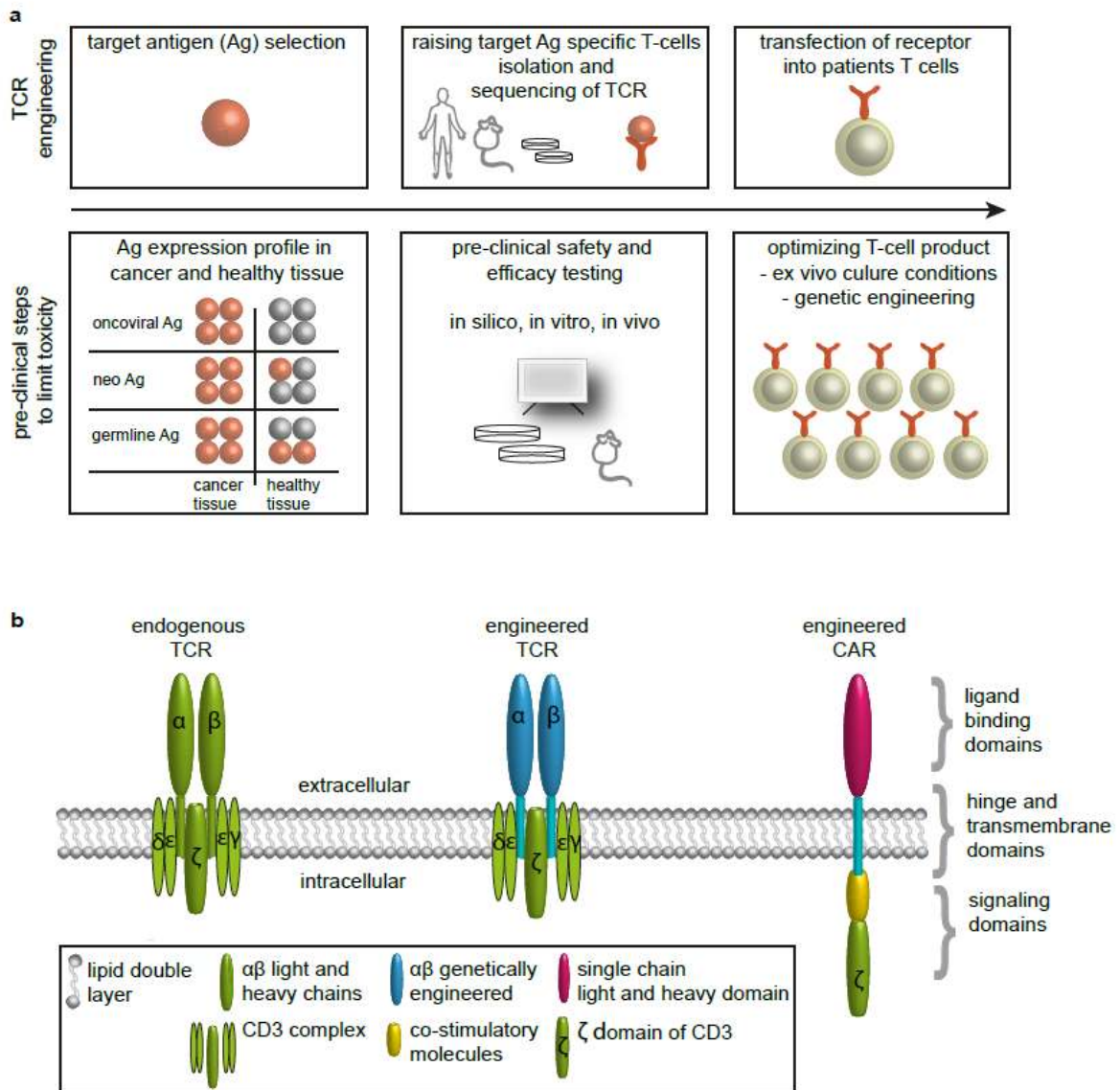


Table 1

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: adoptive cell therapy, CAR: chimeric antigen receptor, TCR: T-cell receptor, TIL: tumor infiltrating lymphocytes, IL-2: interleukin 2, G1-4: grading of symptom severity according to CTCAE (Common Terminology Criteria for Adverse Events).

ACT	Component	Toxicity	Management
TIL/TCR/ CAR	Fludarabine	myelosuppression, thrombopenia, anemia, neutropenia gastrointestinal side effects, anorexia, nausea vomiting malaise, fever, chills, fatigue, and weakness neurological side effects: paresthesia and visual disturbances myelosuppression, anemia, thrombopenia, anemia, neutropenia gastrointestinal side effects, anorexia, nausea vomiting reversible alopecia	standard clinical practice, empiric broad-spectrum antibiotics and growth-factor support in case of fever standard clinical practice standard clinical practice standard clinical practice standard clinical practice standard clinical practice
	Cyclophosphamide	gastrointestinal side effects, anorexia, nausea vomiting reversible alopecia	standard clinical practice standard clinical practice
	TIL infusion	urological side effects cystitis, haematuria and haemorrhagic cystitis acute cytokine release by the infused cells, *see also cytokine release syndrome under CAR T cells fever, skin reactions, dyspnea autoimmune complications from attacking 'self-antigens' also expressed by some normal tissues, and off-target toxicities	standard clinical practice G1 continue TIL infusion G2 stop TIL infusion until symptoms are G1 G3 symptoms stop of infusion
		uveitis vitiligo	
		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 respiratory insufficiency with pulmonary edema or pleural effusion renal failure due to hypotension	(references 42,46), avoid steroids as long as possible supportive care e.g. nasal oxygen, diuretics, intubation supportive care, e.g. hydric support, pletefing/inger/lactal supportive care, treatment of myocardial infarction according to guidelines supportive care
		arrhythmias (supraventricular and ventricular), angina pectoris, myocardial infarction hepatic dysfunction hematologic abnormalities, including anemia, leukopenia, and thrombocytopenia, are common during IL-2 therapy, but are rarely severe or dose limiting. constitutional symptoms, chills, fever, malaise and fatigue gastrointestinal symptoms, nausea, vomiting or diarrhea, bleeding	supportive care supportive care e.g. pethidine in case of G2/G3 chills supportive care supportive care
		neurological adverse events: confusion, dizziness, disorientation and somnolence. Altered sleeping patterns, agitation and paranoia. Rare: leukoencephalopathy endocrine and metabolic disorders: Thyroid dysfunction, mainly hypothyroidism, subclinical presentation. Hyperthyroidism is less frequently. Potassium, calcium, magnesium, and phosphorus levels commonly decrease dermatological effects, erythema, pruritus on the face and neck and progress to the trunk and extremities within three days	supportive care supportive care supportive care
		Cytokine release syndrome (can be observed in all ACT, but most frequently seen in CAR ACT)	
CAR/TCR		general, depending on grade, according to Lee 2015 (reference 105) and Neepalu 2018 (reference 99) fever	acetaminophen, ibuprofen, and a cooling blanket fluid bolus, if refractory anti-IL-6 (tocilizumab or siltuximab), if refractory vasopressor, TNF antibody etanercept nasal oxygen, intravenous tocilizumab (8 mg/kg) intravenous tocilizumab (8 mg/kg)
		hypotension hypoxemia neurotoxicity	intensive care support, tocilizumab, corticosteroids
CAR	CAR	CAR- T-cell-related encephalopathy syndrome (CRES), toxic encephalopathic state with symptoms of confusion and delirium, and occasionally seizures and cerebral edema luminal haemorrhagic lymphohistiocytosis (HLH) (also known as macrophage-activation syndrome (MAS)) severe immune activation, lymphohistiocytic tissue infiltration, and immune-mediated multiorgan failure	intensive care support, dexamethasone, etoposid, atemlizumab

Table 2

Selected clinical trials using TCR ACT, encountered toxicities and their management. Column headings are: reference, trial ID, the treated disease (DISEASE), the major histocompatibility complex (MHC) used, the receptor origin, details of receptor generation, the T-cell origin, the number (n) of patients, the observed treatment response, treatment related toxicities, the mechanism of toxicity, toxicity management, if IL-2 (interleukin 2) was administered (yes/no).

MART-1: melanoma-associated antigen recognized by T cells, gp100: glycoprotein 100, NY-ESO-1: New York esophageal squamous cell carcinoma 1, CEA: carcinoembryonic antigen, MAGE-A3: Melanoma-associated antigen 3, LAGE1: Cancer/testis antigen 2, WT1: Wilms tumor protein, TIL: tumor infiltrating lymphocytes, n.a.: not applicable, autol.: autologous, PBL: peripheral blood lymphocytes, AML: acute myeloid leukemia, MDS: myelodysplastic syndrome, CR: complete response according to RECIST, PR: partial response according to RECITS, SD: stable disease according to RECIST, HD: high dose

Reference	trial ID	disease	MCH	antigen	receptor	receptor generation	T cell origin	n patients	treatment	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/CDER	melanoma	class 1	MART-1-1:27-35 epitope	human	DNA sequence obtained from TIL from responding melanoma	auto T cells	16	PR 2/15	no reported toxicities and G3 ear toxicity, 1/20 skin tox, 1/20 eye tox, 10/20 ear tox, and G3 ear/1/6 skin tox, 4/16 eye tox, 5/16 ear tox	n.a.	n.a.	no
Johnson et al. [37]	Phase I, NCI-07-C-0174, and NCI-07-C-0175	melanoma	class 1	gp100:154-162 peptide	human	TIL from melanoma	auto PBL	20	PR 6/20	CR 1/16, PR 2/16	on-target toxicity, expression of target antigen in uvea, ear, skin	oculocorticosteroids	yes HD
Johnson et al. [37]	Phase I, NCI-07-C-0174, and NCI-07-C-0175	melanoma	class 1	gp100:154-162 peptide	mouse	TIL from melanoma	auto T cells	16	CR 1/16, PR 2/16	CR 1/16, PR 2/16	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/CDER	various epithelial cancers	class 1	p63	mouse	-272 peptide sequence was synthesized	auto T cells	14	PR 1/0	no reported toxicities	n.a.	n.a.	yes HD
Robbins et al. [65]	Phase I, approved by the NIH IRB, NCI IRB, NIH RAC, and FDA/CDER	melanoma and synovial sarcoma	class 1	NY-ESO-1	mouse	In vitro affinity selected TCR (by amino acid substitution)	auto T cells	17 total (11 melanoma, 6 synovial sarcoma)	Melanoma CR 2/11, PR 3/11; synovial sarcoma PR 4/6	no reported toxicities	n.a.	n.a.	yes HD
Parthunur et al. [59]	Phase I, NCT00222806	colorectal	class 1	CEA	mouse	In vivo (CEA sequence from COS7 CEA expressing cells)	auto T cells	3	PR 1/3, CR 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)	auto T cells	9 total (7 melanoma, 1 synovial sarcoma, 1 esophageal)	PR 4/7, synovial sarcoma CR 1/1; esophageal carcinoma NS 0/1	3 patients developed mental disturbances and 2 died from neurotoxicity and/or encephalopathy	on-target toxicity with unexpected MART-1A3 expression in the brain	high dose steroids, levetiracetam, and carbamazepine	yes HD
Linde et al. [58]	Phase I, NCT01356401 and NCI01352286	melanoma and myxoma	class 1	MAGE-A3	human	affinity enhanced	auto T cells	2	Short-term regression 0/4	2/2 patients died from neurotoxicity and cardiogenic shock	cross-reactivity with presented epitope of an unrelated protein (E1A) expressed in the heart	high dose steroids, levetiracetam, and carbamazepine	not described
Chodon et al. [56]	Phase I, NCT00910650	melanoma	class 1	MART-1	human	no information	auto T cells	14	minimal tumor regression 0/14	2/14 G3 respiratory distress	not investigated, most likely not TCR related	high dose corticosteroids, infliximab	yes, HD
Kagayama et al. [60]	Phase I, UMIN Clinical Trials Registry ID: UMIN000002326	esophageal cancer	class 1	MAGE-A4	human	human MAGE-A4 specific CTL clone	auto T cells	10	nCR or CR 14/20	Grade 3 or lower AEs nCR 2/20, PR nCR 3/20, CR 1/20, and CR 4/20 with PD 1/20	no reported toxicities	n.a.	no
Rappoport et al. [63]	Phase I, NCT01352286	multiple myeloma	class 1	NY-ESO-1/LA5E1	human	affinity enhanced	auto T cells	20	nCR 1/20, PR nCR 3/20, CR 1/20, and CR 4/20 with PD 1/20	Grade 3 or lower AEs nCR 2/20, PR nCR 3/20, CR 1/20, and CR 4/20 with PD 1/20	not related to TCR lymphocyte infiltrate in colon lower than normal tissue	not described	no
Tamura et al. [64]	www.umin.ac.jp/UMIN00011519	AML and high risk MDS	class 1	WT1	human	CTL clone, used RACE	auto PBL	8	4/8 PR or SD	1/8 fatal (G1), 1/8 severe (G1), 1/8 neurotoxicity (G1), 1/8 dermatitis (G1), 1/8	not related to TCR	not reported	no
2017_AOC_Lu	Phase I, NCT012111850	cervical cancer, esophageal, urothelial osteosarcoma	class 2	MAGE-A3	human	MAGE-A3-specific TCRs were isolated from a patient (PR) and an effector	auto PBL	17	4/17 with PR at highest dose level (n=3)	PR 4/17, CR 2/17, transient pruritus, pruritus elevations G3/G4	not investigated, could be TCR related	not reported	yes, HD

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