



Adoptive CD8⁺ T cell therapy against cancer: Challenges and opportunities

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ABSTRACT

Cancer immunotherapy is a new and promising option for cancer treatment. Unlike traditional chemo- and radiotherapy, immunotherapy activates host immune system to attack malignancies, and this potentially offers long-term protection from recurrence with less toxicity in comparison to conventional chemo- and radiation therapy. In adoptive CD8⁺ T cell therapy (ACT), large numbers of tumor-specific T cells are sourced from patients and expanded in vitro and infused back to patients. T cells can be expanded from naturally-induced tumor-specific CD8⁺ T cells isolated from tumor infiltrating lymphocytes (TIL) or genetically-modified autologous circulating CD8⁺ T cells. The engineered T cells expressed tumor-specific antigen receptors including chimeric antigen receptors (CARs) and T cell receptors (TCRs), prepared from cultured B and T cell clones, respectively. The most successful ACT, anti-CD19 chimeric antigen receptor T (CAR-T) cell therapy directed against B cell lymphoma, is already approved for use based on evidence of efficacy. Efficacy of solid tumors is not yet forthcoming. This review summarizes current technology developments using ACT in clinical trials. In this review, differences between various ACT approaches are discussed. Furthermore, resistance factors in the tumor microenvironment are also considered, as are immune related adverse effects, critical clinic monitoring parameters and potential mitigation approaches.

1. Introduction

CD8⁺ T cells are the major immune cells to conduct immune surveillance to detect antigens derived from cancer cells and developing malignancies. CD8⁺ T cells are activated by TCR antigen recognition, followed by fast proliferation and differentiation into cytotoxic T lymphocytes (CTL), thus to eliminate cancer cells in the cell-cell contact manner. Lymphocytes are often found in the tumour lesion. The tumour infiltrating lymphocytes (TIL) contain immune cells specific to the

tumour. The ex vivo cultured expanded cancer-specific CD8⁺ T cells can be infused back to the patient to control the tumour since 1988. This process is defined as adoptive cell-based therapy (ACT) since 1988. Initial ACTs were performed using cells expanded from TIL (Fig. 1A). More recently, gene-engineering technology enables the consistent generation of a large amount of cancer-specific T cells by transferring antigen receptor into in vitro activated T cells, such as TCR engineered T cells (TCR-T) and Chimeric Antigen Receptor T-cell (CAR-T), for cancer immunotherapy [1]. (Fig. 1A and B). In comparison to TIL based

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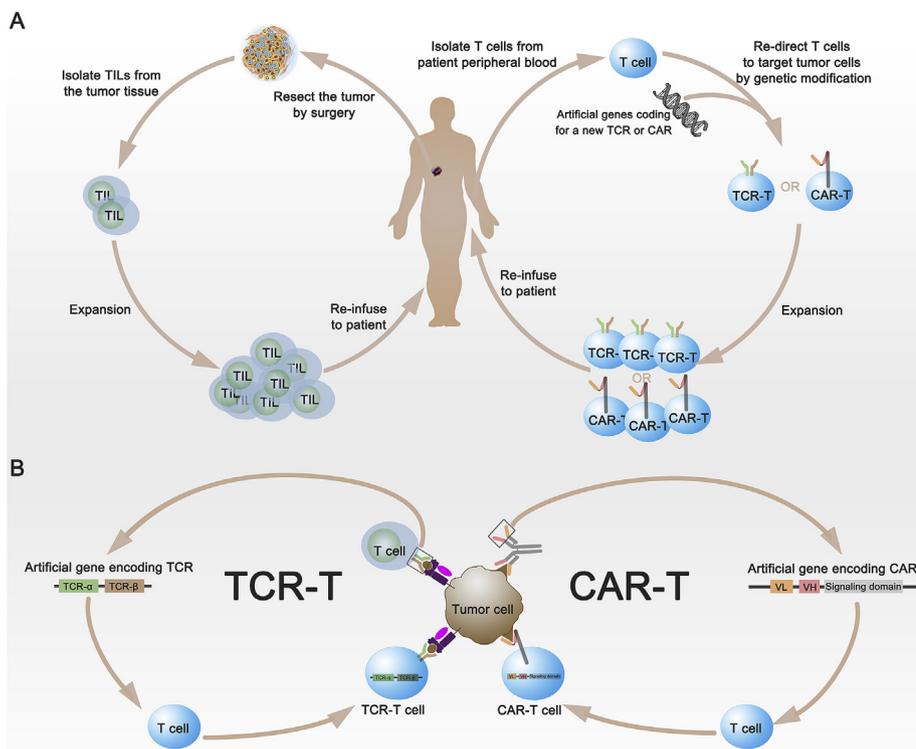


Fig. 1. Adoptive cell therapy is currently represented by three general approaches. TILs are derived from surgical excision of tumor, and TCR- and CAR-modified T cells are produced from peripheral blood lymphocytes with genetic engineered antigen receptors. A. The universal procedure of ACT. B. The different binding pattern of TCR-T and CAR-T.

ACT, TCR-T and CAR-T are much more consistent and robust process to produce large amount of cancer specific T cells. While the antigen receptors may derived from the TIL from certain patients, the use of the identified receptor can be extended to many patients with similar cancer. TCR-T and CAR-T revolutionized the ACT, and have generated promising results in some cancer types, so far. However, some problems remain to be resolved for broad use of ACT in cancer clinic, such as safety, efficacy, and persistence. In this review, we summarized the progress and the challenges of ACT in clinical usage.

2. The milestones of ACT to cancer

In ACT, autologous CD8⁺ T cells are derived from patients' blood, tumor-associated lymph nodes or fresh tumor samples. The cancer specific T cells are expanded in vitro, and infused back into the patients. The tumor infiltrating lymphocytes (TILs), harvested from the liver and pulmonary metastases of a MCA-105 sarcoma patient, is the earliest ACT pioneered by Steven Rosenberg in 1988 [2]. Later, the multimeric peptide-MHC technology and antigen-dependent IFN- γ -secretion assay were used to isolate tumor-specific T cells from tumor sample and blood. Rosenberg and others firstly achieved cancer control by the adoptive transfer of autologous TILs after IL-2 administration for melanoma with 60% objective response rate and the response range from 2 to 13 month [3]. In 2002, lymphodepletion before TIL injection was realized to promote the transferred cell proliferation, functional maturation and be critical to cancer regression after ACT [4]. Nevertheless, a key process, in vitro expansion of tumor-specific T cells from TIL, remains to be technically challenging. In 2006, the first therapy using genetically modified CD8⁺ T cells with the TCR recognizing MART1 melanoma antigen was reported to cause regression of metastatic melanoma in two patients but the transferred T cells engrafted well in all patients [5]. In 2011, TCR-T therapy specific a newly identified tumor antigen, NY-ESO-1, achieved success in treating synovial sarcoma with an objective response rate of 67% [6]. A distinctive type of antigen receptor, chimeric antigen receptor (CAR) targeting the B lymphocytes antigen CD19, was developed to treat advanced B cell lymphoma in 2010. CD19-specific CAR-T has shown great clinical

efficacy in treating follicular lymphoma, large-cell lymphomas, chronic lymphocytic leukemia, and acute lymphocytic leukemia [7–10]. In 2014, the FDA approved CD19-specific CAR-T therapy for relapsed/refractory ALL. In 2017, two commercial CAR-Ts were approved by the FDA for treating B-cell malignancies: Novartis's CAR-T (Kymriah) for children and young adults with refractory B-cell acute lymphoblastic leukemia (ALL), and Kite's CAR-T (Yescarta) for adults with certain types of large B-cell lymphoma [11] (Fig. 2). Until Dec. 2018, there are total of almost 600 clinic trials with engineered CD8 T cells immunotherapy worldwide. USA and China are the top two countries in the numbers of clinical trials in CAR-T and TCR-T-cell therapy (Fig. 3A). The clinical trials in China mainly located in 5–6 large cities with more prospective local economy (Fig. 3B and C). According to unpublished data, the cost of 30,000USD in China for every patient in the trial is cheaper than the cost of 50,000USD in US, except human resource cost. With the recent rapid progress in identification of tumor-specific antigen, CAR-T and TCR-T clinical trials are now actively targeting many hematological cancers and variety types of solid tumors. The CAR-T therapies predominantly target the hematological cancers, e.g. lymphoma and multiple myeloma, as anti CD19 CAR-Ts have shown their great efficacies in disease control, including disease free survival in some cases. TCR-T therapy are more targeting to solid tumors including Lung, liver, melanoma, head and neck cancer and lymphoma (Fig. 4).

2.1. TCR engineered T cells (TCR-T)

TCR-Ts are genetically modified T cells expressing both α and β chain of a TCR specific to a tumor antigen. The source of TCR can be from a person carrying at least one same particular HLA allele as the patient to be treated or from humanized mice with a same HLA allele immunized with tumor antigens. Theoretically, the safest source of TCR is the T cell repertoire from an HLA fully matched individual. Utilizing the autologous circulating lymphocytes as recipient cells, the TCR-Ts can be rapidly expanded through a stimulation using anti-CD3, anti-CD28 antibodies and IL-2 in vitro. TCR-Ts recognize the complex formed by tumor antigen-derived peptide and class I HLA. While the recognition is restricted by HLA type, TCR-T can recognize both cell

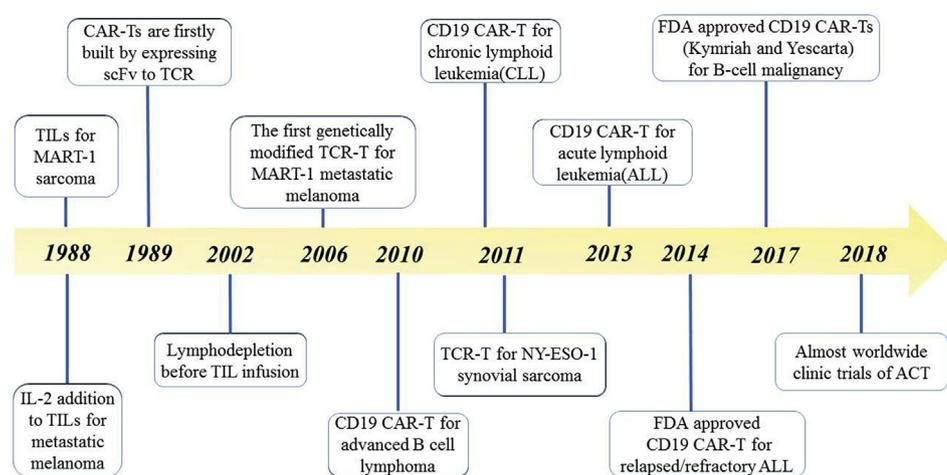


Fig. 2. A brief history of ACT. Figure depicted key events in the development of ACT in treating cancer from the year 1988–2018. Important milestones include 1) initial pioneer work by Steven Rosenberg using TIL to treat metastatic sarcoma; 2) transfer of genetically modified T cells expression cancer cell specific antigen receptor since 2006; 3) FDA first approval of CAR-T use in clinic in 2014.

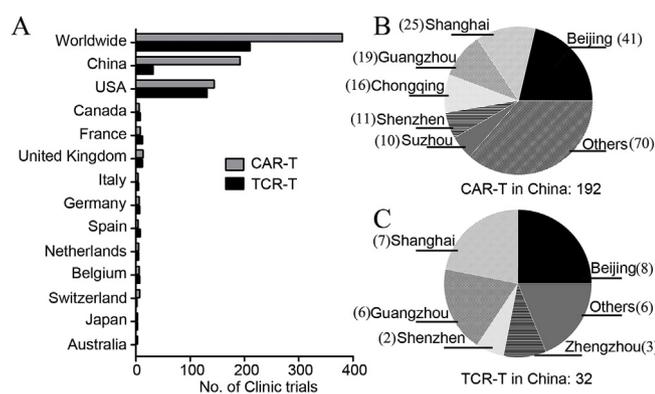


Fig. 3. The geometric distribution of clinical trials of CAR-T and TCR-T. Regional economic status strongly influences the access to ACT in cancer clinic. (A). The global distribution of clinical trials with CAR-T and TCR-T cells. United States and China are the top 2 countries of clinic trials of engineered T cells (CAR-T and TCR-T), take almost 90% of the total number of trials worldwide, suggesting that strong economic support is a major factor influencing the patient's access to ACT therapy. Even within China, current CAR-T and TCR-T trials are preferentially located in few cities with better economic environment (B and C). Among them, three super-sized cities Beijing, Shanghai and Guangzhou are the most advanced cities in clinic trials of engineered T cells, together taking about 50% of total number of trial in China. Innovations aiming for cost cutting for the ACT therapy will be a major challenge for its clinic application.

surface and intracellular tumor antigen [12]. The first clinical trial conducted by Morgan and his partners shown that the infusion of MART-1 melanoma antigen-specific TCR-Ts led to objective regression in two patients with metastatic melanoma, in 2006 [5]. TCR-Ts targeting a number of tumor antigens are currently under clinical trials including MAGE-A3, MAGE-A4, GD2, mesothelin, gp100, MART1,

CD19, AFP, CEA, NY-ESO-1, HER2, HPV, EBV, etc. NY-ESO-1 is the most promising target for many cancer immunotherapy [12–14] (Table 1).

2.2. CAR-T

CAR-Ts are constructed with an extracellular part of antibody-derived single-chain variable fragment (scFv) to T-cell receptor or co-receptors' intracellular signaling domains, pioneered by Zelig Eshhar and Gideon Gross in 1989 [15]. CAR-Ts only recognize cell surface antigens but not restricted by HLA type. Types of antigens recognized by CAR-T can be extended to carbohydrates and glycolipids which often change in cancer cells [16]. Additional engineering of CAR to include intracellular signaling motifs from costimulatory molecules such as CD28, 4-1BB, OX40, and ICOS improves CAR-Ts proliferation and functions such as cancer cell killing activity and cytokine production [11]. Comparing to TCR-Ts, CAR-Ts are more universally applicable to patients with various HLA types and to cancer type lacking HLA expression, which is a common strategy for cancer immune escape. The clinical trials of CAR-T involve the tumor targets of CD19, CD22, CD23, CD30, ROR-1, CAIX, PSMA, MUC1, FRz, meso-RNA, CEA, CD213a2, HER2, and yield encouraging results in treating multiple malignancies [17] (Table 2).

3. The challenges and opportunities of adoptive CD8⁺ T cells immunotherapy

3.1. The tumor-antigen specificity and mutation

Genetically modified T cells carrying cloned tumor antigen receptors have been developed to generate a large quantity of tumor-specific CD8⁺ T cell for therapeutic use. Both viral vectors (adenovirus, retroviruses and lentiviruses) and non-viral systems (Sleeping Beauty [18] transposon system and CRISPR [19]) have been used to transfer antigen receptor into autologous CD8⁺ T cells in vitro. All cancers

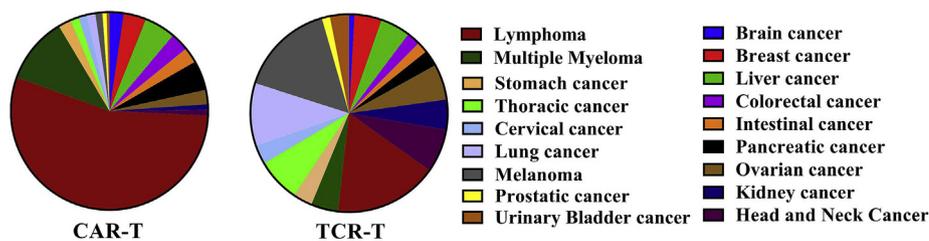


Fig. 4. The composition of cancer types in CAR-T and TCR-T trials. The hematological malignancies (lymphoma and multiple myeloma) are the major cancer types for ACT therapy trials. A diversified spectrum of solid tumors is also been tested, among which pancreatic cancer and breast cancer are the top three trials in CAR-T. TCR-T therapy has been seen with better efficacy in treating solid tumors, thus more diversified cancer types have been trialed for TCR-Therapy in comparison to CAR-T therapy. Major cancer types including lymphoma, thoracic cancer, cervical cancer, head and neck cancer and colorectal cancer.

Table 1
Selected TCR-T trails against common tumor antigen for tumors.

Target	Cancer Type	Identifier
NY-ESO-1	Synovial Sarcoma	NCT02869217, NCT03250325, NCT03462316, NCT03399448, NCT01343043, NCT02319824, NCT02457650, NCT03450122, NCT01477021, NCT02992743
	Melanoma	NCT02869217, NCT03399448, NCT02457650, NCT01967823, NCT02062359, NCT00670748, NCT01457131, NCT01350401
	Multiple Myeloma	NCT03399448, NCT02457650, NCT03506802, NCT02588612, NCT01892293
	Lung Cancer	NCT02869217, NCT03029273, NCT02457650, NCT01967823, NCT02588612
	Ovarian cancer	NCT02869217, NCT03159585, NCT03691376, NCT02457650, NCT03017131, NCT03017131
	Liver Cancer	NCT02869217, NCT03159585, NCT01967823
	Breast cancer	NCT03159585, NCT02457650, NCT01967823
	Esophageal Cancer	NCT02869217, NCT03159585, NCT02457650
	Bladder cancer	NCT02869217, NCT03159585
	Melanoma	NCT01273181, NCT03139370, NCT02153905, NCT02111850
	Renal Cancer	NCT01273181, NCT03139370, NCT02153905, NCT02111850
	MAGE-A3	Breast Cancer
Cervical Cancer		NCT02153905, NCT02111850
Bladder Cancer		NCT02153905, NCT02111850
MART-1	Melanoma	NCT00720031, NCT02424916, NCT00612222, NCT00509288, NCT01495572, NCT00512889, NCT00910650

result from mutations in the genomic DNA sequence of cancer cells [20]. The data from the world's largest and most comprehensive resource (TCGA database) for seeking the impact of somatic mutations in human cancers show that there are 2,002,811 coding region mutations, over six million noncoding mutations, 10,534 gene fusions, 61,299 genome rearrangements, 695,504 abnormal copy number segments and 60,119,787 abnormal expression variants in 1,029,547 tumor samples, involving almost all human genes [21]. Genetic mutations drive cancer development; but also benefit the cancer immunotherapy by providing neoantigens which are absent from the normal tissue. Accumulated results from clinical trials indicated that the tumor mutation burden (TMB) is a predictive biomarker for treatment response of ACT and immune checkpoint inhibition therapy [22]. While immune checkpoint inhibitors (ICI) therapies generate a better outcome for patients with high TMB [23], patients with low TMB respond better to personalized CAR-T or TCR-T therapy [24]. Because a significant fraction of antigenic mutations in human tumors are not shared between patients, most of the neoantigens are private antigens thus require personalized treatment. Combining whole-exome sequencing (WES) and bioinformatics analysis, personalized neoantigen-specific CAR-T cells were developed [25].

Primary tumors can be genetically different from the associated metastatic lesions or local recurrences even for the very same patient. Cancer cells in the different region of the same primary tumor lesions are also genetically different [26]. Nearly 70% of all somatic mutations found on multiregional sequencing were geometrically heterogeneous and thus not detectable in every sequenced region [27]. Single cell sequencing technology revealed that cancer stem cells are also genetically heterogeneous, making ACT even more challenging [28].

Under the selection pressure of ACT, the cancer cells may evolve to escape the recognition by CD8⁺ T cells due to epitope mutation, leading to tumor recurrence. In 2015, Stephan A. Grupp reported that most of the relapse of pediatric B-cell acute lymphoblastic leukemia (B-ALL) after the CAR-T-19 treatment is from the selection of CD19 variant with the mutated CAR-T-19 epitope [29]. Ideal neoantigens to target are those essential to the cellular transformation, cancer cell growth, and invasion with minimal normal tissue toxicity. Comparing to neoantigen CAR-T, neoantigen TCR-T relies on not only identification of neoantigen, but also the identification for HLA restricted neoepitopes and their corresponding TCR [30,31]. The key to success is that the T cell should carry an antigen receptor with high specificity to tumor neoantigen. The affinity between the antigen receptor and its antigen might also play an essential role in therapeutic efficacy. The TCR repertoire of naturally primed CD8⁺ T cells includes mostly TCRs with medium to low affinity, some of which are cross-reactive to autoantigens [32]. Nevertheless, lower affinity TCR may facilitate the priming of long lasting immune memory [33], including stem cells like memory T cells (Tscm). Mysteriously, natural TIL therapy seemed only succeeding in controlling melanoma and Hodgkin's disease but failed in other tumor types with identifiable tumor-associated antigens [34]. This could be a result of selective expanding of high affinity CTL clones under the in vitro culture condition. It is still unclear what is the optimal TCR affinity in determining the therapy outcome. As most of antibodies have much higher affinity than TCR, we speculate that CAR-T would be less favorable in treating solid tumor. The differences between TCR-T and CAR-T are listed in Table 3.

3.2. The influence of immunosuppressive tumor microenvironment (TME) to ACT

Multiple redundant cellular and molecular immune suppressive mechanisms in the TME cause CD8⁺ T cells dysfunction and exhaustion. Both cancer cells and host cells in the tumor play a role in inhibiting the anti-tumor immune response. Intratumor host accessory cells include cancer-associated fibroblast [35], endothelial cells [36], pericytes [37], immunosuppressive immune cells (Treg, tumor-

Table 2
Selected CAR-T trails against common tumor antigen for tumors.

Target	Cancer types	Identifier
Mesothelin	Lung Cancer	NCT03198052,NCT03356808,NCT01583686,NCT03054298,NCT02414269
	Pancreatic cancer	NCT01897415,NCT03497819,NCT03638193,NCT02465983,NCT03267173,NCT01583686,NCT02706782 NCT02580747,NCT02159716
	Ovarian Cancer	NCT03608618,NCT01583686,NCT03054298,NCT02580747,NCT02159716,NCT03692637
	Breast cancer	NCT02792114,NCT02580747,NCT02414269
	Pleural Mesothelioma	NCT01355965,NCT01583686,NCT03054298,NCT02580747,NCT02159716,NCT02414269
	Cervical Cancer	NCT03356795,NCT01583686
	Unnamed solid tumor	NCT02930993,NCT03545815,NCT03030001,NCT03182803,NCT03747965,NCT03615313
HER-2	Brain cancer	NCT03696030,NCT03500991,NCT02442297,NCT03389230,NCT02713984
	Lung Cancer	NCT03198052,NCT03740256,NCT02706392
	Breast Cancer	NCT02713984,NCT02547961,NCT03740256,NCT02706392,NCT02792114
	Pancreatic cancer	NCT03267173,NCT03740256,NCT01688609,NCT02713984
	Gastric Cancer	NCT03740256,NCT02713984
	Colorectal Cancer	NCT02713984,NCT03740256
	Bladder Cancer	NCT03740256
	Esophageal Cancer	NCT03740256
	Sarcoma	NCT00902044,NCT03740256
	Metastatic melanoma	NCT00924287
MUC-1	Lung Cancer	NCT03525782,NCT03356808,NCT03198052,NCT02587689
	Esophageal Cancer	NCT03706326
	Liver cancer	NCT03633773,NCT02587689
	Cervical Cancer	NCT03356795
	Brain cancer	NCT02617134
	Gastric Carcinoma	NCT02617134
	Colorectal Carcinoma	NCT02617134
	Breast cancer	NCT02587689
Pancreatic cancer	NCT02587689	

Table 3
The comparison of CAR-T and TCR-T.

	CAR-T	TCR-T
Antigen binding domain	Single chain variable fragment (ScFv)	Engineered TCR
Binding domain resource	Murine	Human or murine
Binding affinity	High	Low or Medium
MHC-I restriction	No	Yes
Co-stimulators requirement	No(form 3rd generation)	Yes
Manufacturing	Easy	Hard
FDA approved therapy	Hematological malignancy	None
Cytokine release syndrome	Serve, even fatal neurotoxicity	Medium

associated macrophages, tolerogenic dendritic cells, Breg, myeloid-derived suppressor cells, mast cells) [38,39]. These accessory cells create immunosuppressive TME via pathways including 1) creating and reinforcing immune checkpoints [40]; 2) secreting immune suppressive cytokines e.g. transforming growth factor-beta (TGF- β) and IL10 [41]; 3) Physical elements(stiff ECM) [42]; 4) Chemical elements unfavorable to immune activation [43].

Immune checkpoints are key regulatory points of the immune system to maintain immune tolerance to self. Activation of immune checkpoint(s) renders exhaustion of T cells by functional inactivation or physical deletion. Tumors exploit the immune checkpoints to evade immune destruction and cause tumor-specific T cell exhaustion. Most of the immune checkpoint molecules are cell surface receptors on activated T cells such as PD-1 [44], CTLA4 [45], and LAG3 [46] etc. and contain tyrosine-based inhibitory motifs (ITIMs) [47] as well as immunoreceptor tyrosine-based inhibitory switch motifs (ITSMs) [48]. The inhibition of PD-1/PD-L1 and CTLA-4 effectively blocked the T cell exhaustion and apoptosis, and facilitated the anti-tumor immune response, which was granted with Nobel Prize in 2018. FDA approved immune checkpoint inhibition therapy against melanoma, NSCLC, RCC, HNC, bladder cancer, HCC, urothelial cancer, cervical cancer and stomach cancer. Ligands for these immune checkpoint receptors are expressed in TME thus trigger the T cells exhaustion in TME. Immune

checkpoints can be blocked using antibodies against immune checkpoint receptors to prevent the interaction between the receptors and their ligands [49]. IDO is a unique immune checkpoint protein by promoting tryptophan degradation via the kynurenine pathway and can be blocked using small molecule inhibitors. By converting tryptophan to kynurenine, IDO promotes immune suppression via two distinctive molecular mechanisms, 1) depletion of an essential amino acid, tryptophan, to activate cellular stress pathways via GCN2 [50] and 2) generating downstream immune suppressive metabolites such as 3-hydroxyanthranilic acid [51] and xanthurenic acid [52]. Details of these mechanisms are extensively discussed in reviews [53]; thus we will not discuss this further due to the space limitation. Some immune checkpoint blockers are approved to be used in treating multiple cancer types, and combination therapies with ACT together are currently under clinic trials for various cancers, e.g. combination therapy of ACT with *anti*-CTLA4 (NCT02027935) or *anti*-PD1 (NCT02652455) [54,55].

Immune suppressive cytokines play important roles in immune tolerance to tumor. TGF- β , IL10 [56], VEGF [57], and prostaglandin E2 (PGE2) [58] are all recognized as major players in suppressing immunity using non-redundant mechanisms, including suppressing anti-tumor immunity of CTL, conventional dendritic cells, pDC, Natural killer cells, but supporting pro-tumor immunity of TAM, MDSC, Treg [59,60].

The stiff ECM together with tumor metabolic state create a physical acidic, hypoxia [61] local environment filled with polyamines produced via activated arginase pathway [62,63]. This shelter the tumor antigen from immune surveillance, trap the cytotoxic T cells from infiltrating into tumor [64], and promote type 2 tumor-associated macrophage polarization and Treg activation [65,66].

The hostile metabolite situation in TME conducts a chemical barrier against ACT. The balance of nutrients and oxygen controls ACT destiny. The cancer cells won the competition of the consumption of glucose and amino acid against immune cells [67]. Besides of immune cells starving, HIF-1 α in hypoxia induced glycolysis and lactate, which both facilitate PD-L1 on tumor cells and stromal cells and PD-1 on CTL [68].

3.3. The short persistence of adoptively transferred tumor-specific T cells

The longevity of ACT is an important factor for long-lasting tumor control effect. Current methodology uses initial potent activating stimuli, including CD3/CD28 stimulation, IL-2 or allogeneic feeder cells, to produce T cells which can be transfected to carry tumor-specific CAR or TCR. This process generates a large number of functional CTLs with high level of perforin and granzyme. These CTLs are effective in controlling tumor in the short term while long term efficacy is variable due to complex immunosuppressive tumor microenvironment [69]. This is particularly problematic in treating solid tumors. It seems to be that the efficacy of initial tumor control by ACT is associated with high-affinity T cell engraftment, but this may not be true if a long-lasting antitumor T cell persistence is required. Low affinity T cells with the capability becoming stem memory T cells (Tscm) were reported to produce better and long lasting protective effects in mouse model of HCC [33]. Generating long lasting memory T cells including Tscm probably is the holy grail of ACT to reach the goal of long lasting tumor control or eradication. The number of memory T cells required for initial adoptive transfer therapy will be significantly lower than the current practice as memory cells can be primed *in vivo* using vaccine to regenerate CTL as needed. Current approaches to induce Tscm-like ACT include 1) Cytokines addition: IL-7, IL-15, IL-21 [70]; 2) using inhibitors for AKT, mTORC and PI3K [71,72]; 3) activation of NOTCH [73]; 4) Weak TCR signaling during activation [74]; 5) providing additional costimulation e.g. 4-1BB, ICOS [75]; 6) altered metabolism status [76,77]. These potentially persistent tumor specific T cells could control cancer and prevent recurrence for a long time [78,79].

3.4. The immune related toxicity of ACT therapy

Toxicities from immune-related adverse effects (irAEs) were widely reported in many ACT cases including cytokine release syndrome (CRS), CAR-T-related encephalopathy syndrome (CRES), off-target/off-tumor toxicity. Unlike the immediate adverse effect of other chemotherapeutic drugs, the adverse effects of ACT happen weeks after T cells infusion and affect multiple organs [80].

3.4.1. The CRS with uncontrollable inflammatory cytokines

CRS is the most common life-threatening toxicity due to uncontrollable release of proinflammatory cytokines. CRS is characterized by high fever, skin rash, hypotension, hypoxia, cardiac dysfunction, kidney failure, electrolyte abnormalities and neurologic symptoms, even death [80]. The use of Glucocorticoids and/or antibodies blocking IL-1 receptor (Anakinra) and IL-6 receptor (Tocilizumab), but not IFN- γ and TNF- α , could effectively reverse CRS [81,82].

3.4.2. The on-target/off-tumor toxicity relates to antigens both on tumour and normal cells

The on-target/off-tumor toxicity is common in both CAR-T and TCR-T therapy. Many target antigens for cancer therapies are also present on normal cells, such CD19 on B cells, MART-1 on melanocytes, CAIX on biliary duct epithelium and CEA on normal gastrointestinal epithelial cells. Activated T cells targeting these antigens can recognize the same antigens expressed in normal tissues and cause damage. The CD19 CAR-T therapy always induces transient or prolonged lack of B cells, resulting in adaptive immunoglobulin deficiency [83]. Severe toxicity was reported for multiple tissues damage including the skin, eyes, and ears in a late stage clinical trial enrolled 36 patients with metastatic melanoma received MART-1 or gp100-specific TCR-T cells [84,85].

3.4.3. The cross-reactive off-target/off-tumor toxicities

Off-target/off-tumor toxicities are caused by cross-reactivity of antigen receptor to autoantigens not associated with tumors. Tumor antigen MAGE-A3-specific TCR-T reacts with a few known autoantigens including MAGE-A12 expressed in brain and titin expressed in cardiac

muscle. An early-stage clinical trial using MAGE-A3-specific TCR-T resulted in two death from total nine patients due to severe neurological toxicity and two cardiovascular-related deaths in another clinical trial [86,87].

3.4.4. The neurotoxicity of ACT

The irAEs related neurotoxicity including confusion, delirium, expressive aphasia, obtundation, myoclonus, and seizure has been reported in patients after receiving ACT. It is unclear how the tumor targeting immune response causes the neurological toxicities because the central nervous system (CNS) is usually well isolated from peripheral tissue by the tightly controlled blood-brain barrier. The severity of CRS, on target/off-tumor and off-target/off-tumor, may together contribute to the neurotoxicity [88]. It is worthwhile to mention that elevated kynurenine production by inducing indoleamine 2, 3 dioxygenase (IDO) during infection led to neuropathic pain [89]. Same mechanisms probably contribute to irAEs, such as kynurenine production being often increased in cancer patient and further enhanced by therapy [90]. CAR-T-related encephalopathy syndrome (CRES) is the most severe neurological syndrome and its development is critically dependent on monocytes-derived IL-1 and IL-6. Neutralizing these two cytokines reversed CRES effectively [81]. The inflammatory cytokines may activate endothelial cells of the blood-brain barrier and disrupt the barrier integrity, and endothelial cell activation in the central nervous system might drive the CAR-T therapy associated neurotoxicity [91].

3.4.5. The prevention and treatment for the irAEs

Efforts have been made to improve the safety of ACT tumor therapy. The first line drugs against irAEs are glucocorticoid and dexamethasone, with supportive care [92]. Some antagonists to inflammatory cytokines also decrease the toxicity [43]. Recently, self-amplifying catecholamine loop was found as a self-amplifier of CRS, and inhibiting key steps in the catecholamine synthesis pathway with metyrosine effectively reduced the CRS toxicity in mouse [93].

The on-demand cell destruction of engineered T cells is designed with co-transfer of suicide genes together with antigen receptors. Commonly used suicide genes include herpes simplex virus thymidine kinase (HSV-TK), inducible caspase-9 (iCasp9). Insertion of HSV-TK increases the T cells' susceptibility to ganciclovir. The strategy was developed to control graft-versus-host disease (GVHD) after allogeneic transplantation or ease immunologic response after CTLs infuse into immune-deficient hosts. iCasp9 is a genetically engineered human caspase-9 protein with the endogenous activation domain replaced by a controllable dimerization domain from human FK506-binding protein. A mutation (F36V) was introduced to allow specific binding to a bioinert chemical induction of dimerization (CID) drug, AP1903. Upon binding to AP1903, iCasp9 activates and leads to quick apoptotic cell death of CTLs [18,94]. Introducing these suicide genes made the CAR-T and TCR-T more controllable, but remains to be challenging in clinic. HSV-TK is immunogenic, and the induction of cell death is a slow process [95]. Thus, it is hard to reverse strong acute CRS toxicities, although HSV-TK renders the cells sensitive to ganciclovir [96]. iCasp9 is activated by a CID that is not widely available and extensively tested. The efficacy and safety of the broad usage of these suicide genes in cancer ACT need to be further evaluated.

4. Conclusion

After decades of research, the power of CD8⁺ T cells has finally been wielded in our battle against cancer. Clinical application of ACT in tumor therapy starts to achieve encouraging results. Recent technology advances such as bioinformatics, deep sequencing in single-cell level, structure biology, and genetic-manipulation etc. continuously supply new weapons to the old foot soldier CD8⁺ T cells. Nevertheless, many challenges remain to be solved to overcome the scientific (Fig. 5), and economic (Fig. 3B and C) barriers, with the global high price tag of

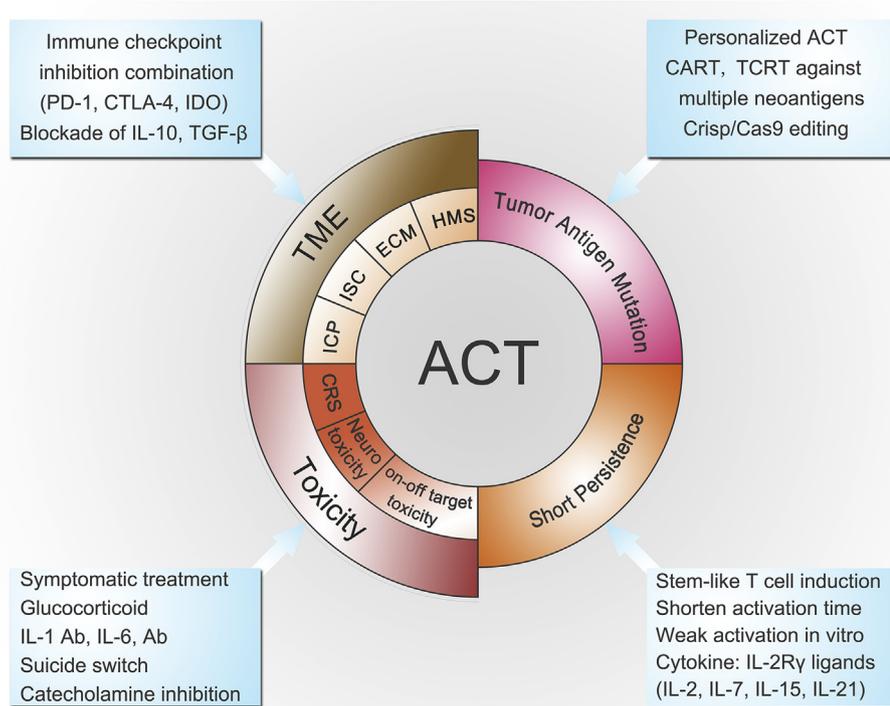


Fig. 5. Optimization strategy for successful ACT therapy in clinic. Four major factors are to be improved when apply ACT therapy in cancer clinic. (1) Tumor antigen mutation may not always fit an available generic antigen receptor. Development of personalized ACT by identifying tumor neo-antigens and their specific antigen receptors using Nextgen sequencing plus bioinformatics analysis will better tailor the therapy from individual to serve broader patient population. More efficient targeted gene delivery technologies such as Crispr/Cas9 gene editing will accelerate service to bypass long process of viral vector production, increase both safety and efficiency. (2) During tumor progression, cancer cells change normal developmental procedure to setup a supportive but immunosuppressive tumor microenvironment (TME) comprised of immune cells, fibroblasts, pericytes, and endothelial cells, often covered within extracellular matrix (ECM), with abnormality of metabolism and hypoxia. Short persistence of transferred immune cells and immune suppressive TME pose major threats for successful therapy and long lasting protection. Combination therapies to include ICI agents and/or blocking antibodies against immune suppressive cytokines are expected to greatly improve ACT therapies. Selection of long lasting memory T cells to carried the cancer targeting antigen receptor are expected to offer long term protection as well as reducing cell

number required for initial transfer. (3) ACT therapy toxicities, sometime lethal adverse effects, are already well known. Efforts have been taken to develop cancer targeting immune cells with sophisticated genetically engineer including safe-keep mechanisms, but this approach is yet to be demonstrated successful. There is a strong need to develop a standardized guideline to recognize and treat ACT therapy adverse effects. Most of acute symptoms related ACT therapy toxicities are treatable if recognized on time. Long-term consequences, in particular neurotoxicity are yet to be understood. TME: Tumor microenvironment. ICP: Immune Checkpoint. ISC: Immunosuppressive cytokines. ECM: Hostile Metabolic State. CRS: Cytokine release syndrome.

Kymriah (475,000 USD in US, 307,000 USD in Japan, 373,000 USD in UK), to establish a universal and viable therapeutic platform.

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List of Abbreviation

- ACT adoptive CD8⁺ T cell therapy
- TIL tumor infiltrating lymphocytes
- CAR chimeric antigen receptor
- CAR-T chimeric antigen receptor T cells
- TCR T cell receptor
- TCR-T TCR engineered T cells
- CTL Cytotoxic T cells
- MART-1 Melanoma antigen recognized by T-cells 1
- CRISPR clusters of regularly interspaced short palindromic repeats
- HLA human leukocyte antigen
- MAGE-A Melanoma-associated antigen
- NY-ESO-1 New York esophageal squamous cell carcinoma 1
- HER2 human epidermal growth factor receptor 2
- FR α anti-alpha folate receptor
- HPV Human papillomavirus
- HBV Hepatitis B virus
- EBV Epstein-Barr Virus
- ScFv antibody-derived single-chain variable fragment
- ICOS inducible T cell costimulator
- ROR1 receptor tyrosine kinase like orphan receptor 1
- CA IX Carbonic anhydrase IX
- PMSA Prostate specific membrane antigen

- MUC1 Mucin 1
- CEA carcinoembryonic antigen
- TMB tumor mutation burden
- WES whole-exome sequencing
- irAEs immune-related adverse effects
- CRS cytokine release syndrome
- CRES CAR-T-related encephalopathy syndrome
- IDO indoleamine 2, 3 dioxygenase
- HSV-TK herpes simplex virus thymidine kinase
- iCasp9 inducible caspase-9
- GVHD graft-versus-host disease
- CID chemical induction of dimerization
- TME tumor microenvironment
- ECM extracellular matrix
- PD-1 Programmed cell death protein 1
- CTLA-4 cytotoxic T-lymphocyte-associated protein 4
- LAG-3 Lymphocyte-activation gene 3
- ITIM tyrosine-based inhibitory motifs
- ITSM immunoreceptor tyrosine-based inhibitory switch motif (ITSMs)
- GCN2 general control nonderepressible 2
- VEGF Vascular endothelial growth factor
- Tscm stem-like memory T cells
- PI3K phosphatidylinositol 3-kinase
- mTORC The mammalian target of rapamycin

Declarations

Ethics approval and consent to participate. These issues are not applicable for this review.

Consent for publication

Not applicable.

Availability of data and materials

The dataset supporting the conclusions of this article is included within the article **Competing interests**.

The authors declare that they have no competing interests.

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Authors' contributions

SW and WW designed the study. XTJ, YX, MFL coordinated and drafted the manuscript. HX, LH, ALM edited and finalized the drafting of the manuscript. All authors read and approved the final manuscript.

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