



## Review

## Chimeric antigen receptor -T cell therapy: Applications and challenges in treatment of allergy and asthma

Abdolreza Esmaeilzadeh<sup>a,b</sup>, Safa Tahmasebi<sup>c</sup>, Seyyed Shamsadin Athari<sup>a,\*</sup><sup>a</sup> Department of Immunology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran<sup>b</sup> Cancer Gene Therapy Research Center (CGRC), Zanjan University of Medical Sciences, Zanjan, Iran<sup>c</sup> Department of Immunology, Health Faculty, Tehran University of Medical Sciences, Tehran, Iran

## ARTICLE INFO

## Keywords:

Adoptive cell therapy  
CAR-T cell  
Allergy  
Asthma  
T regulatory cells  
Immunotherapy

## ABSTRACT

Despite the current advancements, cancer treatment approaches have limitations restricting their cure rate. Immunotherapy techniques are among novel and promising cancer therapeutic approaches. Therapeutic antibodies and adoptive cell therapy (ACT) are the main branches of immunotherapy. T lymphocytes and genetically engineered cells are among important cells which can be used in ACT. This review has focused on recent advances in engineered cell-based immunotherapy based on T lymphocytes with chimeric antigen receptors (CARs). CARs are recombinant receptors expressing T cell signaling domains with or without co-stimulatory molecules. CAR-T cells are expanded ex vivo and re-infused to patients in order to improve their therapeutic efficacy. Nowadays, the beneficial function of CAR-T cell therapy has been indicated in various diseases including hematological malignancies, solid tumors, autoimmune diseases, and allergic diseases such as asthma. Furthermore, antigen-specific T regulatory cells (T<sub>regs</sub>) and gene-edited T cells seem to be beneficial in controlling inflammation in allergic asthma. In fact, dysregulated function of T<sub>regs</sub> is responsible for dominance of T helper 2 immune response and progression of allergic asthma. CAR-T<sub>reg</sub> cells can also be designed and reproduced using iT<sub>reg</sub> population to manage asthma. In addition, universal CAR-T cells can be modified to selectively target multiple antigens. The fourth generation CAR-T cells (i.e. TRUCK cells) represent novel strategies to cure asthma and allergic diseases as well. Despite the advantages of CAR-T cells, their applications can be associated with some unwanted reactions such as cytokine storm, anaphylaxis, neurotoxicity, etc. For clinical application, there is a need to prevent and manage these complications by optimizing ACT protocols.

**Abbreviations:** Ag, antigen; Ab, antibody; ACT, adoptive cell therapy; AHR, airway hyper responsiveness; BALf, broncho alveolar lavage fluid; B-ALL, B-cell acute lymphoblastic leukemia; BBIR, biotin-binding immune receptors; Bak, Bcl-2 homologous antagonist killer; Bax, Bcl-2-associated X protein; Bcl-xL, B-cell lymphoma-extra large; CAAR, creating chimeric autoantibody receptor; CAR, chimeric antigen receptor; Cas, CRISPR associated protein; CCL, Chemokine ligand; CCR, chemokine receptors; CD, cluster of differentiation; CRISPR, clustered regularly interspaced short palindromic repeats; CRS, cytokine release syndrome; CTLA, cytotoxic T-lymphocyte-associated protein; DC, dendritic cells; DGK, diacylglycerol kinase; dsDNA, double-strand DNA; EGFR, epidermal growth factor receptor; eBP, epsilon binding protein; FOXP, Forkhead box P; GE, genetically engineered; GM-CSF, granulocyte-macrophage colony-stimulating factor; GMP, good manufacturing practices; GVHD, graft-versus-host-disease; HLA, human leukocyte antigen; IBS, irritable bowel syndrome; ICOS, inducible costimulator; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; IND, investigational new drug; iP, immunoproteasome; ITAM, immune-receptor tyrosine-based activation motif; iTreg, induced Treg; iTTr35, IL-35 induced Treg; JAK, The Janus kinase; LAG, lymphocyte activation gene; LAP, Latency associated peptide; MCP, monocyte chemotactic protein; MDSC, myeloid-derived suppressor cells; MHC, major histocompatibility complex; MIP, macrophage inflammatory protein; mTOR, mammalian target of rapamycin; NFAT, nuclear factor of activated T cell; NF-κB, nuclear factor kappa-B; NK, natural killer; nTreg, natural Treg; OVA, ovalbumin; PBMC, peripheral blood mononuclear cells; PD, programmed death receptor; PD-L, programmed death receptor ligand; PG, prostaglandin; PI, phosphoinositide; RANK, receptor activator of nuclear factor kappa-B; RANKL, receptor activator of nuclear factor kappa-B ligand; scFv, single-chain variable fragment; SHP, Src homology region 2 domain-containing phosphatase; STAT, signal transducers and activators of transcription; SUPRA, split universal and programmable; TALEN, transcription activator-like effector nucleases; TCR, T cell receptor; TGF, transforming growth factor; Th, T helper; TIGIT, T Cell Immunoreceptor with Ig and ITIM Domains; TIM, T-cell immunoglobulin and mucin-domain containing (T-cell membrane protein); TLR, toll-like receptor; TNF, tumor necrosis factor alpha; TRAF, tumor necrosis family receptor-associated factor; TRUCK, T cell redirected for universal cytokine killing; TRAC, T Cell Receptor Alpha Constant; Treg, regulatory T cell; UniCAR-Treg, Universal CAR-Treg cell; UniCAR-T, Universal CAR-T cell; VEGF, vascular endothelial growth factor; VH, variable heavy; VL, variable light; ZFN, zinc-finger nuclease

\* Corresponding author.

E-mail addresses: [SS.Athari@gmail.com](mailto:SS.Athari@gmail.com), [SS.Athari@zums.ac.ir](mailto:SS.Athari@zums.ac.ir) (S.S. Athari).<https://doi.org/10.1016/j.bioph.2019.109685>

Received 19 September 2019; Received in revised form 28 October 2019; Accepted 16 November 2019

0753-3322/ © 2019 The Author(s). Published by Elsevier Masson SAS. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

During the recent decade, immunotherapy has taken very big strides forward from research laboratories to successful clinical trials. Therapeutic target-specific antibodies and cell therapy are the major branches of immunotherapy. ACT as an advanced immunotherapy technique has a significant role in stimulation and augmentation of immune system [1–3]. GE) cells have also been successfully developed as a new ACT strategy in recent years.

T lymphocytes which recognize and react against specific antigens, induce immune regulatory cytokines, and activate other immune cells have been presented as promising cells to be applied in ACT. CAR-T cells are manipulated lymphocytes expressing reengineered TCR combined with different co-stimulatory and signaling molecules. Actually, CARs are recombinant receptors including an antigen-binding domain named ScFv and a signaling domain with or without co-stimulatory domains. CAR-T cells which role as living drugs can be administrated by a single infusion and present advantages such as HLA-independent Ag recognition, simple construction of TCR complex, minimal risk of autoimmunity or GVHD, recognition of multiple antigens including proteins, carbohydrates and glycolipids, induction of strong and durable immune responses, and ability to be incorporated with high-affinity recombinant antibodies. Using CAR-T cells has resulted in favorable outcomes in hematological cancers; however, the therapeutic outcomes in solid tumors have been unsatisfactory due to the immunosuppressive features of solid tumors microenvironment. Efforts have been conducted to improve the safety and efficiency of CAR-T cells. The therapeutic roles of CAR-T cells have also been investigated in diseases such as infections, autoimmunity, as well as allergy and asthma.

The importance of immune suppressing regulatory T cells has been noted in the prevention of allergy, asthma, autoimmune diseases, and graft rejection. CAR-T cells can also be genetically engineered to carry a regulatory domain (i.e. CAR-T<sub>reg</sub>) to assist treating diseases such as allergy and asthma [4–6].

This review has focused on recent advancements in engineered cell-based immunotherapy and especially CAR-T cells and their roles in treatment and control of allergic diseases, particularly asthma. Here, we reviewed the molecular properties of CARs, as well as future perspectives and potential clinical applications of CAR-T cell therapy in allergic diseases and asthma.

## 2. CAR-T cells

CAR T-cells are genetically engineered peripheral lymphocytes containing a recombinant receptor targeting a specific antigen. These GE-T Cells are expanded ex vivo and re-infused into patients. CARs are recombinant receptors which are incorporated with co-stimulatory molecules. Inhibitory molecules are disabled in CARs to improve their activity [4,5]. Several subtypes of T cells including CD8<sup>+</sup> and CD4<sup>+</sup> T lymphocytes have been engineered as CAR-T cells and applied *in vivo*. CAR-T cells were first introduced by Eshar in 1989 who suggested re-directing CAR to armor T cells toward tumor sites [6,7]. The Novartis's CTL019 [B-cell acute lymphoblastic leukemia (B-ALL)], Kite's KTE-C19 (large B-cell lymphoma), and Juno's JCAR015 (B-ALL) have been among first-ever used FDA approved CAR-T cells [8].

### 2.1. The structure and signaling properties of CAR-T cells

CARs include a subtype of engineered TCRs. Engineered TCRs are generated by transferring  $\alpha$  and  $\beta$  chains from a known TCR to react with an antigen presented by a specific MHC. Multiple clinical trials have investigated the therapeutic potential of TCR-engineered T cells [9,10]. CARs; on the other hand, are chimeras of domains derived from antibodies and TCRs.

CARs are constituted from four essential domains. The first domain is the antigen recognition domain (i.e. CAR ecto-domain) conferring the

antigen-specificity of the receptor. The second domain is antigen binding domain containing a ScFv which is derived from a monoclonal antibody. This domain determines target specificity of the receptor obviating the need for antigen presentation by MHC. Nevertheless, co-expression of two separate variable heavy (VH) and variable light (VL) chains of the antibody is required to generate non-HLA restricted antigen recognition ability. The third domain is a spacer or hinge which gives flexibility to the ScFv part and ensures the stability of CARs on cell surface. The last domain of CARs is a transmembrane domain also contributing to the flexibility of CARs and in fact is a part of the hinge domain. The hinge and transmembrane domains are predominantly structural components of CARs; however, they also may promote functional roles by physical separation of the targeting moiety from the cell membrane [11–13]. Human IgG-derived spacer is commonly used as the spacer to stabilize CARs on cell surface [14,15]. In the transmembrane domain of CARs, CD3 $\zeta$  is required to facilitate dimerization and association of the receptor with other signaling and co-stimulatory molecules such as CD28 or CD8 [16,17]. Co-stimulatory molecules can also accompany the cytoplasmic signaling domain of CARs which generates downstream signals required for T cell activation, function, and proliferation [4]. The most commonly used signaling domains include CD3 $\zeta$  (CD3 zeta), CD28, 4-1BB (CD137), OX40 (CD134) and ICOS [18]. Recent studies have shown that the number of co-stimulatory domains can affect the phosphorylation of signaling proteins (i.e. LAT, ZAP-70, ERK, SYK and LCK) [19–21]. Therefore, masking/unmasking the phosphorylation sites on ITAM of CD3 $\zeta$  chains or other co-stimulatory molecules such as CD28 can have a crucial role in signal transduction of CARs [22,23]. Furthermore, the configuration of the signaling chains can influence the clustering of receptors and alter the initiation and/or the maintenance of signal transduction process [24,25]. Some studies have shown that signaling of TCRs is triggered by the separation of bulky phosphatases (i.e. CD45 and CD148) from immunological synapses which are formed between the membranes of T lymphocytes and target cells [26–28]. The formation of the immunological synapse is also essential for activation of CARs, and these recombinant receptors recruit similar biophysical and biochemical signaling events to activate T cells. Similar to B cell receptors (BCRs); however, CARs harbor fewer subunits and ITAMs. They can also bind to antigens with high affinity and in a MHC independent way [29,30].

### 2.2. CAR-T cells generations

CARs are classified into four generations based on the number of their cytoplasmic co-stimulatory signaling domains. In the first generation of CAR-T cells, the  $\zeta$  chain of the TCR/CD3 complex constituted from a single T cell activating domain generating sufficiently potent signals from the ITAMs (i.e. signal-1) [31,32]. The first generation of CARs did not contain co-stimulatory molecules. On the other hand, the second generation of CARs contained one co-stimulatory molecule (i.e. CD28) on the surface. It is notable that co-stimulatory signals (i.e. signal-2) are required for activation and proliferation of T-cells. The third generation of CAR-T cells contained two co-stimulatory molecules including CD27, CD28 (for IL-2 production), 4-1BB (cytokine release and T cell survival), OX40, ICOS (potentiate cytotoxicity and Th17 differentiation), and DAP10 or CD244 (2B4) [33–36]. The fourth generation of CAR-T cells constituted of ScFv-CD3 $\zeta$ , CD28, and 4-1BB. These cells are known as TRUCK. They recruited NFAT to induce gene cassettes containing IL-12 gene promoter (Figs. 1 & 2 and Table 1) [4,37].

### 2.3. Production of CAR-T cells

For producing CAR-T cells, isolated autologous PBMCs are initially activated with anti-CD3 and anti-CD28 monoclonal antibodies and then engineered to express CARs using viral vectors as powerful gene transfer systems. CAR-T cells are subsequently expanded ex vivo using

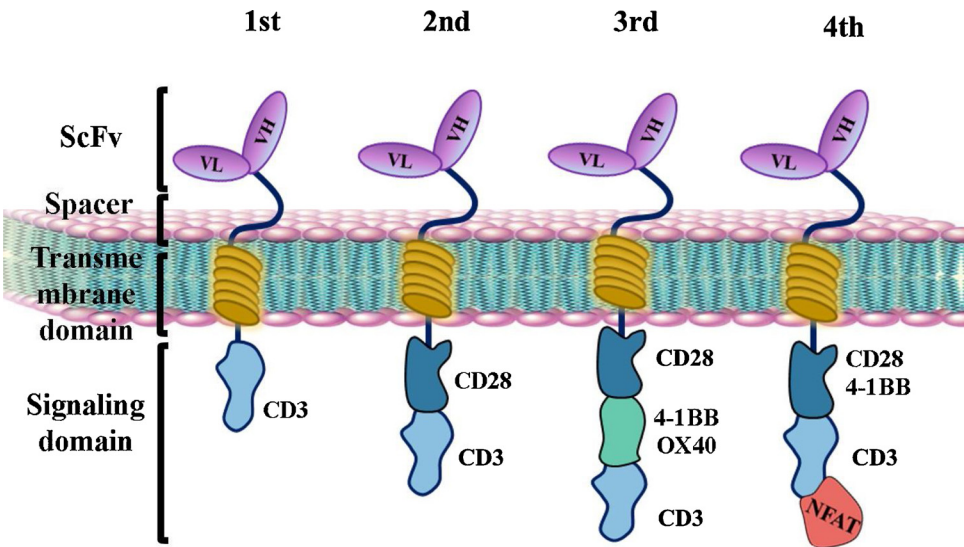


**Fig. 1.** All generations of CAR-T cells. In each new generation, signaling and applications are renewed. The first generation of CAR-T cell has CD3 $\zeta$  chain as intracellular domain and in the second generation of CAR-T cell, CD28 or CD 137 (4-1BB) are added to CD3 as co- stimulatory molecules. In the third generation of CAR-T cell, the second co- stimulatory moleculeOX-40 (CD134), is added. In the fourth generation, the nuclear factor of activated T cell (NFAT) to induce cassette containing IL-12 genes promoter is added.

cytokines and antibody coated beads and re-infused to patients following lymphodepletion [38,39]. Non-viral gene transfers techniques such as the Sleeping Beauty & PiggyBac transposon/transposase and electroporation of naked DNA plasmid have low integration efficiency. Electroporation of mRNA is another valuable and safe strategy to produce CARs [40–42].

The administration of CAR-T cells follows three steps including lymphocyte apheresis and collection, genetic manipulation, and re-infusion. These steps necessitate the availability of an apheresis center, as well as product-shipping and receipt-coordinating facilities. Also, all management guidelines should be applied in pre-and post-infusion care (Fig. 2).

According to the FDA’s guidelines, manufacturing procedure of CAR-T cells must attain Investigational New Drug (IND) approval and

| The molecules of all generations of the CAR-T cell. |                                                                                                                |
|-----------------------------------------------------|----------------------------------------------------------------------------------------------------------------|
| generation                                          | molecules                                                                                                      |
| First                                               | CD3 $\zeta$                                                                                                    |
| Second                                              | CD3 $\zeta$ and CD28 or CD 137                                                                                 |
| Third                                               | CD3 $\zeta$ and CD28 or CD 137 and CD134                                                                       |
| Fourth                                              | CD3 $\zeta$ and CD28 or CD 137 and CD134 and NFAT and with other applicable molecules according to the purpose |
| In the future                                       | Special uniCAR-Treg against asthma                                                                             |

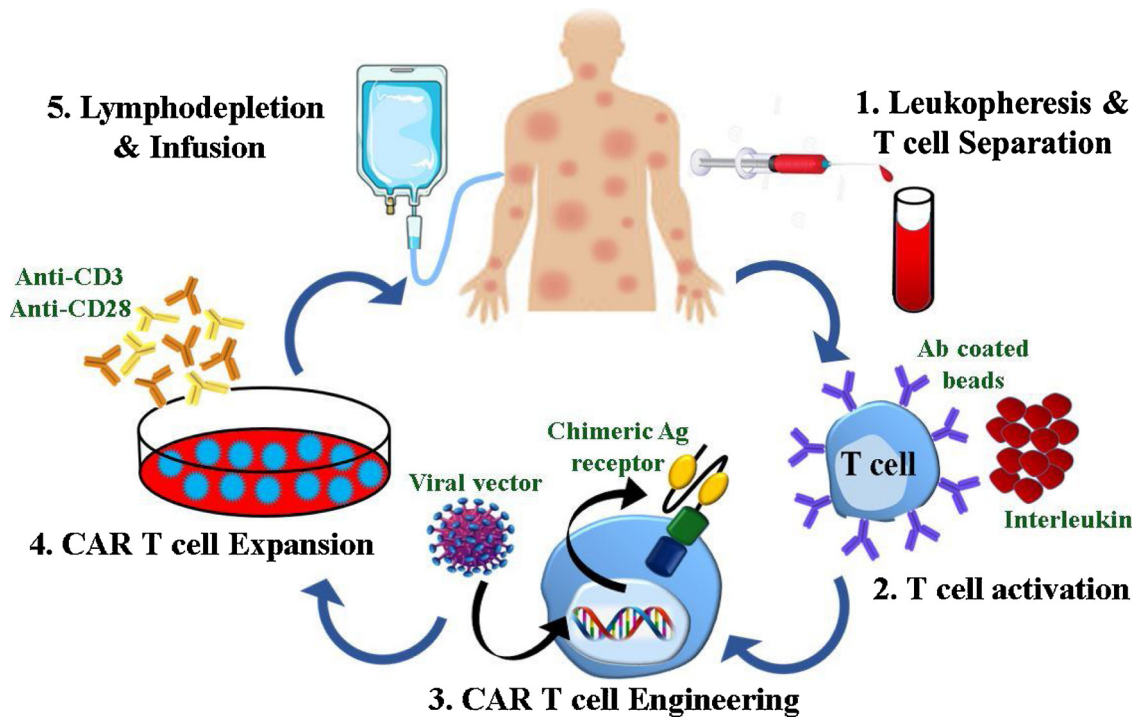


**Fig. 2.** CAR-T cell application process. Separation, design, harvest and administration of CAR-T cell for human diseases. Firstly, T cells are separated from patients (leukopheresis), harvested, activated. The CAR is designed and then engineered and produced in T cells. After expansion of produced CAR-T cell, the cells are administrated to the patients.

be executed according to Good Manufacturing Practices (GMP) protocols including the facility, staff and training, general considerations (cell collection, T cell enrichment, activation, transduction, expansion, cryopreservation), manufacturing, release testing (immunology, identity, purity, potency, microbiological testing, stability testing, product release and distribution), commercialization, and cost-effective considerations [43].

Due to the low immunogenicity and permanent transgene expression, retroviral and lentiviral vectors are commonly used to express CARs in T cells. Lentiviral vectors are particularly suitable for integrating large DNA sequences [44,45]. The protocols for generating and expanding CAR-T cells for clinical application should be rationally designed and therapeutically optimized.

### 3. Application of CAR-T cells in treatment of diseases

The therapeutic efficiency of CAR-T cells has been more commonly investigated in hematological malignancies and solid tumors than other diseases. Although these investigations have demonstrated hopeful results in hematological tumors, the success rate in solid tumors has been unsatisfactory [46]. This has been attributed to the presence of immunosuppressive factors, physical barriers, hypoxia, nutrient deficiency, inhibitory checkpoints, antigen heterogeneity, and poor cell trafficking within the microenvironment of solid tumors. For this reason, restructuring CAR-T cells to overcome these challenges is necessary to treat solid tumors [47].

CAR-T cells have also been applied for treating inflammatory diseases such as irritable bowel syndrome (IBS) and autoimmune diseases such as Pemphigus vulgaris. In the recent case, chimeric autoantibody receptor (CAAR) T cells with the specificity of Dsg3 autoantigen kill autoimmune B cells without induction widespread immune suppression. CARs constructed based on neutralizing antibodies with a single chain as binding domain have been applied to treat type 1 human immunodeficiency virus (HIV-1) infection resulting in enhanced killing of both the virus and infected cells *in vitro* [48,49]. Details of experimental studies and clinical trials on the application of specific CAR-T cells have been demonstrated in Table 2, Fig. 3, and Table 3.

#### 3.1. The challenge of suppressive immune cells

The suppressive immune cells such as regulatory T cells ( $T_{reg}$ ) can suppress immune responses mediated by effector T cells such as Th2 lymphocytes which are suppressed by CD41/FOXP31 positive  $T_{reg}$ s [50,51]. Many soluble factors, such as TGF- $\beta$ , prostaglandin E2 (PGE2), vascular endothelial growth factor (VEGF), adenosine, and Indoleamine 2, 3-dioxygenase (IDO) which are produced by  $T_{reg}$ s can potentially inhibit the activation of a variety of immune cells. In particular, immunomodulatory factors including immunosuppressive cytokines such as IL-10 can suppress T lymphocytes, and therefore can be incorporated in CAR-T cells to suppress the immune responses against these recombinant cells [52,53]. TGF- $\beta$  can also be used in CAR-T cell therapy of allergic diseases. As this modulatory factor is involved in balancing Th1/Th2 immune responses; however, its application should be carried out with caution to avoid a shift toward Th2 [54]. Other potential suppressors of immune cells include programmed death receptor 1 (PD-1) or its ligand (PD-L1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), Lymphocyte-activation gene 3 (LAG-3), and T Cell Immunoreceptor with Ig and ITIM Domains (TIGIT), [55–57].

#### 3.2. Applications of CAR-T cells in the treatment of fungal allergy

Antifungal CAR-T cells can be produced using the extracellular domain of the pattern-recognition receptor; dectin-1, as the antigen-binding domain to treat fungal allergies such as Aspergillus asthma. This domain should be able to recognize carbohydrate epitopes on the

surface of Aspergillus opportunistic fungus. This phenomenon demonstrated that pattern-recognition receptors can serve as antigen-binding domains of CARs. Furthermore, CAR- $T_{reg}$ s may be used along with antifungal CAR-T cells to promote tolerance to engineered cells in the treatment of non-fungal allergic diseases [58,59].

#### 3.3. CAR- $T_{reg}$ cells as suppressors of allergic asthma

CAR-T cytotoxic cell therapy has impressive efficacy in the treatment of malignancies, especially hematologic neoplasms. On the other hand,  $T_{reg}$ -based therapy is one of the effective strategies in the treatment of allergic diseases and asthma in which Th2 immune response dominates. Redirecting CAR- $T_{reg}$ s cells toward inflamed airways can be an effective strategy to effectively manage allergic diseases and asthma [60].

Asthma is a complicated chronic respiratory disease which is characterized with airway inflammation and AHR, wheezing, cough, dyspnea, and reversible airway obstruction [61,62]. Asthma imposes a very high economic and social burden on the health system and society. Bronchodilators and anti-inflammatory drugs can control symptoms in most patients but cannot cure asthma completely. Actually, there are currently no curative therapies available for asthma. Allergic asthma is associated with Th2 immune response which can be modulated by the action of CD4+ regulatory T cells ( $T_{reg}$ s) [60,63–65].

$T_{reg}$ s regulate multiple immune responses by inducing anergy and suppressing immune cells. In allergy and asthma,  $T_{reg}$ s maintain the Th2 immune response which finally leads to the development of allergic response [66,67].  $T_{reg}$ s usually modulate immune cells and suppresses local immune responses through induction of suppressor cytokines [68–70].  $T_{reg}$ s can suppress the function of effector T cells by binding to B7 molecules on the surface of antigen-presenting cells (APCs) through CTLA-4 [71,72]. Additionally, these cells can kill cytotoxic and helper lymphocytes via the granzyme-perforin-pathway [73,74]. Through expressing CD25 (i.e. IL-2R),  $T_{reg}$ s can also scavenge IL-2 which is required for T cell proliferation and differentiation [75].

Based on their origins,  $T_{reg}$ s are categorized into either natural ( $nT_{reg}$ ) or induced ( $iT_{reg}$ ) classes [67]. The  $nT_{reg}$ s which are also known as thymus-derived  $T_{reg}$ s ( $tT_{reg}$ ) are derived from thymocytes and express transcription factor forkhead box P3 (Foxp3). These cells obtain their phenotypic characteristics during thymus selection and induce immune tolerance to autoantigens. The  $nT_{reg}$ s express CD4, CD25, and Foxp3 and show low expression of CD127 [66].

The  $iT_{reg}$  cells, also known as peripherally induced  $T_{reg}$ s ( $pT_{reg}$ ), are derived from peripheral lymphoid tissues (i.e. outside the thymus), [76]. While  $nT_{reg}$ s mainly regulate immune responses against self-antigens,  $iT_{reg}$ s modulate immune responses against foreign antigens. Therefore,  $iT_{reg}$ s can be utilized to design CAR- $T_{reg}$  cells for managing allergic diseases and asthma. Thus far, there are no specific cell surface markers for identifying  $iT_{reg}$ s [67,77,78].

The  $iT_{reg}$ s secrete suppressive cytokines such as TGF- $\beta$ , IL-10, and IL-35, as well as cell-lysing molecules such as granzymes A and B [79]. IL-35 is capable of inducing  $T_{reg}$  differentiation and is particularly secreted by  $T_{reg}$ s but not by other effector T cells [80]. The IL-35-induced- $T_{reg}$ s ( $iTr35$ ) are newly identified  $T_{reg}$ s promoting immunosuppression in allergy and asthma by producing IL-35 and IL-10 cytokines. More importantly,  $iTr35$  cells do not express Foxp3 and are highly stable in comparison with TGF- $\beta$ -induced- $iT_{reg}$ s [81,82]. Studies have shown that dysregulated function of  $T_{reg}$ s is the main reason for Th2 immune response dominance and development of asthma [83,84]. Some animal studies have reported that the depletion of  $T_{reg}$ s before sensitization aggravated the inflammation of airways and AHR. Re-infusion of  $T_{reg}$ s into OVA-sensitized and challenged asthmatic mice significantly suppressed AHR, eosinophilic infiltration and production of IL-5 and IL-13 in the BALF [85,86].

Foxp3 gene mutations have been associated with immune dysregulation, higher IgE level, eosinophilia, and allergy [87,88]. Mutated

**Table 2**

Targeted Ags with CARs construct were used in clinical trials studies for treatment of diseases such as malignancy. All of the clinical trials were done on the cancer and tumor problems. According to our search, there is no clinical trial on CAR-T cell therapy for allergy and asthma. ALL: Acute lymphocytic leukemia, AML: Acute myeloid leukemia, CLL: Chronic lymphocytic leukemia, DLBCL: Diffuse large B-cell lymphoma, HL: Hodgkin lymphoma, MCL: Mantle cell lymphoma, MM: Multiple myeloma, NHL: Non-Hodgkin lymphoma, FL: Follicular Lymphoma.

| NO | Targeted Ag                      | Tumor type                                                                                                                      | CAR-receptor                                                                                             | Reference |
|----|----------------------------------|---------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|-----------|
| 1  | MART-1/HLA-A2                    | Melanoma                                                                                                                        | DMF4-TCR and DMF5-TCR (from TIL)                                                                         | 1         |
| 2  | MAGE-A3/HLA-A2<br>MAGE-A3/HLA-A1 | Melanoma, synovial cell sarcoma, esophageal cancer                                                                              | 1D3-TCR (from patient, codon optimized and murinized)<br>TCR (from immunized mice and affinity enhanced) | 1         |
| 3  | CEA/HLA-A2                       | Melanoma, Myeloma                                                                                                               | a3a-TCR (from patient and affinity-enhanced)                                                             | 1         |
| 4  | NY-ESO-1/HLA-A2                  | Colorectal carcinoma                                                                                                            | TCR (from immunized mice and affinity enhanced)                                                          | 1         |
| 5  | CAIX                             | Melanoma, Myeloma                                                                                                               | 1G4-TCR (from patient and affinity-enhanced)                                                             | 1         |
| 6  | CD171                            | Renal cell carcinoma                                                                                                            | FcR $\gamma$                                                                                             | 1         |
| 7  | CD19                             | Neuroblastoma                                                                                                                   | CD3 $\zeta$                                                                                              | 1         |
|    |                                  | NHL, CLL                                                                                                                        | CD28-CD3 $\zeta$                                                                                         | 5         |
|    |                                  | NHL, ALL, CLL                                                                                                                   | CD28-CD3 $\zeta$                                                                                         |           |
|    |                                  | ALL                                                                                                                             | CD28-CD3 $\zeta$                                                                                         |           |
|    |                                  | CD191 malignancy                                                                                                                | CD28-CD3 $\zeta$                                                                                         |           |
|    |                                  | ALL                                                                                                                             | 4-1BB-CD3 $\zeta$                                                                                        |           |
|    |                                  | ALL, CLL                                                                                                                        | CD28-CD3 $\zeta$                                                                                         |           |
|    |                                  | ALL, NHL                                                                                                                        | 4-1BB-CD3 $\zeta$                                                                                        |           |
|    |                                  | CLL                                                                                                                             | CD28-CD3 $\zeta$                                                                                         | 1         |
|    |                                  | CLL                                                                                                                             | 41BB-CD3 $\zeta$                                                                                         |           |
|    |                                  | CLL                                                                                                                             | CD28-CD3 $\zeta$                                                                                         |           |
|    |                                  | Lymphoma (FL, DLBCL, NHL)                                                                                                       | 41BB-CD3 $\zeta$                                                                                         |           |
|    |                                  |                                                                                                                                 | 4-1BB-CD28-CD3 $\zeta$                                                                                   |           |
|    |                                  |                                                                                                                                 | CD28-CD3 $\zeta$                                                                                         |           |
| 8  | CD20                             | Multiple Myeloma                                                                                                                | 41BB-CD3 $\zeta$                                                                                         | 5         |
|    |                                  | CD201 malignancy                                                                                                                | 4-1BB-CD3 $\zeta$                                                                                        |           |
|    |                                  | CD20-positive malignancies                                                                                                      | CD3 $\zeta$ or 4-1BB-CD3 $\zeta$                                                                         | 37        |
| 9  | CD22                             | ALL                                                                                                                             | CD28-CD3 $\zeta$                                                                                         | 5         |
|    |                                  |                                                                                                                                 | 4-1BB-CD3 $\zeta$                                                                                        |           |
|    |                                  |                                                                                                                                 | CD28-4-1BB-CD3 $\zeta$                                                                                   |           |
|    |                                  | DLBCL, FL, MCL                                                                                                                  | CD28-CD3 $\zeta$                                                                                         | 5         |
|    |                                  | FL, NHL, DLBCL, B-ALL                                                                                                           | CD28-CD3 $\zeta$                                                                                         | 37        |
| 10 | CD33                             | AML                                                                                                                             | 4-1BB-CD3 $\zeta$                                                                                        | 5, 37     |
| 11 | CD123                            | AML                                                                                                                             | CD28-CD3 $\zeta$                                                                                         | 5, 37     |
| 12 | PSMA                             | Prostate cancer                                                                                                                 | CD3 $\zeta$ and CD28                                                                                     | 37        |
| 13 | CD133                            | AML, ALL                                                                                                                        | 4-1BB-CD3 $\zeta$                                                                                        | 5         |
| 14 | CD138                            | MM                                                                                                                              | 4-1BB-CD3 $\zeta$                                                                                        | 5, 37     |
| 15 | k-chain                          | CLL                                                                                                                             | CD28-CD3 $\zeta$                                                                                         | 5         |
| 16 | ROR1                             | CLL, ALL                                                                                                                        | CD28-CD3 $\zeta$                                                                                         | 5, 37     |
| 17 | Ig $\kappa$                      | CLL, low-grade B-cell malignancies                                                                                              | CD3 $\zeta$ and CD28                                                                                     | 37        |
| 18 | CD30                             | HL, NHL                                                                                                                         | CD3 $\zeta$ and CD28                                                                                     | 37        |
| 19 | LeY                              | AML                                                                                                                             | CD3 $\zeta$ and CD28                                                                                     |           |
| 20 | BCMA                             | MM                                                                                                                              | CD3 $\zeta$ and 4-1BB                                                                                    | 37        |
| 21 | FR $\alpha$                      | Ovarian cancer                                                                                                                  | FcR $\gamma$                                                                                             | 28        |
| 22 | Mesothelin                       | Malignant pleural mesothelioma                                                                                                  | CD3 and 4-1BB                                                                                            | 37        |
|    |                                  | Pancreatic cancer Metastatic pancreatic adenocarcinoma, epithelial ovarian cancer and malignant epithelial pleural mesothelioma |                                                                                                          |           |
|    |                                  | Mesothelioma and malignant pleural disease                                                                                      | CD3 $\zeta$ and CD28                                                                                     |           |
|    |                                  | Mesothelioma, pancreatic and ovarian cancer                                                                                     | CD3 $\zeta$ , CD28 and 4-1BB                                                                             |           |
|    |                                  | Mesothelioma Murine                                                                                                             | 4-1BB- CD3 $\zeta$                                                                                       | 5         |
| 23 | FAP                              | Mesothelioma                                                                                                                    | CD3 $\zeta$ and CD28                                                                                     | 37        |
| 24 | EGFRvIII                         | Glioma                                                                                                                          | CD3 $\zeta$ and 4-1BB                                                                                    | 37        |
|    |                                  | Glioma                                                                                                                          | CD3 $\zeta$ , CD28 and 4-1BB                                                                             |           |
| 25 | CEA                              | Liver metastases                                                                                                                | CD3 $\zeta$ and CD28                                                                                     | 37        |
|    |                                  | Colorectal cancer                                                                                                               | CD3 $\zeta$ and CD28                                                                                     | 5         |
| 26 | CD171                            | Neuroblastoma                                                                                                                   | CD3 $\zeta$ and 4-1BB or                                                                                 | 37        |
|    |                                  |                                                                                                                                 | CD3 $\zeta$ , CD28 and 4-1BB                                                                             |           |
| 27 | GD2                              | Neuroblastoma, osteosarcoma and melanoma                                                                                        | CD3 $\zeta$ , OX40, CD28                                                                                 | 37        |
|    |                                  | Neuroblastoma                                                                                                                   |                                                                                                          |           |
|    |                                  | Sarcoma                                                                                                                         | CD3 $\zeta$ , OX40, CD28                                                                                 |           |
|    |                                  | Neuroblastoma                                                                                                                   | EBV specific T-cells transduced with CD3 $\zeta$ CAR construct                                           | 1         |
|    |                                  | Neuroblastoma, Osteosarcoma, and Melanoma                                                                                       | OX40-CD28- CD3 $\zeta$ -iCasp9                                                                           |           |
| 28 | Glypican-3                       | Advanced-stage hepatocellular carcinoma                                                                                         | CD3 $\zeta$ , CD28 and 4-1BB                                                                             | 37        |
| 29 | HER2                             | Sarcoma                                                                                                                         | CD3 $\zeta$ and CD28                                                                                     | 37, 1     |
|    |                                  | Glioblastoma                                                                                                                    | CD3 $\zeta$ and CD28                                                                                     | 37        |
|    |                                  | Glioblastoma multiforme                                                                                                         |                                                                                                          |           |
|    |                                  | Colorectal cancer                                                                                                               | CD28-4-1BB-CD3 $\zeta$                                                                                   | 1         |

(continued on next page)

Table 2 (continued)

| NO | Targeted Ag | Tumor type           | CAR-receptor     | Reference |
|----|-------------|----------------------|------------------|-----------|
| 30 | IL-13       | Glioma               | RαCD3ζ and 4-1BB | 37        |
| 31 | CAIX        | Renal cell carcinoma | FcRγ             | 28        |
| 32 | IL13Rα2     | Glioblastoma         | 41BB-CD3ζ        | 28        |

*Foxp3* can lead to  $T_{reg}$  deficiency, decreased TGF- $\beta$  and IL-10 levels, and mitigated airway inflammation and AHR in asthma.

In a clinical trial, infusion of *in vitro* expanded specific OVA- $iT_{reg}$ s to 20 patients with Crohn's disease induced immune tolerance in the patients [89] indicating the role of antigen-specific  $T_{reg}$ s in prevention and control of asthma symptoms. In fact, CAR- $T_{reg}$  cells provide a viable strategy against asthma pathogenic and etiologic mechanisms. However, there is a need to divulge regulatory mechanisms of  $T_{reg}$ s to design CAR- $T_{reg}$  cells to treat and manage asthma.

According to the study of Skuljec et al. in 2017 on animal models of asthma, accumulation of CAR- $T_{reg}$ s in the lung and tracheobronchial lymph nodes reduced airway AHR, eosinophilic infiltration, mucus production, and other inflammatory cells infiltrates in the lung. Also, they showed that CAR- $T_{reg}$ s were more efficient than  $T_{reg}$ s in controlling severe allergic asthma [60].

Antigen-specific  $T_{reg}$ s seem to be more efficient than polyclonal  $T_{reg}$ s in controlling inflammation in allergic asthma. To confer specificity,  $T_{reg}$ s should be engineered *ex vivo* applying MHC-independent CARs harboring antibody-derived binding domains for the antigen [90–92]. While CAR-T cells have been extensively studied in the treatment of hematologic malignancies in clinical trials, studies on the therapeutic role of CAR- $T_{reg}$ s in other diseases such as colitis and multiple sclerosis have been limited to animal models [92,93].

Antigen-specific CAR- $T_{reg}$ s recognize the intended antigen which is expressed in airways and the lung and redirect these cells toward the target tissues. CAR- $T_{reg}$  cells can accumulate and become activated in the inflamed airways of the asthmatic lung where they can control

Table 3

The number of CAR-T cell therapy clinical trials were done until Feb. 2019 in the world.

| World | East Asia     | China              | 153 |
|-------|---------------|--------------------|-----|
|       |               | Japan              | 4   |
|       |               | Taiwan             | 1   |
|       | North America | United States      | 92  |
|       |               | Canada             | 3   |
|       |               | Ontario            | 2   |
|       |               | Quebec             | 2   |
|       | Europe        | United Kingdom     | 10  |
|       |               | Italy              | 6   |
|       |               | Netherlands        | 6   |
|       |               | Germany            | 6   |
|       |               | France             | 6   |
|       |               | Belgium            | 5   |
|       |               | Spain              | 4   |
|       |               | Austria            | 3   |
|       |               | Norway             | 2   |
|       |               | Sweden             | 2   |
|       | Pacifica      | Australia          | 2   |
|       |               | Victoria           | 1   |
|       | North Asia    | Russian Federation | 1   |

allergic inflammation [60].  $T_{reg}$ s incorporated with CARs containing SCA431scFv-mIgG-CD4(tm)-CD28-CD3ζ have been applied for treatment of asthma. The signals derived from CD28/CD3ζ ensure strong stimulation of the engineered CAR- $T_{reg}$ s. Intravenous infusion of  $10^6$  CAR- $T_{reg}$ s to OVA-challenged mice completely prevented the

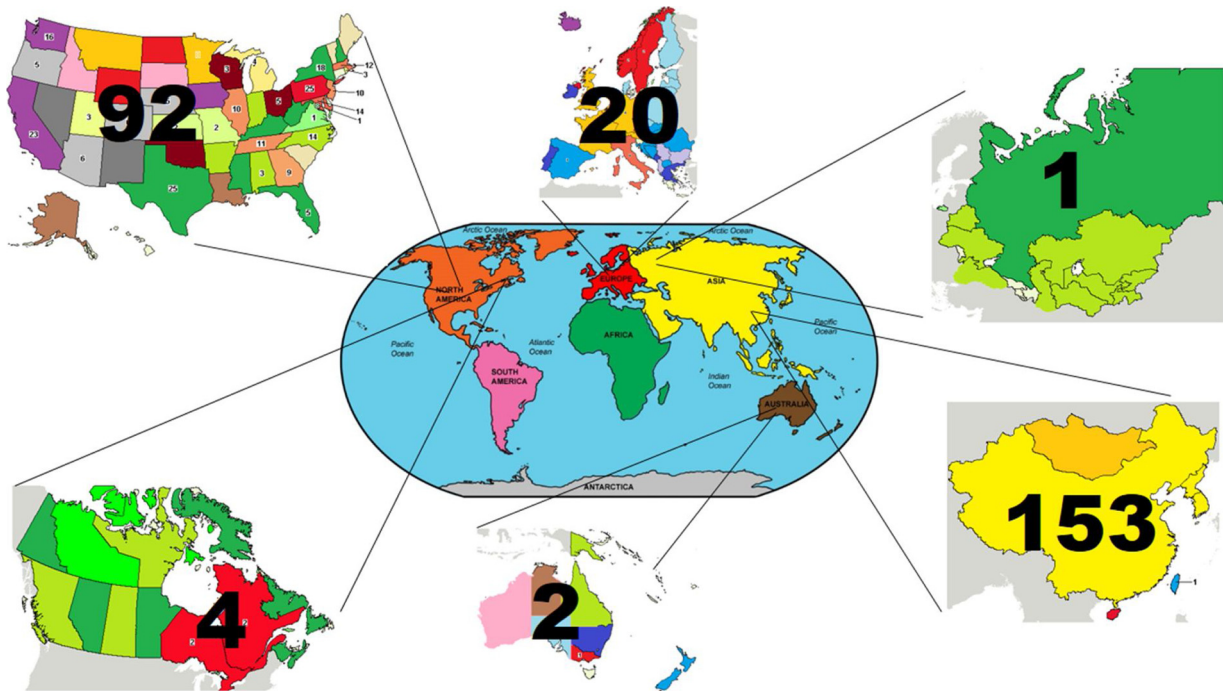


Fig. 3. Some Antigens were used in studies for clinical trials of CAR-T cell therapy until Jan. 2019 which are showed in map. Clinical trials have been done in East Asia, North America, Europe, Pacifica and North Asia. There are 20 clinical trials in Europe, 1 clinical trial in North Asia, 155 clinical trials in Pacifica and East Asia and 96 clinical trials in North America. These maps show that in the recent decades CAR-T cell therapy can attract deeper attentions to the immunotherapy and clinical trials.

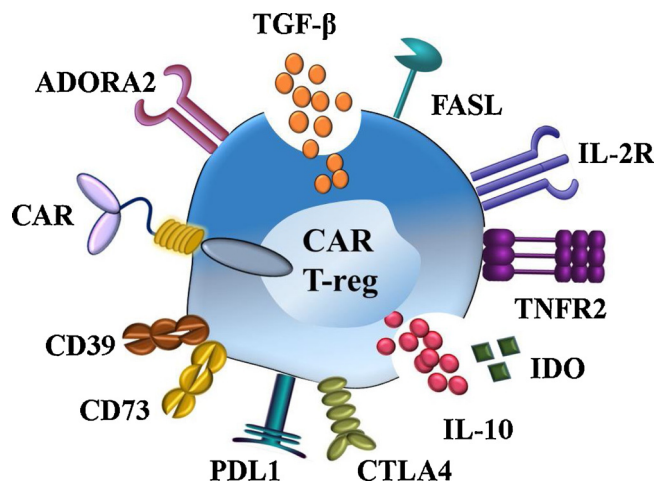


**Fig. 4.** CAR-Treg cells are new instrument which can use in allergic asthma as suppressor agent. In this cell, CD3 and CD28 is produced on Treg cell with suppressory molecules such as FASL and CTLA-4. The current cell can produce regulatory cytokines (IL-10 and TGF- $\beta$ ) and can prevent T cell activation with IL-2 attraction by CD25 (IL-2R). Other molecules, ADORA2, CD39, CD73, IDO, PDL1 and TNFR2 can silent effector function of immune cells. The uni-CAR-Treg cells are applicable to the many peoples with low potency of immunological rejection. In the future, the generation of CAR-T cells would be produced with special and unspecial molecules according to the focused purposes.

development of AHR, eosinophilic inflammation, Th2 cytokine production, and elevation of serum IgE level. This was while infusion of T<sub>regs</sub> without CARs had a respectively very low therapeutic effect. Furthermore, infusion of  $5 \times 10^6$  polyclonal CD4+CD25+ T<sub>regs</sub> successfully suppressed airway inflammation in animal model of allergic asthma. On the other hand, a 10-fold lower number of allergen-specific T<sub>regs</sub> produced similar therapeutic effect (Fig. 4) [94,95].

Signal transduction by CD28-CD3 $\zeta$  complex amplifies the function of CAR-T<sub>regs</sub> by directing these cells toward the target tissue. Also, the expression of suppressive cytokines (i.e. TGF- $\beta$  and IL-10) was increased following the activation of CARs which subsequently can augment IL-10 production and induce apoptosis and suppress proliferation of responder T cells (by upregulating cyclic AMP or by CD39 and CD73-dependent peri-cellular generation of adenosine). These events are probably involved in the regression of asthma presentations [94,96–98].

In another study, the therapeutic role of the first and second (CD137/CD3 $\zeta$ ) generations of CAR-T cells targeting IL13R $\alpha$  was investigated in patients with glioblastoma and malignant glioma [99]. Modified IL13R $\alpha$ 2-targeted CAR-T cells (IL13BB $\zeta$ ) were constructed by incorporating 4-1BB (as co-stimulatory molecule) and a mutated IgG4-Fc linker (to reduce off-target Fc-receptor interactions) [100–102]. Considering the role of IL-13 in the pathogenesis of allergic asthma, IL-13 receptor can be a good target to construct CAR-T cells for treating asthma. Selection of appropriate target antigen to redirect T<sub>reg</sub> cells to airways in asthma requires more studies [103].

The fourth generation of CAR-T cells (i.e. TRUCK cells) can be designed against asthma biomarkers to induce type 1 cytokines (i.e. Th1 immune response) and suppress type 2 cytokines (i.e. Th2 immune response) [37,104,105]. Immune-regulatory molecules have important roles in the balance of immune responses in the lung and the immunogenicity of allergens in asthma. Therefore, regulating immune-stimulatory checkpoints in immune surveillance pathway through application of immunosuppressive molecules and cells can be beneficial in the treatment of asthma. These approaches; however, can be problematic in immune-deficient and immunocompromised patients. Therefore, there is a need for augmenting immunocompetent responses in these patients.

### 3.4. CAR-T cell derived co-stimulatory signals and cytokines

Cytokines can be induced by the application of a bicistronic vector in the engineered CAR-T cells to overcome the inhibitory effects of T<sub>regs</sub> and regulate the shift of Th1/Th2 responses [106–108]. Also, TCR mediated T cell anergy and exhaustion can be controlled via incorporation of co-stimulatory molecules during CAR-T cell construction [109,110]. For this purpose, many co-stimulatory (and inhibitory) molecules have been identified. CD80, CD86, CD40 L, 4-1BBL, CD40, CD28, and 4-1BB are commonly used co-stimulatory adaptors [111–113], and PD-1, CTLA-4, T cell membrane protein 3 (TIM-3), and lymphocyte activating gene 3 (LAG-3) are among common in used immunosuppressors [114,115].

### 3.5. CARs targeting IgE receptors in allergic asthma

A IgE receptor can be used to construct CARs with scFvs to treat allergic asthma. The scFvs domain decreases clustering and spontaneous signaling from CARs. In humans, there are three different IgE-receptors including low affinity IgE receptor or Fc $\epsilon$ R2 (CD23), low affinity galectin-3 or epsilon binding protein (eBP), and high-affinity receptor for IgE or Fc $\epsilon$ R1 [116,117].

Soluble CD23 (sCD23) which is derived from membrane bound CD23 on B cells enhances IgE production and asthma severity. Therefore, reducing sCD23 level by CAR-T cell therapy can present therapeutic implication in patients with allergic diseases including asthma [117,118]. Nevertheless, more studies are required to validate this application.

Fc $\epsilon$ R1 consists of an  $\alpha$  chain which binds to IgE Fc region with high affinity. The  $\beta$  and  $\gamma$  chains constitute ITAM signaling domains. Soluble Fc $\epsilon$ R1 is a candidate as modulator of IgE-mediated responses to construct CARs targeting Fc $\epsilon$ R1 and to prevent binding of IgE to its receptor and subsequent immune responses [116–118]. In the development of fifth generation of CAR-T cells, Fc $\epsilon$ R1 can be used along with other receptors in the structure of CAR-T cells to control asthma progression (i.e. allergotherapy). Also, constructing CAR-T<sub>reg</sub> cells using anti-IL-5 may be useful to trap IgE and control asthma attacks.

Circulating IgE in biological liquids and tissues can bind CARs and block their interactions with IgE in target tissues. For resolving this problem, low affinity Fc $\epsilon$ R1 $\alpha$  mutants can be employed [119,120].

In recent studies, CD28 costimulatory molecule has been applied to design third-generation CAR-T cells. These cells are able to prevent 4-1BB signaling via TRAF2-dependent activation of NF- $\kappa$ B pathway, to augment FAS-mediated apoptosis in CAR-T cells, to activate PI3Kinase/Akt and Bcl-XL expression, and finally to enhance Fc $\epsilon$ R1 $\alpha$ -based CAR-T cells persistence [19,119–121].

### 3.6. CARs with Fc $\epsilon$ R1 $\alpha$ for mIgE

In recent decades, the prevalence of allergic diseases and asthma has dramatically increased. Allergic asthma cannot be controlled by available medications and patients often suffer from poorly controlled symptoms and frequent asthma attacks [61,62,65]. In a predisposed individual, exposure to an allergen leads to IgE synthesis in membrane form (mIgE) by cytokines activated B cells that can secrete and then strongly binds to its high affinity receptor (Fc $\epsilon$ R1), on allergic immune cells such as mast cells and basophils (allergic sensitization process). The Fc $\epsilon$ R1 consists of an  $\alpha$  chain that binds to Fc region of the IgE with high affinity,  $\beta$  and  $\gamma$  chains containing the ITAM signaling domains. Subsequent exposure of the same allergen causes allergen-induced crosslinking of the IgE-Fc $\epsilon$ R1 cell surface complex, degranulation of effector cells and release of bio-reactive mediators (histamine, cytokines and leukotrienes) that are responsible for the symptoms of allergy and asthma. Therefore, IgE plays a central role in the pathogenesis of allergic diseases. The omalizumab as a recombinant humanized IgE-specific monoclonal Ab prevents the IgE-Fc $\epsilon$ R1 binding and reduce

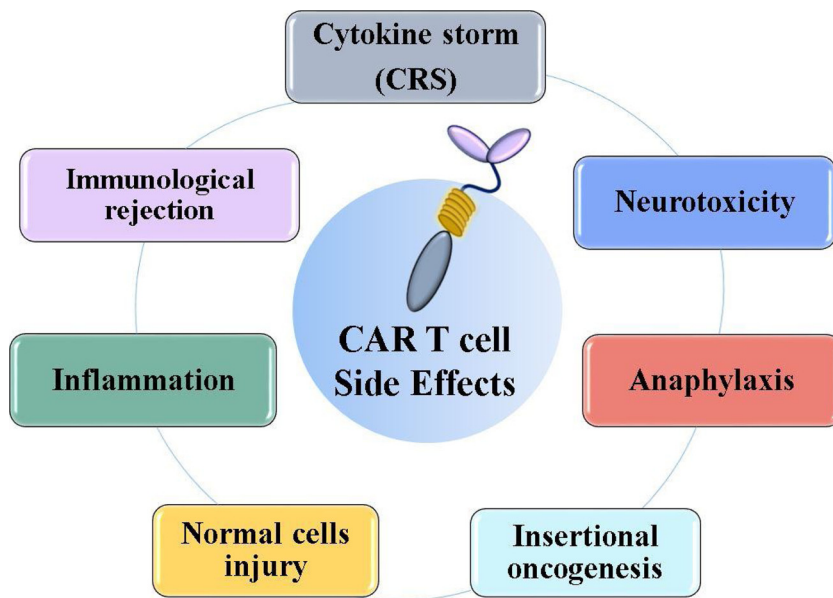


**Fig. 5.** Some of main side effects of CAR-T cell therapy. CRS and neurotoxicity are main side effects of CAR-T cell therapy and in the patients with cancer, other two problems, tumor lysis syndrome (TLS) and on-target off tumor toxicity can be appeared. In the clinical trial of CAR-T cells for other diseases include asthma and allergy, insertional oncogenesis, anaphylaxis, normal cell injury, inflammation, immunological rejection (not in uniCAR) and maybe other side effects would be observed.

circulating levels of IgE. Currently, omalizumab is benefit treatment of severe allergic asthma in non-responding patients. But omalizumab requires frequent administration at high doses and is not cost-effective [122–125]. Because, IgE plays a main role in allergic response, therefore, targeting the IgE-expressing B cells represents an effective therapeutic approach. Immunotherapy with T cells which have IgE-specific CARs has long-term potential to suppressing the IgE related responses through specific killing of IgE-expressing B cells. For producing of these cells, CD8 + T cells should be used.

Targeting IgE-expressing cells may be effective to achieve long term suppression of allergy reactions. For this, T cells are isolated and engrafted with ERs that are specific for molecular markers on inflammatory cells in asthma through recognition membrane-bound IgE (mIgE) [122–124]. CARs incorporated with extracellular domain of FcεRI α chain (FcεRIα) have been designed to bind to mIgE; however, secreted IgE bound to FcεRII on cell surface cannot be recognized by these CARs [122,125]. Dana et al. in 2018 designed CAR-T cells targeting cells expressing mIgE and FcεRI-expressing mast cells, basophils and eosinophils to treat allergic diseases by preventing cellular degranulation and anaphylaxis reaction.

The first-generation FcεRIα-based CAR was constructed and was able to bind IgE, and mediated specific responses to target cells. However, this approach limitation was the lack of co-stimulatory signaling domains. To improve FcεRIα-based CAR-T cell persistence, the 2<sup>nd</sup> and 3<sup>rd</sup> generations of FcεRIα-based CARs can be constructed with co-stimulatory domains, CD28 and/or 4-1BB for the second-generation, and both CD28 and 4-1BB for the third generation. In designing of CAR, the WT FcεRIα extracellular domain can be fused with a CD3ζ intracellular signaling domain (the first generation) or with the 4-1BB and/or CD28 co-stimulatory domain (the second and third generation) CARs [19,21,31,34,36,37,116,118,121,122]. Future studies can be led to the development of adoptive T cell therapy for allergic asthma using FcεRIα-based CAR-T cells. Additionally, memory phenotype of the CD8+ CAR-T cells can persist and allow long-term monitoring and elimination of target cells.

#### 4. CAR-T cells side effects

CAR-T cell therapy, as an effective and new strategy, has some side effects including tissue inflammation, neurotoxicity, hypoplasia in target cells, heart and pulmonary vasculature toxicity, cholangitis, injury to bile duct epithelial cells, and anaphylactic shock [126–128].

CRS, also known as “cytokine storm”, is another important side effect of CAR-T cell therapy characterized with sudden and dramatic increase in the serum levels of various cytokines [129–131] including IFN-γ, IFN-α, IL-1, IL-2, IL-6, GM-CSF, IL-8, IL-10, IL-12, TNF-α, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP) 1α, etc. leading to supra-physiological immune activation [132–134]. Main symptoms of CRS are hypotension, hypoxia, fever, fatigue, myalgias, malaise, nausea, hypoxia, coagulopathy, capillary leakage, tachycardia, tachypnea, hemophagocytic lymphohistiocytosis/macrophage activation syndrome, organ toxicity, pulmonary edema, pneumonitis, and renal insufficiency [135–139]. Neurotoxicity is a side effect of CAR-T cell therapy presenting with encephalopathy, anxiety, headache, aphasia, delirium, cerebral edema, tremor, unconsciousness, confusion, and seizures [140–142]. Neurotoxicity has also been reported after administration of monoclonal antibodies such as blinatumomab and CD3/CD19 bi-specific antibody [143,144]. In addition, CAR-T cell therapy may lead to the elevation of acute phase proteins such as C-reactive protein (CRP) and ferritin [143,145]. The variable regions of most CARs are derived from murine antibodies which can be immunogenic, especially after infusion of large number of autologous CAR-T cells (e.g.  $2 \times 10^6$  cells/kg of body-weight), resulting in immunological rejection. Therefore, constructing CARs using complete human antibodies (HuCAR) is required in such conditions [146,147]. Development of IgE alloantibodies against CAR-T cells and anaphylactic shock are other important problems of CAR-T cell therapy [148,149] (Fig. 5).

##### 4.1. Preventing toxicities of CAR-T cell therapy

Appropriate selection of target antigens is particularly essential to prevent CAR-T cell therapy side effects. Another approach is to develop CAR-T cells that recognize highly specific-antigens in the target tissue. Using inducible promoter constructs to regulate the expression of CARs on T lymphocytes is another viable approach. Corticosteroids and monoclonal antibodies can be used to prevent CRS; however, these drugs can attenuate and suppress immune functional cells. Although mild and moderate CRS are usually self-limited, severe CRS needs to be managed and prevented by administration of either steroids or tocilizumab (either alone or in combination with dexamethasone and levetiracetam) [150,151]. Recent preclinical studies have suggested that ibrutinib (Bruton's tyrosine kinase inhibitor) and ruxolitinib (JAK/STAT pathway inhibitor) can prevent CRS [143,152,153]. The toxicity

as well as the efficiency of CAR-T cell therapy, are influenced by the T-cell dosage and the duration of CARs expression on T cells. Repeated infusions of CAR-T cells increase the risk of immune rejection [148]. CAR-T cell mediated GVHD is another challenge that can be treated with alemtuzumab [154–156].

#### 4.2. Increasing CAR-T-Cell effector function

Incorporation of co-stimulatory molecules, as well as cytokine and chemokine receptors are useful strategies to enhance the persistence, reduce the exhaustion, and increase the efficacy of CAR-T cells [157,158]. Another way to promote immune function of CAR-T cells is to augment the transgenic expression of cytokines promoting T cell proliferation and effector functions [157,159,160]. In this approach; however, the side effects caused by overexpression of cytokines should be monitored. Cytokines The expression of cytokines can be modulated through regulation of the balance between stimulatory and inhibitory signals.

To avoid antigen escaping, it is amenable to produce CARs targeting two separate antigens through constructing two tandemly arranged scFvs coupled with the same signaling endo-domain (i.e. Tan-CAR) [161,162]. Also, it is possible to exploit Fc $\gamma$  receptor III (CD16) expressing CAR-T or natural killer (NK) cells to produce universal CAR-T cells recognizing wide range of antigens. Universal CAR-T cells should lack HLA-A, B and C which are replaced with HLA-E to prevent being recognized and lysed by the host's NK cells [163,164].

#### 4.3. CAR-T cell trafficking augmentation

After being infused, *ex vivo*-engineered and expanded CAR-T cells migrate through the blood circulation and then enter into target sites. Engineered CAR-T cells expressing specific chemokine receptors have enhanced ability to migrate to the target tissues. Some studies have shown that engineered CAR-T cells expressing ICOS co-stimulatory domain persist and survive longer than CAR-T cells containing CD28 and 4-1BB co-stimulatory molecules [165,166]. Constitutive expression of CD40 L on CAR-T cells can increase the proliferation and cytokine secretion ability of the cells [165,167]. Accordingly, engineered CAR-T cells secreting Th1 cytokines can be used to treat allergic diseases by shifting immune responses toward Th1. Nevertheless, the risk of serious side effects due to cytokines overexpression must be considered.

#### 4.4. Negative regulators of CAR-T cells function

The effector functions of CAR-T cells can be modulated by incorporating dominant-negative receptors inducing suppressive cytokines such as IL-10 and TGF- $\beta$ . Regulatory molecules such as PD-L1, PD-1, and CTLA-4 can be applied to suppress CAR-T cell activity [168,169]. Other suppressive strategies include siRNA and CRISPR-Cas9 mediated silencing approaches to selectively inactivate effector genes in the target cells [170].

#### 4.5. Gene editing in CAR-T cells

TCR engineered lymphocytes can be generated by transduction of viral vectors encoding TCR gene. Due to MHC-restricted antigen recognition, TCR-engineered T cells can only be used in a subset of patients. On the other hand, CAR engineered T cells provide MHC-independent antigen recognition mechanisms. CAR engineered T cells can be designed using current gene editing tools including Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the CRISPR-Cas9 system. These systems bind to specific genomic loci and break double-strand DNA (dsDNA). Subsequently, the repair of the broken DNA leads to various genetic modifications and gene rearrangements [171–173].

MHC-I molecules should be removed to minimize the risk of

immune reaction against CAR-T cells. To avoid GVHD; on the other hand, antibody-TCRs complexes ought to be eliminated from CAR-T cells by efficient multiplex gene editing strategies. As the TCR  $\alpha$  chain is encoded by a single TRAC gene while TCR  $\beta$  chain is expressed by two TCRB genes, knocking out a single TRAC gene is the simplest strategy to eliminate functional TCR. MHC-I molecules can be removed from T cell surface by knocking out  $\beta$ 2 microglobulin which is essential for the formation of MHC heterodimers and their expression on cell surface. Removing HLA-I molecules from T cells; however, may induce NK cell response against these cells. For resolving this issue, one solution is to induce the expression of other HLA-I molecules such as HLA-G or HLA-E on the engineered T cells [174–176]. Using T cells with a known TCR specificity can reduce the risk of unexpected CAR-T cell therapy toxicities. Nevertheless, some allergens contain no proteins in their structures obviating the need for MHC-dependent antigen recognition pathway.

For constructing universal CAR-T cells, eliminating the endogenous TCR using gene-editing techniques [(e.g. CRISPR/Cas9 or TALENs or ZFNs)] is important [58,177–179].

### 5. Universal CAR-T and T<sub>reg</sub> cells

Unlike the conventional CAR-T cells, universal CAR-T cells are capable of selectively targeting multiple antigens. In comparison with conventional CAR-T cells, universal cells have different extracellular domains and structures linked to soluble antigens and ligands. Universal CAR-T cells are activated through binding of their extracellular domains to soluble antigens [180,181]. The biotin-avidin “lock-key” mechanism has been used to generate CARs harboring avidin as the extracellular domain which is linked to an intracellular signaling domain (i.e. biotin-binding immune receptors (BBIR) system). The BBIR containing dimeric avidin (as the recognition domain) identifies and binds to a variety of biotinylated antigen-specific molecules (ScFv, mAbs and tissue-specific ligands). The dcAv BBIR specifically binds to target antigens in the presence of extremely low concentrations of biotinylated molecules. The genetically modified T cells recognize antigens after the addition of matched biotinylated antibodies. For increasing the flexibility of CARs, the split universal and programmable (SUPRA) CAR system has been designed. This is a two-component receptor system consisting of a universal receptor and a separate ScFv domain targeting specific antigens. Both of these components are attached to separate leucine zipper adaptors. The “lock-key” structure of SUPRA CAR with leucine zipper adaptors can be expanded without further modification of the CAR-T cells meaning that their target antigens can be switched. Furthermore, the potency of T cell signaling, the extent of T cell activation, and toxicity of SUPRA CAR-T cells can be managed by manipulating signaling domains of effector cells [180,181].

Another advantage of universal CAR-T cells is the possibility of using allogeneic instead of autologous T cells to engineer CARs. Therefore, GVHD can be better controlled using universal CAR-T cells than conventional CAR-T cells. To decrease the risk of side effects and increase the safety of using universal CAR-T cells, it is suggested to apply human-derived ScFv [180,181]. Most studies have focused on the role of universal CAR-T cells in cancer therapy, and there is insufficient data about the therapeutic implications of these cells in asthma and allergic diseases.

T<sub>regs</sub> exert immuno-suppressive functions in both contact dependent and independent manners, and CAR-T<sub>regs</sub> have high expression of immunomodulatory CTLA-4 and LAP molecules. So, uniCAR-T<sub>regs</sub> have been recently developed incorporating a peptide epitope-conjugated ScFv to target cell surface structures [182].

### 6. Concluding remarks

The knowledge on the role of CAR-T cell therapy in the treatment of

asthma and allergic diseases has increased in recent years. Novel engineering strategies enable producing CAR-T cells with superior clinical efficiency *in vivo*.

The efficacy of CAR-T cell therapy, as an important immunotherapeutic strategy, can be promoted by applying novel gene editing strategies such as ZFN, TALEN, and CRISPR-Cas9 to target disease-specific genes including those involved in asthma pathogenesis. Although CAR-T cell therapy through gene editing mechanisms is a major achievement in immunotherapy, information about the safety of gene-edited T cells is insufficient. In this regard, improving the specificity of gene editing systems and reducing their off-target functions are major challenges.

The application of universal CAR-T cells for treating immunologic and non-immunologic diseases has resulted in promising results. Nevertheless, achieving best outcomes in CAR-T cell therapy requires all protocols to be standardized and optimized.

CRS and neurotoxicity are two potential serious side effects of CAR-T cell therapy necessitating the consideration of prophylactic protocols. In this regard, CAR-T cells can be engineered to express appropriate suppressive molecules to manage immune reactions in target organs. The role of  $\gamma\delta$  (Gamma Delta) T cells in CAR-T cell therapy should be investigated in future studies. The CAR-T cell therapy, as an adoptive immune cell therapy, presents a potential new revolution in cancer therapy as well. However, information about the role of CAR-T cell therapy in the treatment of asthma and other allergic diseases is inadequate requiring more studies.

There are several approaches to use the CAR-T cell in allergic asthma. Fungal asthma is very dangerous disease with low prevalence. For this reason, treatment of this disease is very important. Antifungal CAR-T cells with dectin-1 as the antigen-binding domain of CAR can be used to treat fungal asthma.  $T_{regs}$  usually suppresses local immune responses by anti-inflammatory cytokines and may have this role in bronchi environment.  $T_{regs}$  can suppress the function of effector Th2 cells by CTLA-4. In addition, through expressing IL-2R,  $T_{regs}$  can also scavenge IL-2, which is required for T cell proliferation. On the other hand, CAR- $T_{regs}$  may be used along with allergic asthma to promote tolerance in Th2 cells and prevent IL-4, IL-5 and IL-13 secretions by Th2 cells. Redirecting CAR- $T_{regs}$  cells toward inflamed airways can be suppressed IgE overproducing, eosinophilic inflammation and mucus hypersecretion. IgE receptor can be used to construct CARs with ScFvs to treat allergic asthma. CARs incorporated with Fc $\epsilon$ RI $\alpha$  bind to mIgE. Therefore, designed CAR-T cells targeting Fc $\epsilon$ RI-expressing mast cells, basophils and eosinophils can be used to treat allergic diseases. Furthermore, reducing sCD23 level by CAR-T cell therapy can control allergic reactions in asthma. UniCAR-T cells have capability of selectively targeting multiple antigens and are activated through binding of their extracellular domains to soluble antigens. The BBIR containing dimeric avidin binds to a variety of biotinylated Ag-specific molecules, which can be used in CARs structure. With this approach, uniCAR-T cells can be designed to targeting the IL-4, IL-5, IL-13, and IL-35 in a cell simultaneously.

## Author contributions

AE and SA contributed to the hypothesis, data gathering, corresponding, scientific and structural editing, designing figures, and verifying the manuscript before submission. ST contributed to data gathering and designing figures.

## Funding resources

This research has not received any specific grant from funding agencies (in the public, commercial and not-for-profit sectors).

## Declaration of Competing Interest

The authors declare that the current research was conducted in the absence of any commercial or financial relationships which could be construed as a potential conflict of interest.

## References

- [1] D.M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy, *Nat. Rev. Cancer* 12 (2012) 252–264.
- [2] S. Spranger, Mechanisms of tumor escape in the context of the T-cell-inflamed and the non-T-cell-inflamed tumor microenvironment, *Int. Immunol.* 28 (2016) 383–391.
- [3] P.C. Tumeh, et al., PD-1 blockade induces responses by inhibiting adaptive immune resistance, *Nature* 515 (2014) 568–571.
- [4] Aaron J. Smith, John Oertle, Dan Warren, Dino Prato, Chimeric antigen receptor (CAR) T cell therapy for malignant cancers: summary and perspective, *J. Cell. Immunother.* 2 (2016) 59e68.
- [5] Mohanraj Ramachandran, Anna Dimberg, Magnus Essand, The cancer-immunity cycle as rational design for synthetic cancer drugs: novel DC vaccines and CAR-T cells, *Semin. Cancer Biol.* 45 (2017) 23–35.
- [6] G. Gross, T. Waks, Z. Eshhar, Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity, *Proc. Natl. Acad. Sci. U. S. A.* 86 (24) (1989) 10024–10028.
- [7] M. Vasekar, S. Rizvi, X. Liu, K.E. Vrana, H. Zheng, Novel immunotherapies for hematological malignancies, *Curr. Mol. Pharmacol.* 9 (3) (2016) 264–271.
- [8] L. DeFrancesco, CAR-T's forge ahead, despite Juno deaths, *Nat. Biotechnol.* 35 (2017) 6–7.
- [9] D.A. Schmid, M.B. Irving, V. Posevitz, Evidence for a TCR affinity threshold limiting maximal CD8T cell function, *J. Immunol.* 184 (9) (2010).
- [10] R. Debets, E. Donnadieu, S. Chouaib, G. Coukos, TCR-engineered T cells to treat tumors: seeing but not touching? *Semin. Immunol.* 28 (2016) 10–21.
- [11] G. Gross, T. Waks, Z. Eshhar, Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity, *Proc. Natl. Acad. Sci. U. S. A.* 86 (1989) 10024–10028.
- [12] Z. Eshhar, T. Waks, G. Gross, D.G. Schindler, Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors, *Proc. Natl. Acad. Sci. U. S. A.* 90 (1993) 720–724.
- [13] R.D. Guest, R.E. Hawkins, N. Kirillova, E.J. Cheadle, J. Arnold, A. O'Neill, J. Irlam, K.A. Chester, J.T. Kemshead, D.M. Shaw, M.J. Embleton, P.L. Stern, D.E. Gilham, The role of extracellular spacer regions in the optimal design of chimeric immune receptors: evaluation of four different scFvs and antigens, *J. Immunother.* 28 (2005) 203–211.
- [14] H. Almasbakh, E. Walseng, A. Kristian, M.R. Myhre, E.M. Suso, L.A. Munthe, J.T. Andersen, M.Y. Wang, G. Kvalheim, G. Gaudernack, J.A. Kyte, Inclusion of an IgG1-Fc spacer abrogates efficacy of CD19 CAR-T cells in a xenograft mouse model, *Gene Ther.* 22 (2015) 391–403.
- [15] M. Hudecek, D. Sommermeyer, P.L. Kosasih, A. Silva-Benedict, L. Liu, C. Rader, M.C. Jensen, S.R. Riddell, The non-signaling extracellular spacer domain of chimeric antigen receptors is decisive for *in vivo* antitumor activity, *Cancer Immunol. Res.* 3 (2015) 125–135.
- [16] J.S. Bridgeman, R.E. Hawkins, S. Bagley, M. Blaylock, M. Holland, D.E. Gilham, The optimal antigen response of chimeric antigen receptors harboring the CD3zeta transmembrane domain is dependent upon incorporation of the receptor into the endogenous TCR/CD3 complex, *J. Immunol.* 184 (2010) 6938–6949.
- [17] Lynsey M. Whilding, John Maher, CAR-T cell immunotherapy: the path from the by-road to the freeway? *Mol. Oncol.* 9 (2015) 1994 e2018.
- [18] A.T. Gacerez, B. Arellano, C.L. Sentman, How chimeric antigen receptor design affects adoptive T cell therapy, *J. Cell. Physiol.* 231 (12) (2016) 2590–2598.
- [19] H. Karlsson, et al., Evaluation of intracellular signaling downstream chimeric antigen receptors, *PLoS One* 10 (2015) e0144787.
- [20] M. Sadelain, et al., The basic principles of chimeric antigen receptor design, *Cancer Discov.* 3 (2013) 388–398.
- [21] J. Eyquem, et al., Targeting a CAR-T to the TRAC locus with CRISPR/Cas9 enhances tumour rejection, *Nature* 543 (2017) 113–117.
- [22] J. Dobbins, et al., Binding of the cytoplasmic domain of CD28 to the plasma membrane inhibits Lck recruitment and signaling, *Sci. Signal.* (2016) 9 ra75.
- [23] C. Xu, et al., Regulation of T cell receptor activation by dynamic membrane binding of the CD3 $\epsilon$  cytoplasmic tyrosine based motif, *Cell* 135 (2008) 702–713.
- [24] P.A. van der Merwe, et al., Why do some T cell receptor cytoplasmic domains associate with the plasma membrane? *Front. Immunol.* 3 (2012) 29.
- [25] H. Zhang, et al., Basic residues in the T-cell receptor  $\zeta$  cytoplasmic domain mediate membrane association and modulate signaling, *Proc. Natl. Acad. Sci. U. S. A.* 108 (2011) 19323–19328.
- [26] J.R. James, R.D. Vale, Biophysical mechanism of T-cell receptor triggering in a reconstituted system, *Nature* 487 (2012) 64–69.
- [27] S.-P. Cordoba, et al., The large ectodomains of CD45 and CD148 regulate their segregation from and inhibition of ligated T-cell receptor, *Blood* 121 (2013) 4295–4302.
- [28] C.T. Ellebrecht, et al., Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease, *Science* 353 (2016) 179–184.
- [29] D.T. Harris, D.M. Kranz, Adoptive T cell therapies: a comparison of T cell receptors

- and chimeric antigen receptors, *Trends Pharmacol. Sci.* 37 (2016) 220–230.
- [30] N.E. Harwood, F.D. Batista, Early events in B cell activation, *Annu. Rev. Immunol.* 28 (2010) 185–210.
  - [31] N.M. Haynes, M.B. Snook, J.A. Trapani, L. Cerruti, S.M. Jane, M.J. Smyth, P.K. Darcy, Redirecting mouse CTL against colon carcinoma: superior signaling efficacy of single-chain variable domain chimeras containing TCR-zeta vs Fc epsilon RI-gamma, *J. Immunol.* 166 (2001) 182e187.
  - [32] B.A. Irving, A. Weiss, The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor associated signal transduction pathways, *Cell* 64 (1991) 891e901.
  - [33] Wei Li, Fengxiao Zhang, Chenhui Ju, Suying Lv, Kai Huang, The role of CD27-CD70 signaling in myocardial infarction and cardiac remodeling, *Int. J. Cardiol.* (2018) S0167-5273(18)35497-4.
  - [34] R.J. Brentjens, E. Santos, Y. Nikhamin, R. Yeh, M. Matsushita, K. La Perle, A. Quintas-Cardama, S.M. Larson, M. Sadelain, Genetically targeted T cells eradicate systemic acute lymphoblastic leukemia xenografts, *Clin. Cancer Res.* 13 (2007) 5426–5435.
  - [35] C. Carpenito, M.C. Milone, R. Hassan, J.C. Simonet, M. Lakhal, M.M. Suhoski, A. Varela-Rohena, K.M. Haines, D.F. Heitjan, S.M. Albelda, R.G. Carroll, J.L. Riley, I. Pastan, C.H. June, Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains, *Proc. Natl. Acad. Sci. U. S. A.* 106 (2009) 3360–3365.
  - [36] D.G. Song, Q. Ye, C. Carpenito, M. Poussin, L.P. Wang, C. Ji, M. Figini, C.H. June, G. Coukos, D.J. Powell Jr., In vivo persistence, tumor localization, and antitumor activity of CAR-engineered T cells is enhanced by costimulatory signaling through CD137 (4-1BB), *Cancer Res.* 71 (2011) 4617e4627.
  - [37] M. Chmielewski, H. Abken, Tucks: the fourth generation of CARs, *Expert Opin. Biol. Ther.* 15 (2015) 1145–1154.
  - [38] C. Geldres, B. Savoldo, G. Doti, Chimeric antigen receptor redirected T cells return to the bench, *Semin. Immunol.* 28 (2016) 3–9.
  - [39] C.J. Turtle, L.A. Hanafi, C. Berger, et al., CD19 CAR-T cells of defined CD41:CD81 composition in adult B cell ALL patients, *J. Clin. Invest.* 126 (2016) 2123–2138.
  - [40] P. Kebriaei, H. Singh, M.H. Huls, et al., Phase I trials using sleeping beauty to generate CD19-specific CAR-T cells, *J. Clin. Invest.* 126 (2016) 3363–3376.
  - [41] P.V. Manuri, M.H. Wilson, S.N. Maiti, et al., piggyBac transposon/transposase system to generate CD19-specific T cells for the treatment of B-lineage malignancies, *Hum. Gene Ther.* 21 (2010) 427–437.
  - [42] B.G. Till, M.C. Jensen, J. Wang, et al., CD20-specific adoptive immunotherapy for lymphoma using a chimeric antigen receptor with both CD28 and 4-1BB domains: pilot clinical trial results, *Blood* 119 (2012) 3940–3950.
  - [43] Adrian P. Gee, GMP CAR-T cell production, *Best Pract. Res. Clin. Haematol.* 31 (2018) 126–134.
  - [44] J. Scholler, T.L. Brady, G. Binder-Scholl, et al., Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells, *Sci. Transl. Med.* 4 (2012) 132ra53.
  - [45] G.P. Wang, B.L. Levine, G.K. Binder, et al., Analysis of lentiviral vector integration in HIV1 study subjects receiving autologous infusions of gene modified CD41 T cells, *Mol. Ther.* 17 (2009) 844–850.
  - [46] Reza Elahi, Elnaz Khosh, Safa Tahmasebi, Abdolreza Esmailzadeh, Immune cell hacking: challenges and clinical approaches to create smarter generations of chimeric antigen receptor t cells, *Front. Immunol.* 9 (2018) 1717.
  - [47] S. Tahmasebi, R. Elahi, A. Esmailzadeh, Solid tumors challenges and new insights of CAR-T cell engineering, *Stem Cell Rev.* (2019), <https://doi.org/10.1007/s12015-019-09901-7>.
  - [48] Olivia Wilkins, Allison M. Keeler, Terence R. Flotte, CAR-t-Cell therapy: progress and prospects, *Hum. Gene Ther.* 28 (2) (2017) 61–66.
  - [49] C. Ellebrecht, V. Bhoj, A. Nace, et al., Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease, *Science* 353 (2016) 179–184.
  - [50] S. Sakaguchi, M. Miyara, C.M. Costantino, D.A. Hafler, FOXP3 regulatory T cells in the human immune system, *Nat. Rev. Immunol.* 10 (2010) 490–500.
  - [51] Q. Zhou, M.E. Munger, S.L. Highfill, et al., Program death-1 signaling and regulatory T cells collaborate to resist the function of adoptively transferred cytotoxic T lymphocytes in advanced acute myeloid leukemia, *Blood* 116 (2010) 2484–2493.
  - [52] Soyeon Kim and, Edmund K. Moon, Development of novel avenues to overcome challenges facing CAR-T cells, *Transl. Res.* 187 (2017) 22–31.
  - [53] J.Y. Shin, I.H. Yoon, J.S. Kim, B. Kim, C.G. Park, Vascular endothelial growth factor-induced chemotaxis and IL-10 from T cells, *Cell. Immunol.* 256 (2009) 72–78.
  - [54] H. Maeda, A. Shiraishi, TGF-beta contributes to the shift toward Th2-type responses through direct and IL-10-mediated pathways in tumor-bearing mice, *J. Immunol.* 156 (1996) 73–78.
  - [55] M.A. Postow, M.K. Callahan, J.D. Wolchok, Immune checkpoint blockade in cancer therapy, *J. Clin. Oncol.* 33 (2015) 1974–1982.
  - [56] D.M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy, *Nat. Rev. Cancer* 12 (2012) 252–264.
  - [57] X. Liu, R. Ranganathan, S. Jiang, et al., A chimeric switch-receptor targeting PD1 augments the efficacy of second-generation CAR-T cells in advanced solid tumors, *Cancer Res.* 76 (2016) 1578–1590.
  - [58] Ze Nan L. Chang, Yvonne Y. Chen, CARs: synthetic immunoreceptors for Cancer therapy and beyond, *Trends Mol. Med.* 23 (5) (2017) 430–450 May.
  - [59] P.R. Kumaresan, et al., Bioengineering T cells to target carbohydrate to treat opportunistic fungal infection, *Proc. Natl. Acad. Sci. U. S. A.* 111 (2014) 10660–10665.
  - [60] Jelena Skuljec, Markus Chmielewski, Christine Happle, Anika Habener, Mandy Busse, Hinrich Abken, Gesine Hansen, Chimeric antigen receptor-redirected regulatory T cells suppress experimental allergic airway inflammation, a model of asthma, *Front. Immunol.* 8 (1125) (2017).
  - [61] Seyyed Shamsadin Athari, Seyyed Moehyadin Athari, The importance of eosinophil, platelet and dendritic cell in asthma, *Asian Pac. J. Trop. Dis.* 4 (1) (2014) 41–47.
  - [62] Mehdi Taghavi, Alireza Khosravi, Esmail Mortaz, Donya Nikaein, Seyyed Shamsadin Athari, Role of pathogen-associated molecular patterns (PAMPs) in immune responses to fungal infections, *Eur. J. Pharmacol.* 808 (2017) 8–13.
  - [63] K.F. Chung, S.E. Wenzel, J.L. Brozek, A. Bush, M. Castro, P.J. Sterk, et al., International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma, *Eur. Respir. J.* 43 (2) (2014) 343–373.
  - [64] D.H. Strickland, P.G. Holt, T regulatory cells in childhood asthma, *Trends Immunol.* 32 (9) (2011) 420–427.
  - [65] S.S. Athari, S.M. Athari, F. Beyzay, M. Movassaghi, E. Mortaz, M. Taghavi, Critical role of Toll-like receptors in pathophysiology of allergic asthma, *Eur. J. Pharmacol.* 808 (2017) 21–27.
  - [66] Sheng-tao Zhao, Chang-zheng Wang, Regulatory T cells and asthma, *Biomed. Biotechnol. Res. J.* 19 (9) (2018) 663–673.
  - [67] N. Komatsu, M.E. Mariotti-Ferrandiz, Y. Wang, et al., Heterogeneity of natural Foxp3+ T cells: a committed regulatory T-cell lineage and an uncommitted minor population retaining plasticity, *Proc. Natl. Acad. Sci. U. S. A.* 106 (6) (2009) 1903–1908.
  - [68] K. Nakamura, A. Kitani, I. Fuss, et al., TGF-β1 plays an important role in the mechanism of CD4+CD25+ regulatory T cell activity in both humans and mice, *J. Immunol.* 172 (2) (2004) 834–842.
  - [69] L.W. Collison, C.J. Workman, T.T. Kuo, et al., The inhibitory cytokine IL-35 contributes to regulatory T-cell function, *Nature* 450 (7169) (2007) 566–569.
  - [70] N.S. Stanilov, L. Miteva, G. Cirovski, et al., Increased transforming growth factor β and interleukin 10 transcripts in peripheral blood mononuclear cells of colorectal cancer patients, *Contemp. Oncol.* 20 (6) (2016) 458–462.
  - [71] S. Read, V. Malmström, F. Powrie, Cytotoxic T lymphocyte associated antigen 4 plays an essential role in the function of CD25+CD4+ regulatory cells that control intestinal inflammation, *J. Exp. Med.* 192 (2) (2000) 295–302.
  - [72] B.T. Liang, C. Workman, J. Lee, et al., Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II, *J. Immunol.* 180 (9) (2008) 5916–5926.
  - [73] D.C. Gondek, L.F. Lu, S.A. Quezada, et al., Cutting edge: contact-mediated suppression by CD4+CD25+ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism, *J. Immunol.* 174 (4) (2005) 1783–1786.
  - [74] W.J. Grossman, J.W. Verbsky, W. Barchet, et al., Human T regulatory cells can use the perforin pathway to cause autologous target cell death, *Immunity* 21 (4) (2004) 589–601.
  - [75] A.M. Thornton, E.M. Shevach, CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production, *J. Exp. Med.* 188 (2) (1998) 287–296.
  - [76] M.S. Jordan, A. Boesteanu, A.J. Reed, et al., Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide, *Nat. Immunol.* 2 (4) (2001) 301–306.
  - [77] M. Miyara, Y. Yoshioka, A. Kitoh, et al., Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor, *Immunity* 30 (6) (2009) 899–911.
  - [78] S. Deaglio, K.M. Dwyer, W.D. Gao, et al., Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression, *J. Exp. Med.* 204 (6) (2007) 1257–1265.
  - [79] S. Sakaguchi, K. Wing, Y. Onishi, et al., Regulatory T cells: how do they suppress immune responses? *Int. Immunol.* 21 (10) (2009) 1105–1111.
  - [80] E. Bardel, F. Larousse, P. Charlot-Rabiega, et al., Human CD4+CD25+ Foxp3+ regulatory T cells do not constitutively express IL-35, *J. Immunol.* 181 (10) (2008) 6898–6905.
  - [81] Peng Gao, Zhenzhong Su, Xuejiao Lv, Jie Zhang, Interleukin-35 in asthma and its potential as an effective therapeutic agent, *Mediators Inflamm.* 2017 (2017) 5931865.
  - [82] B.M. Olson, J.A. Sullivan, W.J. Burlingham, Interleukin 35: a key mediator of suppression and the propagation of infectious tolerance, *Front. Immunol.* 4 (2013) 315.
  - [83] A.J. Baatjes, S.G. Smith, R. Watson, et al., T regulatory cell phenotypes in peripheral blood and bronchoalveolar lavage from non-asthmatic and asthmatic subjects, *Clin. Exp. Allergy* 45 (11) (2015) 1654–1662.
  - [84] D. Hartl, B. Koller, A.T. Mehlhorn, et al., Quantitative and functional impairment of pulmonary CD4+CD25hi regulatory T cells in pediatric asthma, *J. Allergy Clin. Immunol.* 119 (5) (2007) 1258–1266.
  - [85] A. Joetham, M. Schedel, B.P. O'Connor, et al., Inducible and naturally occurring regulatory T cells enhance lung allergic responses through divergent transcriptional pathways, *J. Allergy Clin. Immunol.* 139 (4) (2017) 1331–1342.
  - [86] I.P. Lewkowich, N.S. Herman, K.W. Schleifer, et al., CD4+CD25+ T cells protect against experimentally induced asthma and alter pulmonary dendritic cell phenotype and function, *J. Exp. Med.* 202 (11) (2005) 1549–1561.
  - [87] C.L. Fu, Y.H. Chuang, L.Y. Chau, et al., Effects of adenovirus expressing IL-10 in alleviating airway inflammation in asthma, *J. Gene Med.* 8 (12) (2006) 1393–1399.
  - [88] E. Gambineri, T.R. Torgerson, H.D. Ochs, Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis, *Curr. Opin. Rheumatol.* 15 (4) (2003) 430–435.
  - [89] P. Desreumaux, A. Foussat, M. Allez, et al., Safety and efficacy of antigen-specific regulatory T-cell therapy for patients with refractory Crohn's disease,

- Gastroenterology 143 (5) (2012) 1207–1217.
- [90] M. Chmielewski, A.A. Hombach, H. Abken, Antigen-specific T-cell activation independently of the MHC: chimeric antigen receptor-redirectioned T cells, *Front. Immunol.* 4 (2013) 371.
  - [91] A.A. Hombach, D. Kofler, G. Rappl, H. Abken, Redirecting human CD4+CD25+ regulatory T cells from the peripheral blood with pre-defined target specificity, *Gene Ther.* 16 (9) (2009) 1088–1096.
  - [92] M. Fransson, E. Piras, J. Burman, B. Nilsson, M. Essand, B. Lu, et al., CAR/FoxP3-engineered T regulatory cells target the CNS and suppress EAE upon intranasal delivery, *J. Neuroinflammation* 9 (2012) 112.
  - [93] D. Blat, E. Zigmond, Z. Alteber, T. Waks, Z. Eshhar, Suppression of murine colitis and its associated cancer by carcinoembryonic antigen-specific regulatory T cells, *Mol. Ther.* 22 (5) (2014) 1018–1028.
  - [94] J. Kearley, J.E. Barker, D.S. Robinson, C.M. Lloyd, Resolution of airway inflammation and hyperreactivity after in vivo transfer of CD4+CD25+ regulatory T cells is interleukin 10 dependent, *J. Exp. Med.* 202 (11) (2005) 1539–1547.
  - [95] W. Xu, Q. Lan, M. Chen, H. Chen, N. Zhu, X. Zhou, et al., Adoptive transfer of induced-Treg cells effectively attenuates murine airway allergic inflammation, *PLoS One* 7 (7) (2012) e40314.
  - [96] S. Sakaguchi, K. Wing, Y. Onishi, P. Prieto-Martin, T. Yamaguchi, Regulatory T cells: how do they suppress immune responses? *Int. Immunol.* 21 (10) (2009) 1105–1111.
  - [97] Q. Tang, J.A. Bluestone, The Foxp3+ regulatory T cell: a jack of all trades, master of regulation, *Nat. Immunol.* 9 (3) (2008) 239–244.
  - [98] M. Chmielewski, A.A. Hombach, H. Abken, Antigen-specific T-cell activation independently of the MHC: chimeric antigen receptor-redirectioned T cells, *Front. Immunol.* 4 (371) (2013).
  - [99] Hollie J. Jackson, Sarwish Rafiq, Renier J. Brentjens, Driving CAR-T-cells forward, *Nat. Rev. Clin. Oncol.* 13 (6) (2016) 370–383.
  - [100] X. Wang, A. Naranjo, C.E. Brown, et al., Phenotypic and functional attributes of lentivirus-modified CD19-specific human CD8+ central memory T cells manufactured at clinical scale, *J. Immunother.* 35 (2012) 689–701.
  - [101] C.E. Brown, D. Alizadeh, R. Starr, L. Weng, J.R. Wagner, A. Naranjo, J.R. Ostberg, M.S. Blanchard, J. Kilpatrick, J. Simpson, A. Kurien, S.J. Priceman, X. Wang, T.L. Harshbarger, M. D'Apuzzo, J.A. Ressler, M.C. Jensen, M.E. Barish, M. Chen, J. Portnow, S.J. Forman, B. Badie, Regression of glioblastoma after chimeric antigen receptor T-Cell therapy, *N. Engl. J. Med.* 375 (26) (2016) 2561–2569.
  - [102] M. Jonnalagadda, A. Mardiros, R. Urak, et al., Chimeric antigen receptors with mutated IgG4 Fc spacer avoid Fc receptor binding and improve T cell persistence and antitumor efficacy, *Mol. Ther.* 23 (2015) 757–768.
  - [103] H. Jethwa, A.A. Adami, J. Maher, Use of gene-modified regulatory T-cells to control autoimmune and alloimmune pathology: is now the right time? *Clin. Immunol.* 150 (1) (2014) 51–63.
  - [104] D. Sommermeyer, M. Hudecek, P.L. Kosasih, T. Gogishvili, D.G. Maloney, C.J. Turtle, et al., Chimeric antigen receptor-modified T cells derived from defined CD8(β) and CD4(β) subsets confer superior antitumor reactivity in vivo, *Leukemia* 30 (2016) 492e500.
  - [105] M.T. Stephan, V. Ponomarev, R.J. Brentjens, A.H. Chang, K.V. Dobrenkov, G. Heller, et al., T cell-encoded CD80 and 4-1BBL induce auto- and transcostimulation, resulting in potent tumor rejection, *Nat. Med.* 13 (2007) 1440e9.
  - [106] Janneke E. Jaspers, Renier J. Brentjens, Development of CAR-T cells designed to improve antitumor efficacy and safety, *Pharmacol. Ther.* 178 (2017) 83–91.
  - [107] S. Wilkie, S.E. Burbridge, L. Chiapero-Stanke, A.C.P. Pereira, S. Cleary, S.J.C. van der Stegen, et al., Selective expansion of chimeric antigen receptor-targeted T-cells with potent effector function using interleukin-4, *J. Biol. Chem.* 285 (33) (2010) 25538–25544.
  - [108] S.J.C. van der Stegen, D.M. Davies, S. Wilkie, J. Foster, J.K. Sosabowski, J. Burnet, et al., Preclinical in vivo modeling of cytokine release syndrome induced by ErbBretargeted human T cells: Identifying a window of therapeutic opportunity? *J. Immunol.* 191 (9) (2013) 4589–4598.
  - [109] S.J.C. van der Stegen, M. Hamieh, M. Sadelain, The pharmacology of second-generation chimeric antigen receptors, *Nat. Rev. Drug Discov.* 14 (7) (2015) 499–509.
  - [110] J. Maher, R.J. Brentjens, G. Gunset, I. Rivière, M. Sadelain, Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta/CD28 receptor, *Nat. Biotechnol.* 20 (1) (2002) 70–75.
  - [111] U. Schönbeck, P. Libby, The CD40/CD154 receptor/ligand dyad, *Cell. Mol. Life Sci.* 58 (1) (2001) 4–43.
  - [112] R.M. Spaapen, M.Y.K. Leung, M.B. Fuentes, J.P. Kline, L. Zhang, Y. Zheng, et al., Therapeutic activity of high-dose intratumoral IFN-β requires direct effect on the tumor vasculature, *J. Immunol.* 193 (8) (2014) 4254–4260.
  - [113] X. Yang, X. Zhang, M.L. Fu, R.R. Weichselbaum, T.F. Gajewski, Y. Guo, et al., Targeting the tumor microenvironment with interferon-β bridges innate and adaptive immune responses, *Cancer Cell* 25 (1) (2014) 37–48.
  - [114] J. Larkin, V. Chiarion-Sileni, R. Gonzalez, J.J. Grob, C.L. Cowey, C.D. Lao, et al., Combined nivolumab and ipilimumab or monotherapy in untreated melanoma, *N. Engl. J. Med.* 373 (1) (2015) 23–34.
  - [115] L.B. John, C. Devaud, C.P.M. Duong, C.S. Yong, P.A. Beavis, N.M. Haynes, et al., Anti-PD-1 antibody therapy potentially enhances the eradication of established tumors by gene-modified T cells, *Clin. Cancer Res.* 19 (20) (2013) 5636–5646.
  - [116] Eleonora Dehlink, Barbara Platzter, Alexandra H. Baker, Jessica LaRosa, Michael Pardo, Peter Dwyer, Elizabeth H. Yen, Zsolt Szépfalusi, Samuel Nurko, Edda Fiebigler, A soluble form of the high affinity IgE receptor, Fc-epsilon-RI, circulates in human serum, *PLoS One* 6 (4) (2011) e19098.
  - [117] M. Acharya, G. Borland, A.L. Edkins, L.M. MacLellan, J. Matheson, B.W. Ozzanne, W. Cushley, CD23/FcεRI: molecular multi-tasking, *Clin. Exp. Immunol.* 162 (2010) 12–23.
  - [118] Scott C. Garman, Beth A. Wurzberg, Svetlana S. Tarchevskaya, Jean-Pierre Kinet, Theodore S. Jardetzky, Structure of the Fc fragment of human IgE bound to its high-affinity receptor FcεRIα, *Nature* 406 (2000) 259–266.
  - [119] D.E. Ward, B.L. Fay, A. Adejwon, H. Han, Z. Ma, Chimeric antigen receptors based on low affinity mutants of fcγRI Re-direct t cell specificity to cells expressing membrane IgE, *Front. Immunol.* 9 (2018) 2231.
  - [120] Marcela V. Maus, Andrew R. Haas, Gregory L. Beatty, Steven M. Albelda, Bruce L. Levine, Xiaojun Liu, Yangbing Zhao, Michael Kalos, Carl H. June, T cells expressing chimeric antigen receptors can cause anaphylaxis in humans, *Cancer Immunol. Res.* 1 (2013) 26–31.
  - [121] X. Tang, Y. Sun, A. Zhang, et al., Third-generation CD28/4-1BB chimeric antigen receptor T cells for chemotherapy relapsed or refractory acute lymphoblastic leukaemia: a non-randomised, open-label phase I trial protocol, *BMJ Open* 6 (12) (2016) e013904.
  - [122] Dana E. Ward, Brittany L. Fay, Adebomi Adejwon, Huihui Han, Ma Zhengyu, Chimeric antigen receptors based on low affinity mutants of FcεRI Re-direct T cell specificity to cells expressing membrane IgE, *Front. Immunol.* 9 (2231) (2018).
  - [123] O. Talay, D. Yan, H.D. Brightbill, E.E. Straney, M. Zhou, E. Ladi, et al., IgE(+) memory B cells and plasma cells generated through a germinal-center pathway, *Nat. Immunol.* 13 (2012) 396–404.
  - [124] L.C. Wu, A.A. Zarrin, The production and regulation of IgE by the immune system, *Nat. Rev. Immunol.* 14 (2014) 247–259.
  - [125] M.C. Milone, J.D. Fish, C. Carpenito, et al., Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo, *Mol. Ther.* 17 (8) (2009) 1453–1464.
  - [126] R.A. Morgan, et al., Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2, *Mol. Ther.* 18 (2010) 843–851.
  - [127] R.A. Morgan, et al., Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy, *J. Immunother.* 36 (2013) 133–151.
  - [128] Marcela V. Maus, Andrew R. Haas, Gregory L. Beatty, Steven M. Albelda, Bruce L. Levine, Xiaojun Liu, Yangbing Zhao, Michael Kalos, Carl H. June, T cells expressing chimeric antigen receptors can cause anaphylaxis in humans, *Cancer Immunol. Res.* 1 (2013) 26–31.
  - [129] R.A. Morgan, et al., Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2, *Mol. Ther.* 18 (2010) 843–851.
  - [130] R.J. Brentjens, et al., CD19-targeted T Cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia, *Sci. Transl. Med.* 5 (177) (2013) 177ra38.
  - [131] M.L. Davila, et al., Efficacy and toxicity management of 19-28z CAR-T cell therapy in B cell acute lymphoblastic leukemia, *Sci. Transl. Med.* 6 (2014) 224–225.
  - [132] M.H. O'Hara, C. Stashwick, G. Plesa, J.L. Tanyi, Overcoming barriers of CAR-T-cell therapy in patients with mesothelin-expressing cancers, *Immunotherapy* 9 (2017) 767–780.
  - [133] Y. Zhang, W. Zhang, H. Dai, Y. Wang, F. Shi, C. Wang, et al., An analytical biomarker for treatment of patients with recurrent B-ALL after remission induced by infusion of anti-CD19 chimeric antigen receptor T (CAR-T) cells, *Sci. China Life Sci.* 59 (2016) 379–385.
  - [134] C.M. Wang, Z.Q. Wu, Y. Wang, Y.L. Guo, H.R. Dai, X.H. Wang, et al., Autologous T cells expressing CD30 Chimeric antigen receptors for relapsed or refractory Hodgkin lymphoma: an open-label phase I trial, *Clin. Cancer Res.* 23 (2017) 1156–1166.
  - [135] J. Buechner, S.A. Grupp, S.L. Maude, M. Boyer, H. Bittencourt, T.W. Laetsch, et al., Global registration trial of efficacy and safety of CTL019 in pediatric and young adult patients with relapsed/refractory (R/R) acute lymphoblastic leukemia (ALL): update to the interim analysis, *Clin. Lymphoma Myeloma Leuk.* 17 (2017) S263–4.
  - [136] S.J. Schuster, M.R. Bishop, C. Tam, E.K. Waller, P. Borchmann, J. McGuirk, et al., Global pivotal phase 2 trial of the Cd19-targeted therapy Ctl019 in adult patients with relapsed or refractory (R/R) diffuse large B-cell lymphoma (DLBcl)-an interim analysis, *Hematol. Oncol.* 35 (2017) 27.
  - [137] R.A. Gardner, O. Finney, C. Annesley, H. Brakke, C. Summers, K. Leger, et al., Intent-to-treat leukemia remission by CD19 CAR-T cells of defined formulation and dose in children and young adults, *Blood* 129 (2017) 3322–3331.
  - [138] J.S. Abramson, M.L. Palomba, L.I. Gordon, M.A. Lunning, J.E. Aronson, M. Wang, et al., High durable CR rates in relapsed/refractory (R/R) aggressive B-NHL treated with the CD19-directed CAR-T cell product JCAR017 (TRANSCEND NHL 001): defined composition allows for dose-finding and definition of pivotal cohort, *Blood* 130 (2017) 581.
  - [139] Y. Wang, M. Chen, Z. Wu, C. Tong, J. Huang, H. Lv, et al., CD133-redirectioned chimeric antigen receptor engineered autologous T-cell treatment in patients with advanced and metastatic malignancies, *J. Clin. Oncol.* 35 (2017) 3042.
  - [140] J. Buechner, S.A. Grupp, S.L. Maude, M. Boyer, H. Bittencourt, T.W. Laetsch, et al., Global registration trial of efficacy and safety of CTL019 in pediatric and young adult patients with relapsed/refractory (R/R) acute lymphoblastic leukemia (ALL): update to the interim analysis, *Clin. Lymphoma Myeloma Leuk.* 17 (2017) S263–4.
  - [141] J.S. Abramson, M.L. Palomba, L.I. Gordon, M.A. Lunning, J.E. Aronson, M. Wang, et al., High durable CR rates in relapsed/refractory (R/R) aggressive B-NHL treated with the CD19-directed CAR-T cell product JCAR017 (TRANSCEND NHL 001): defined composition allows for dose-finding and definition of pivotal cohort, *Blood* 130 (2017) 581.
  - [142] S.J. Schuster, M.R. Bishop, C. Tam, E.K. Waller, P. Borchmann, J. McGuirk, et al., Global pivotal phase 2 trial of the Cd19-targeted therapy Ctl019 in adult patients with relapsed or refractory (R/R) diffuse large B-cell lymphoma (DLBcl)-an interim analysis, *Hematol. Oncol.* 35 (2017) 27.

- [143] Zhengguang Wang and, Weidong Han, Biomarkers of cytokine release syndrome and neurotoxicity related to CAR-T cell therapy, Wang and Han Biomarker Res. 6 (4) (2018).
- [144] M.S. Topp, N. Gökbuget, A.S. Stein, G. Zugmaier, S. O'Brien, R.C. Bargou, et al., Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, singlearm, phase 2 study, *Lancet Oncol.* 16 (2015) 57.
- [145] Y. Zhang, W. Zhang, H. Dai, Y. Wang, F. Shi, C. Wang, et al., An analytical biomarker for treatment of patients with recurrent B-ALL after remission induced by infusion of anti-CD19 chimeric antigen receptor T (CAR-T) cells, *Sci. China Life Sci.* 59 (2016) 379–385.
- [146] Jennifer N. Brudno, James N. Kochenderfer, Chimeric antigen receptor T-cell therapies for lymphoma, *Clin. Oncol.* 15 (2018) 31–46.
- [147] Robert Stürups, CAR-T-cell therapy in refractory large B-cell lymphoma, *Lancet Oncol.* 19 (2018) 19.
- [148] M.V. Maus, et al., T cells expressing chimeric antigen receptors can cause anaphylaxis in humans, *Cancer Immunol. Res.* 1 (2013) 26–31.
- [149] G.L. Beatty, et al., Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce antitumor activity in solid malignancies, *Cancer Immunol. Res.* 2 (2014) 112–120.
- [150] R. Gardner, K.J. Leger, C.E. Annesley, C. Summers, J. Rivers, J. Gust, et al., Decreased rates of severe CRS seen with early intervention strategies for CD19 CAR-T cell toxicity management, *Blood* 128 (2016) 586.
- [151] S.S. Neelapu, S. Tummala, P. Kebriaei, W. Wierda, C. Gutierrez, F.L. Locke, et al., Chimeric antigen receptor T-cell therapy – assessment and management of toxicities, *Nat. Rev. Clin. Oncol.* 15 (1) (2018) 47–62.
- [152] S.S. Kenderian, M. Ruella, O. Shestova, et al., Ruxolitinib prevents cytokine release syndrome after CART cell therapy without impairing the anti-tumor effect in a xenograft model, *Blood* 128 (2016) 652.
- [153] M. Ruella, S.S. Kenderian, O. Shestova, et al., Kinase inhibitor ibrutinib prevents cytokine-release syndrome after anti-CD19 chimeric antigen receptor T cells (CART) for B cell neoplasms, *Blood* 128 (2016) 2159.
- [154] L. Poirot, B. Philip, C.S. Mannioui, et al., Multiplex genome edited T-cell manufacturing platform for “off-the-shelf” adoptive T-cell immunotherapies, *Cancer Res.* 75 (2015) 3853–3864.
- [155] Olivia Wilkins, Allison M. Keeler, Terence R. Flotte, CAR-T-cell therapy: progress and prospects, *Hum. Gene Ther.* 28 (2) (2017) 61–66.
- [156] M. Ratner, Off-the-shelf CAR-T therapy induces remission in child with ALL, *Nat. Biotechnol.* 34 (2016) 12.
- [157] S.K. Perna, et al., Interleukin-7 mediates selective expansion of tumor-redifferentiated cytotoxic T lymphocytes (CTLs) without enhancement of regulatory T-cell inhibition, *Clin. Cancer Res.* 20 (2014) 131–139.
- [158] A. Di Stasi, et al., T lymphocytes coexpressing CCR4 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model, *Blood* 113 (2009) 6392–6402.
- [159] W. Peng, et al., Transduction of tumor-specific T cells with CXCR2 chemokine receptor improves migration to tumor and antitumor immune responses, *Clin. Cancer Res.* 16 (2010) 5458–5468.
- [160] H.J. Pegram, et al., CD28z CARs and armored CARs, *Cancer J.* 20 (2014) 127–133.
- [161] M. Hegde, A. Corder, K.K. Chow, M. Mukherjee, A. Ashoori, Y. Kew, Y.J. Zhang, D.S. Baskin, F.A. Merchant, V.S. Brawley, T.T. Byrd, S. Krebs, M.F. Wu, H. Liu, H.E. Heslop, S. Gottschalk, E. Yvon, N. Ahmed, Combinational targeting offsets antigen escape and enhances effector functions of adoptively transferred T cells in glioblastoma, *Mol. Ther. J. Am. Soc. Gene Ther.* 21 (2013) 2087e2101.
- [162] Z. Grada, M. Hegde, T. Byrd, D.R. Shaffer, A. Ghazi, V.S. Brawley, A. Corder, K. Schonfeld, J. Koch, G. Dotti, H.E. Heslop, S. Gottschalk, W.S. Wels, M.L. Baker, N. Ahmed, TanCAR, A novel bispecific chimeric antigen receptor for cancer immunotherapy, *Mol. Ther. Nucleic Acids* 2 (2013) e105.
- [163] K. Kudo, C. Imai, P. Lorenzini, T. Kamiya, K. Kono, A.M. Davidoff, W.J. Chng, D. Campana, T lymphocytes expressing a CD16 signaling receptor exert antibody dependent cancer cell killing, *Cancer Res.* 74 (2014) 93–103.
- [164] G.G. Gornalusse, R.K. Hirata, S.E. Funk, L. Rioloobos, V.S. Lopes, G. Manske, D. Prunkard, A.G. Colunga, L.A. Hanafi, D.O. Clegg, C. Turtle, D.W. Russell, HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells, *Nat. Biotechnol.* 35 (8) (2017) 765–772.
- [165] D. Vestweber, How leukocytes cross the vascular endothelium, *Nat. Rev. Immunol.* 15 (2015) 692–704.
- [166] H.J. Pegram, et al., Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning, *Blood* 119 (2012) 4133–4141.
- [167] M. Chmielewski, A.A. Hombach, H. Abken, Of CARs and TRUCKS: chimeric antigen receptor (CAR) T cells engineered with an inducible cytokine to modulate the tumor stroma, *Immunol. Rev.* 257 (2014) 83–90.
- [168] D.G. Song, Q. Ye, C. Carpenito, M. Poussin, L.P. Wang, C. Ji, M. Figini, C.H. June, G. Coukos, D.J. Powell Jr., In vivo persistence, tumor localization, and antitumor activity of CAR-engineered T cells is enhanced by costimulatory signaling through CD137 (4-1BB), *Cancer Res.* 71 (2011) 4617–4627.
- [169] Farooq Marofi, Ghasem Vahedi, Alireza Biglari, Abdolreza Esmailzadeh, Seyyed Shamsadin Athari, Mesenchymal stromal/stem cells: a new era in the cell-based targeted gene therapy of cancer, *Front. Immunol.* 8 (1770) (2017).
- [170] K. Iwamura, et al., siRNA-mediated silencing of PD-1 ligands enhances tumor-specific human T-cell effector functions, *Gene Ther.* 19 (2012) 959–966.
- [171] B. Savoldo, C.A. Ramos, E. Liu, M.P. Mims, M.J. Keating, G. Carrum, R.T. Kamble, C.M. Bollard, A.P. Gee, Z. Mei, H. Liu, B. Grilley, C.M. Rooney, H.E. Heslop, M.K. Brenner, G. Dotti, CD28 costimulation improves expansion and persistence of chimeric antigen receptoremmodified T cells in lymphoma patients, *J. Clin. Invest.* 121 (2011) 1822e1826.
- [172] A.J. Bogdanove, D.F. Voytas, TAL effectors: customizable proteins for DNA targeting, *Science* 333 (2011) 1843–1846.
- [173] J.A. Doudna, E. Charpentier, Genome editing. The new frontier of genome engineering with CRISPR-Cas9, *Science* 346 (2014) 1258096.
- [174] H. Torikai, A. Reik, F. Soldner, E.H. Warren, C. Yuen, Y. Zhou, D.L. Crossland, H. Huls, N. Littman, Z. Zhang, S.S. Tykodi, P. Kebriaei, D.A. Lee, J.C. Miller, E.J. Rebar, M.C. Holmes, R. Jaenisch, R.E. Champlin, P.D. Gregory, L.J. Cooper, Toward eliminating HLA class I expression to generate universal cells from allogeneic donors, *Blood* 122 (2013) 1341–1349.
- [175] Yongping Zhang, Mu Wei, Haoyi Wang, Gene editing in T cell therapy, *J. Genet. Genom.* 44 (2017) 415–422.
- [176] J. Valton, V. Guyot, A. Marechal, J.M. Filhol, A. Juillerat, A. Duclert, P. Duchateau, L. Poirot, A multidrug-resistant engineered CAR-T cell for allogeneic combination immunotherapy, *Mol. Ther.* 23 (2015) 1507–1518.
- [177] L. Poirot, et al., Multiplex genome-edited T-cell manufacturing platform for ‘off-the-shelf’ adoptive T-cell immunotherapies, *Cancer Res.* 75 (2015) 3853–3864.
- [178] J. Ren, X. Liu, C. Fang, S. Jiang, C.H. June, Y. Zhao, Multiplex genome editing to generate universal CAR-T cells resistant to PD1 inhibition, *Clin. Cancer Res.* 23 (9) (2017) 2255–2266.
- [179] X. Liu, et al., CRISPR- Cas9-mediated multiplex gene editing in CAR-T cells, *Cell Res.* 27 (2017) 154–157.
- [180] Juanjuan Zhao, Quande Lin, Yongping Song, Delong Liu, Universal CARs, universal T cells, and universal CAR-T cells, *J. Hematol. Oncol.* 11 (132) (2018).
- [181] NogaOr Geva, Rotem Gidron, Aloukick Kumar Singh, Rakefet Sidlik Muskateel, Yair Reisner, Towards ‘Off-the-Shelf’ Universal Chimeric Antigen Receptor (CAR) T Cells: mouse anti-3rd party central memory CD8 veto cells prolong functional engraftment of allogeneic genetically modified T cells, *Blood* 128 (2171) (2016).
- [182] Qunfang Zhang, Weihui Lu, Chun-Ling Liang, Yuchao Chen, Huazhen Liu, Feifei Qiu, Zhenhua Dai, Chimeric antigen receptor (CAR) treg: a promising approach to inducing immunological tolerance, *Front. Immunol.* 9 (2018) 2359.