



Remote controlling of CAR-T cells and toxicity management: Molecular switches and next generation CARs

Ehsan Moghanloo^a, Hasan Mollanoori^{b,c}, Mohsen Talebi^d, Salar Pashangzadeh^e,
Fatemeh Faraji^a, Farimah Hadjilooei^a, Habibollah Mahmoodzadeh^{a,f,*}

^a Cancer Research Center, Cancer Institute of Iran, Tehran University of Medical Science, Tehran, Iran

^b Department of Medical Genetics, Iran University of Medical Sciences (IUMS), Tehran, Iran

^c Medical Genetics Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

^d School of Medicine, West Virginia University, USA

^e Department of Immunology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

^f Breast Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:

Remote controlling
CAR-T cells
Toxicity management
Molecular switches

ABSTRACT

Cell-based immunotherapies have been selected for the front-line cancer treatment approaches. Among them, CAR-T cells have shown extraordinary effects in hematologic diseases including chemotherapy-resistant acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and non-Hodgkin lymphoma (NHL). In this approach, autologous *T* cells isolated from the patient's body genetically engineered to express a tumor specific synthetic receptor against a tumor antigen, then these cells expanded *ex vivo* and re-infusion back to the patient body. Recently, significant clinical response and high rates of complete remission of CAR T cell therapy in B-cell malignancies led to the approval of Kymriah and Yescarta (CD19-directed CAR-T cells) were by FDA for treatment of acute lymphoblastic leukemia and diffuse large B-cell lymphoma. Despite promising therapeutic outcomes, CAR T cells also can elicit the immune-pathologic effects, such as Cytokine Release Syndrome (CRS), Tumor Lysis Syndrome (TLS), and on-target off-tumor toxicity, that hampered its application. Ineffective control of these highly potent synthetic cells causes discussed potentially life-threatening toxicities, so researchers have developed several mechanisms to remote control CAR T cells. In this paper, we briefly review the introduced toxicities of CAR-T cells, then describe currently existing control approaches and review their procedure, pros, and cons.

Introduction

Cancer is defined as abnormal activity of different cell cycles protein, which results in cells' uncontrolled proliferation [1]. Cell-based immunotherapies have been selected for the front-line cancer treatment approaches [2]. Recently, different strategies such as monoclonal antibodies (mAbs), tumor vaccines, immune checkpoint blockades, cytokine-induced killers (CIKs), bispecific antibodies, tumor-infiltrating lymphocytes (TIL), and chimeric antigen receptor *T* (CAR-T) have been recruited to overcome cancer [3]. Monoclonal antibodies such as Herceptin and Cetuximab showed desirable effects on patients with malignant tumors. Currently, Food and Drug Administration (FDA) has approved some immune checkpoint blocking agents, including Ipilimumab (anti-CTLA-4mAb), Pembrolizumab, and Nivolumab (anti-PD-1mAb) for melanoma patients [4]. Cell-based immunotherapy relies on using intact and living immune cells that are extracted from the human body and grown

to increase their amount and power or genetically-modified to boost their ability to find and kill tumor cells. *T* cells play a key role (monitoring and killing potentially malignant cells) in the cell-mediated immune response. Various types of therapies have been developed to culture, redirect, and/or enhance *T* cells against tumors. *T* cell-based adoptive immunotherapy is one of them, which includes three models: tumor-infiltrating lymphocytes, *T* cell receptor (TCR)-modified *T* cells, and chimeric antigen receptor *T* cells (CAR-T cells). Compared with CAR-T cells, the efficacy of TILs and TCR-modified *T* cells is not substantial, because they don't modify *T* cells extremely. Besides, their process of generation, little success rate, and dependency on vaccination have been limited the development of these approaches [5].

CARs were described in 1987 by Diamond et al. [6] and shown to have extraordinary effects in hematologic diseases including chemotherapy-resistant acute lymphoblastic leukemia (ALL) [7–11], chronic lymphocytic leukemia (CLL) [12,13], and non-Hodgkin lym-

* Corresponding author at: Breast Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran.

E-mail address: hmahmoudzadeh2020@gmail.com (H. Mahmoodzadeh).

phoma (NHL) [14,15]. However, these modified T cells for cancer immunotherapy of solid tumors have not yielded successful results yet. CARs mostly consist of a single-chain variable fragment of an antibody (ScFv) recognizing tumor antigen, a transmembrane domain, intracellular single-chain tyrosine-based activation motifs (ITAMs) from CD3 zeta chain (CD3 ζ), and a co-stimulatory domain [16]. The activation process of these engineered T cells is totally independent of the major histocompatibility complex (MHC) [17]. Researchers have developed different generations of them composing of (i) CD3 ζ or Fc receptor γ (FcR γ) activating signal in an intracellular motif which results in transient T cell activation [18] (ii) one activating co-stimulatory domain (CD28 or 4-1BB or OX-40) (iii) two or more activating co-stimulatory domains [19,20] (iv) T cells redirected for universal cytokine killing (TRUCKS) that are engineered to produce IL-12 for tumor environment remodeling [21,22]. Within weeks of engineered T cell administration, cytokine production, targeted cells death, and stimulation of T cell proliferation are predicted [23–25]. Some limitations, including poor permeability, difficulties of target selection, and suppressive tumor microenvironment overshadowed the CAR-T cells' clinical outcome [21].

Although CAR-T cells made some progress in the treatment of the hematologic malignancies, some adverse effects, including fatal complications, have been reported in some patients who have received CAR-modified T cells. This review article highlights the different CAR-related toxicities and introduces potential strategies to overcome them.

2. Adverse effects of CAR-T cells

CAR-T cell infusion is not entirely safe; therefore, patients mostly experience some adverse reactions, including on-target on-tumor toxicity, on-target off-tumor toxicity, and other adverse reactions which are listed below.

2.1. On-target on-tumor toxicity

2.1.1. Cytokine Release Syndrome (CRS)

CAR-T cell therapy not only kills tumor cells but also results in the production of a considerable level of cytokines, including tumor necrosis factor-alpha (TNF- α), interferon γ (IFN- γ), IL-6, and IL-10 [24,26]. This cytokine production is called cytokine release syndrome (CRS) and leads to some clinical side effects such as fever, tachycardia, hypotension, and hypoxia, which may finally result in rapid death. CAR-T cell dosage and disease burden are considered as biomarkers that can predict CRS during CAR-T cell therapy [26–28].

2.1.2. Tumor Lysis Syndrome (TLS)

Destruction, of a large number of tumor cells, causes a rapid release of intracellular substances and brings about some metabolic disorders, including hyperuricemia, hyperphosphatemia, hypocalcemia, and metabolic acidosis, which result in acute renal failure and death [25].

2.2. On-target off-tumor toxicity

On-target off-tumor toxicity is an unavoidable side effect caused by the shared expression of the target antigen on normal tissues. For instance, some target antigens, including CD19, CD20, and CD22, are expressed on some normal blood cells that create an obstacle in the application of CAR-T cells in hematologic tumors [11].

2.3. Other adverse effects

The risk of GVHD (graft versus host disease) incidence may be increased by donor-derived CAR-T cells. Maus et al. showed that combined TCR and CAR cells could decrease GVHD risk. Since murine antibodies are the source of the most recognizing domain of CARs, host anti-CAR responses may be present in some patients [29,30]. Maus et al. reported that the application of CAR-T cells derived from murine mAb against

human mesothelin led to acute anaphylaxis. Moreover, a higher level of IgE is a consequence of repeated CAR-T cell infusion [29]. Although there was no evidence of viral vector-induced immortalization of the cells, viral vector-transfected T cells may increase the risk of oncogenesis [31]. Furthermore, neurotoxicity is the other adverse effect of CAR-T cell therapy, which usually includes confusion, delirium, expressive aphasia, obtundation, myoclonus, and seizure [7].

3. Strategies for remote controlling of CAR-T cells

Although CAR-T cell therapy is a promising therapeutic approach, the immune-pathologic effects of this treatment, such as CRS and on-target off-tumor toxicity, have hampered its application [32,33]. CAR-T cells are engrafted and persist indefinitely in a patient's body, so controlling its unpredicted toxicities is vital. Recent advances in synthetic biology provide new methods to control the immune response in order to augment the accuracy of synthetic immune cell therapies by remote and noninvasive control. Sensing, processing, and responding to the dynamic environments by living cells fulfilled using various biological mechanisms. Specifically, by rewiring cellular ligands, receptors, and signaling pathways into bio-circuitry they can sense and respond to multiple inputs, for example, remote stimuli. Accordingly, synthetic immune cells, which genetically engineered with remote-controlled circuits, supply non-invasive and site-specific activation that capable of adjusting the potency, specificity, and safety of immune responses. Remote control of immunity can apply external targeting with signals such as light or heat, or autonomous circuits. For further information looking at the Gamboa et al. review about the remote control mechanisms in synthetic immunity [34]. Several approaches have been developed to diminish these adverse effects that elaborate in three classes, including suicide switches, endogenous switches, and exogenous switches (Fig.1) that are described in the following paragraphs briefly.

Also, Table 1 summarizes the strengths and weaknesses of these approaches, and ongoing clinical trials using these methods are shown in Table 2.

3.1. Suicide switches

Suicide genes are genetically encoded elements integrated into CAR-T cells that allow the elimination of the introduced T cells in case of unexpected toxicities. They are activated by the administration of a pharmaceutical agent [53,54]. These genes include inducible caspase 9 (iC9), truncated EGFR (tEGFR or EGFRt), herpes simplex virus thymidine kinase (HSV-TK), and CD20 [36].

3.1.1. iCasp9

iCasp9 (inducible caspase 9) is a pro-apoptotic safety switch made by the fusion of a mutant FKBP12, a receptor for the immunosuppressant drug FK506, to a modified human caspase 9 using a flexible Ser-Gly-Gly-Gly-Ser-linker [55]. The mutant FKBP12 moiety allows a small molecular chemical inducer of dimerization (CID) (AP1903/AP20187) to attach to it while it cannot bind to the wild-type FKBP12. The modified caspase 9 is a truncated protein without the physiological dimerization domain or caspase recruitment domain (CARD) to minimize basal signaling. Conditional intravenous administration of a CID (such as AP1903) produces crosslinking of the drug-binding domains of this chimeric protein that results in the dimerization of caspase 9, and whereby activates the downstream executioner caspase3 molecules, leading to apoptosis of the cells expressing the fusion protein [33]. *In-vitro* and *in-vivo* experiments show that this safety switch can cause apoptosis of approximately 99% of donor T cells using a 10 nM dose of AP1903 [56].

3.1.2. HSV-TK

The Thymidine kinase (TK) derived from Herpes simplex viruses-1 (HSV-1) (HSV-TK), which has been probably evolved distinctly from

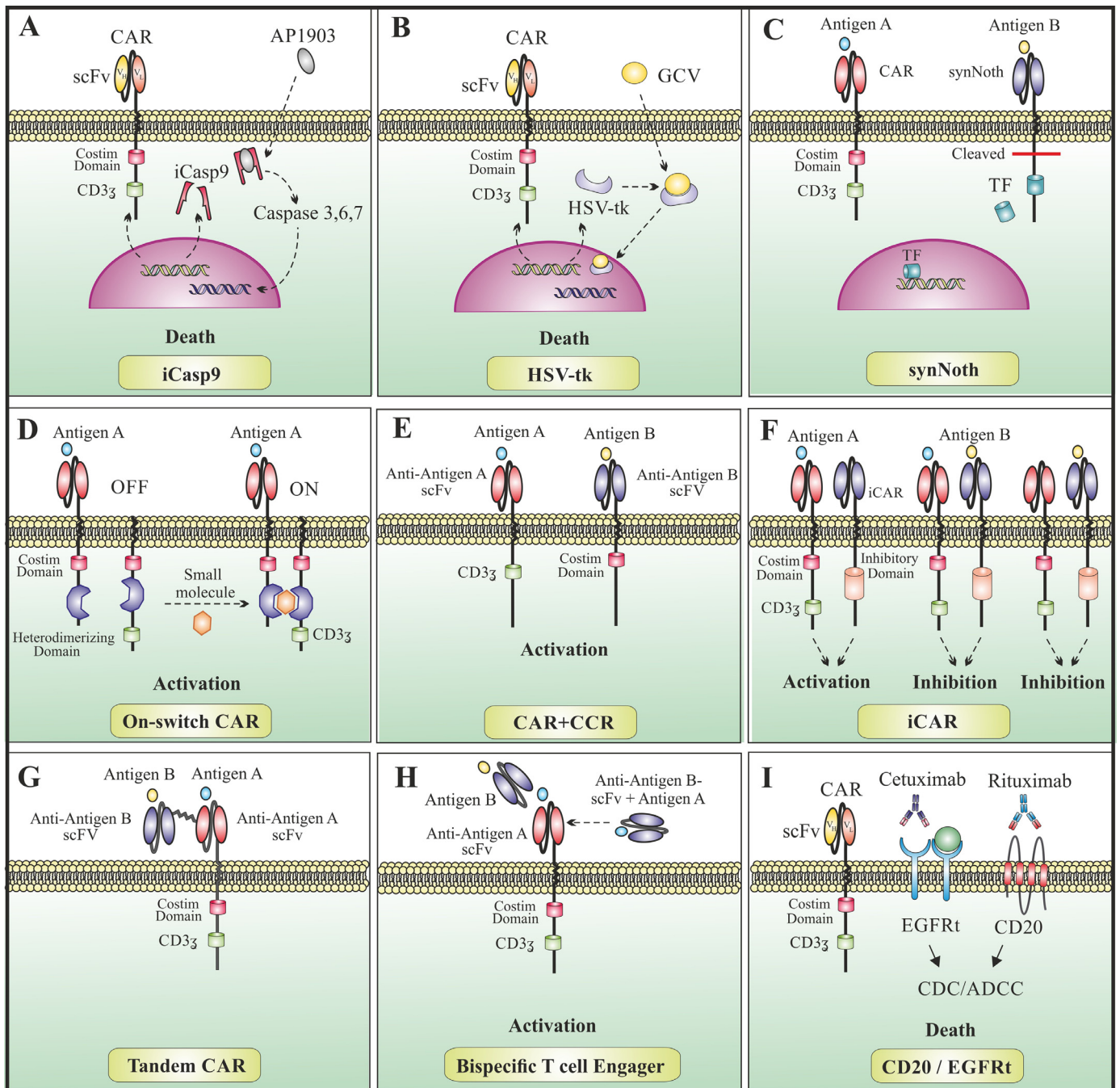


Fig. 1. Safety strategies to overcome CAR-T-cell-related toxicity. A Conditional intravenous administration of AP1903 begins dimerization of caspase9 that activates the downstream executioner caspase3 molecules, resulting in cellular apoptosis of cells expressing the CAR-T cells. B, After the conditional intravenous administration of GCV, HSV-TK catalyzes the phosphorylation of GCV that produces a toxic GCV-triphosphate that causes competitive inhibition of guanosine incorporation with subsequent inhibition of DNA synthesis and death. C, A synNotch receptor recognizes a tumor antigen, then undergoes cleavage, causing the release of the intracellular transcriptional domain that enters the nucleus and activates expression of a CAR-T cell that targeting another tumor antigen. D, Upon administration of a heterodimerizing small molecule and recognition of the antigen, the co-stimulatory domains and the splitting downstream ITAMs joined together that cause activation of CAR-T cell. E, The first moiety provides a CD3 ζ -mediated activation signal after recognition of the first antigen, and the co-stimulatory signal is prepared by secondary moiety after recognition of the second antigen so that CAR-T cells can become completely activated just after dual-antigen recognition. F, The iCAR includes a receptor that is specific to the antigens expressed exclusively on normal tissue (PSMA), and an inhibitory intracellular signaling domain (PD-1 or CTLA-4) to restrict T cell activity so that in the presence of both PSMA and tumor-associated antigen (in healthy cells), iCAR suppresses itself. G, Tandem CARs consist of two tandemly linked scFvs targeting different tumor antigens that are combined with one activation domain. H, administration of bispecific T-cell engager redirects and regulates CAR activity to target antigen-positive tumor tissues. I, CAR-T cells expressing CD20 or EGFRt antigen deleted with an approved monoclonal antibody, such as rituximab or cetuximab through CDC/ADCC.

Table 1
The strengths and weaknesses of various safety strategies of CAR-T cells.

+Molecular switches		Pros	Cones	Efficiency	Reference
Suicide switches	HSV-TK	1. Remarkable function and safety 2. The best studied technique	1. Time-consuming process 2. Premature end of the treatment 3. Immunogenicity 4. Clinical incompatibility of GCV 5. Irreversible depletion 6. Cell-cycle dependency	More than 90%	[32,35–37] [35,36,38,39] [38–40] [38–42]
	iCasp9	1. Human-derived, no immunogenicity 2. Highly effective and acts rapidly 3. Clinical compatibility and optimal bio-distribution 4. Use non-therapeutic agent 5. Long-term outcome	1. Irreversible depletion 2. Premature end of the treatment	> 90% elimination of T cells within 30 min of CID administration	
	CD20	1. Human-derived, no immunogenicity 2. Acts rapidly	1. Limited bio-distribution and tissue penetration of antibody 2. Irreversible depletion 3. Premature end of the treatment 4. Limited capacity of CDC/ADCC in the patients treated with chemotherapy 5. On-target toxicity from mAb 6. Pro-drug infusion reaction	86–97%	
Endogenous switches	EGFRt	1. Human-derived, no immunogenicity 2. Acts rapidly 3. The possibility of in-vivo tracking	1. Limited bio-distribution and tissue penetration of antibody 2. Irreversible depletion 3. Premature end of the treatment 4. Limited capacity of CDC/ADCC in the patients treated with chemotherapy 5. On-target toxicity from mAb 6. Pro-drug infusion reaction	Approximately 83%	
	synNotch	1. Highly controlled custom behaviors of CAR-T cells 2. Localized activity	1. Inability to control the intensity of the CAR T-cell activity 2. Inability to control CAR-T cells in a temporal manner 3. Difficulties in choosing 2 effective antigens		[39,43–45] [39,46] [32,39,47]
	iCAR	1. Discrimination between malignant and healthy cells 2. Regulation of CAR-T cells responses in an antigen-selective manner	1. Inability to control the intensity of the CAR T-cell activity 2. Inability to control CAR T-cells in a temporal manner 3. Potential on-target off-tumor toxicity		
Exogenous switches	Combinatorial Target-Antigen Recognition	1. Preventing tumor antigen loss and tumor escape 2. Increase precise destruction of tumor cells	1. Inability to control the intensity of the CAR T-cell activity 2. Inability to control CAR T-cells in a temporal manner 3. Potential on-target off-tumor toxicity 4. Difficulties in choosing 2 effective tumor antigens		
	Bispecific T Cell Engager	1. Controllable CAR-T cells activity 2. Ability to target different antigens	1. Immunogenicity 2. The limited number of FDA-approved anti-tumor Abs 3. Need more attention to choose small molecules		[48–50] [39,51,52]
	On-switch CAR	1. Controllable CAR-T cells activity 2. Multiple specific cytotoxicity cycles using a small molecule 3. Modular design	1. Need more attention to choose small molecules 2. Neither prevent on-target off-tumor toxicity nor letting spatial control		

Table 2
The clinical trials of the next generation of CAR-T cells in cancer immunotherapy.

Safety strategy	Target	Phase	Stage	Default state (On or Off)	Identifier
HSV-TK EGFRt	CD44v6	Phase1/2	Recruiting	ON	NCT04097301
	CD19	Phase1/2	Recruitingntv	ON	NCT02028455
ICasp9	CD19	Phase1Recruiting	ON	NCT02146924	
	CD19	Phase1	Active, not recruiting	ON	NCT01815749
	CD19	Phase1	Not yet recruiting	ON	NCT03579888
	CD19	Phase1	Active, not recruiting	ON	NCT02051257
	CD19	Phase1/2	Active, not recruiting	ON	NCT01865617
	CD19	Phase1	Recruiting	ON	NCT03103971
	CD19	Phase1	Recruiting	ON	NCT03085173
	CD19	Phase1	Recruiting	ON	NCT02706405
	CD19	Phase1	Active, not recruiting	ON	NCT01683279
	CD19	Phase1	Recruiting	ON	NCT03389230
	CD123	Phase1	Recruiting	ON	NCT02159495
	CD123	Phase1	Recruiting	ON	NCT03114670
	CD123	Phase1/2	Recruiting	ON	NCT04109482
	CD22	Phase1	Active, not recruiting	ON	NCT03244306
	CD22	Phase1	Active, not recruiting	ON	NCT03330691
	CD171	Phase1	Recruiting	ON	NCT02311621
	EGFR	Phase1	Recruiting	ON	NCT03618381
	EGFR	Phase1	Recruiting	ON	NCT03638167
	HER2	Phase1	Recruiting	ON	NCT03500991
	BCMA	Phase1	Active, not recruiting	ON	NCT03070327
	B7H3	Phase1	Recruiting	ON	NCT04185038
	MUC16	Phase1	Recruiting	ON	NCT02498912
	CS1	Phase1	Recruiting	ON	NCT03710421
	GD2	Phase1	Active, not recruiting	ON	NCT01822652
	GD2	Phase1	Active, not recruiting	ON	NCT01953900
	GD2	Phase1	Completed	ON	NCT02107963
	GD2	Phase1	Recruiting	ON	NCT03721068
	GD2	Phase2	Recruiting	ON	NCT02765243
	GD2	Phase1/2	Recruiting	ON	NCT02992210
	GD2	Phase1/2	Recruiting	ON	NCT03373097
	GD2	Phase1	Not yet recruiting	ON	NCT04196413
	CD19	Phase1/2	Recruiting	ON	NCT03016377
	CD19	Phase1	Recruiting	ON	NCT03696784
CD19	Phase1/2	Recruiting	ON	NCT03050190	
CD19	Phase1/2	Recruiting	ON	NCT03373071	
Mesothelin	Phase1/2	Recruiting	ON	NCT02414269	
CD19/CD20Phase1/2 /CD22/CD30/CD38 /CD70/CD123	Recruiting	ON	NCT03125577		
GPC3	Phase1	Not yet recruiting	ON	NCT04377932	
CD19/20	Phase1	Active, not recruiting	ON	NCT03019055	
CD19/20	Phase1/2	Recruiting	ON	NCT03097770	
CD19/20	Phase1/2	Recruiting	ON	NCT04186520	
CD19/22	Phase1/2	Active, not recruiting	ON	NCT03185494	
CD19	Early Phase 1	Not yet recruiting	OFFtv	NCT03824951	
HER2	Phase1	Recruiting	OFF	NCT02442297	
TanCAR					
iCAR					

TK1, the cell-cycle dependent cytosolic TK, can phosphorylate thymidine, various other pyrimidines, and also pyrimidine and purine analogs. Tri-phosphorylated nucleoside analogs are cytotoxic because they interfere with DNA synthesis. Several pro-drugs for the HSV-TK system have been evaluated, including ganciclovir (GCV), acyclovir (ACV), and brivudin (BVDU) and among them, GCV was found to be the most effective pro-drug for this system [57]. By conditional administration of GCV, HSV-TK catalyzes the phosphorylation of GCV that produces a toxic GCV-triphosphate resulting in competitive inhibition of guanosine incorporation with subsequent inhibition of DNA synthesis and cellular death [53].

3.1.3. CD20 and EGFRt safety switches

The other suicide gene-based technology is the co-expression of the CAR-T cell, and a targetable component, a well-known surface antigen such as CD20 or the truncated epidermal growth factor receptor (EGFRt). This approach allows a selective cell removal through the complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) after administration of a specific monoclonal antibody. Rituximab has been used as a clinically approved

monoclonal antibody for CD20 and cetuximab for EGFRt [40,53,58,59]. Using these antibodies has some disadvantages such as limited bio-distribution and tissue penetration and limited CDC/ADCC capacity in the patient that already have been treated by chemotherapy. This issue has been addressed by creating anti-idiotypic CARs recognizing CD19-specific CARs or synthesizing a short peptide epitope (E-tag) in the extracellular domain of the CAR and using an anti-E-tag CAR in order to omit the anti-tumor CARs [60,61].

3.2. Endogenous switches

3.2.1. Combinatorial target-antigen recognition

Because of the few available tumor-specific antigens (TSAs), CAR-T cells always target tumor-associated antigens (TAAs) that are weakly expressed in normal tissues or bystander cells. So, even in successful treatment, probably normal cells are targeted and eradicated. This effect is called on-target off-tumor toxicity, an unavoidable side effect [43]. Adding a second antigen specificity could potentially prevent this toxicity. There are two strategies to combine different target antigens. The first one is constructing two intact CARs into one vector or tandem con-

struction of two scFv domains in one CAR molecule (tandem CAR or TanCAR) that are fully activated when each antigen is engaged [62,63]. Separation of the customary CAR-T cell into two complementary moieties is another approach to raise safety and control T-cell response. The intracellular activating regions of a complete CAR-T cell (CD3 ζ) and co-stimulatory activation domains (CD28 and/or 4-1BB) are transduced separately within two half-baked CAR-T cells. The first moiety provides a CD3 ζ -mediated activation signal after recognition of antigen 1, and the co-stimulatory signal is prepared by the second moiety when antigen 2 is involved. In this approach, engineered T cells can become completely activated just when they encounter with 1^{positive}/2^{positive} target cells, so the dual-antigen binding is necessary for complete CAR-T cell activation [64–66]. In other words, each moiety provides a discrete signal that alone is inadequate to mediate T-cell activation. However, a combination of these signals synergizes and stimulates a complete T-cell response.

3.2.2. Synthetic Notch receptors (*synNotch*)

Among three parts of a wild-type Notch receptor, *synNotch* just retains the core regulatory domain of the cell-cell signaling receptor Notch that cleaves the receptor and releases a transcriptional activator domain. However, the extracellular ligand-binding domain replaced by a synthetic single-chain variable fragment (scFv) and the intracellular transcriptional domain replaced by synthetic intracellular transcriptional domains activates a downstream desired target gene [67]. In this approach, the *synNotch* receptor first recognizes a tumor antigen then undergoes induced trans-membrane cleavage like as the wild-type Notch activation and thereby releasing the intracellular transcriptional domain to enter the nucleus and activates expression of a CAR-T cell that targeted another tumor antigen [43,44].

3.2.3. Inhibitory CAR (*iCAR*)

In human T cells, PD-1 and CTLA-4 intracellular signaling domains are able to reduce TCR signaling, thereby declined T-cell cytokine production and its lysis activity. Through smart use of this ability, Fedorov et al. designed a self-regulating safety switch that allows for discrimination between the tumor and healthy cells. Theoretically, the *iCAR* includes a receptor that is specific to the antigens expressed exclusively on normal tissue (PSMA), and an inhibitory intracellular signaling domain (PD-1 or CTLA-4) to restrict T cell activity. In the presence of both PSMA and CD-19 (healthy cells), the *iCAR* is inhibited by the activity of the intracellular signaling domains (PD-1), but in tumor cells, the absence of PSMA prevents *iCAR*-mediated inhibition of the CAR, thereby T-cell activation and target cell lysis [46].

3.3. Exogenous switches

3.3.1. Bispecific T cell engager

Bispecific T-cell engagers are defined as antibodies or derived proteins with multiple binding sites, each with a unique antigen specificity that allows them to bridge two or more cells by a physical link. One binding site links to an antigen on one given cell, and the other binding site links to an antigen on a different cell [68]. Recently, the folate-FITC conjugate was used as a bispecific small molecule switch in the introduction of more secure CAR-T cells. In this approach, a synthetic CAR was constructed that binds to a fluorescein isothiocyanate (FITC) molecule called "universal" anti-FITC-directed CAR-T cell. The constructed cell does not immediately bind to the antigen on the tumor cells, but it is converted to the effector cell by binding to the small bispecific molecule (folate-FITC conjugate). The CAR is inactive and cannot target normal cells in the lack of folate-FITC conjugate. After the administration of the conjugate, this bispecific T-cell engager redirects and regulates CAR activity to target folate receptor-positive tumor tissues. The alpha isoform of folate receptor (FR) is expressed in the nearly 50% of cancers such as breast, lung, uterus, and ovarian, but it is expressed in very low levels in the normal tissues [49]. So far, several anti-tumor antibodies, including

anti-EGFR, anti-Her2, anti-CD20, anti-CD19, and anti-CD22 antibodies were conjugated to FITC to create different bispecific small molecule Ab-FITC which redirected anti-FITC-CAR-T cells binding to the tumor cells [48,50]. Briefly, in this manner CAR activation and proliferation were rigorously relying on the existence of both bispecific T-cell engager and antigen-positive cells, and also, it was dose titratable with a bispecific small molecule switch that makes it more controllable.

3.3.2. On-switch CAR

The design of on-switch CAR-T cells inspired by the normal T cell activation process in which activation of T cell receptors (TCRs) and a co-stimulatory receptor on the separately expressed polypeptides initiates an immune response [69]. However, in conventional CARs, the antigen recognition domain (scFv), the main signaling motif (such as ITAMs from TCR subunit CD3 ζ), and co-stimulatory motifs are artificially colocalized [70]. So, to imitate normal T cell response, the key signaling modules are distributed into physically separate polypeptides that can be conditionally reassembled when a heterodimerizing small-molecule agent is added. The on-switch CARs consist of two split parts including an extracellular scFv with co-stimulatory domains and a key downstream signaling element (the immunoreceptor tyrosine-based activation motifs (ITAMs) from the T cell receptor CD3 ζ subunit) that each of them contains heterodimerization domains that interact with each other upon binding of a heterodimerizing small molecule. The therapeutic activity of the on-switch CAR-T cell requires a small priming molecule in addition to the cognate antigen, and neither small molecule nor antigen should activate it alone. This CAR provides a small molecule-dependent, titratable, and reversible control over the CAR T cell activity, thereby alleviating toxicity [36,51,52].

4. Concluding remarks

Targeting malignant cells using engineered CAR-T cells is a great advance in the treatment of cancer. This approach is an effective new treatment for hematologic malignancies. So far, two CAR-T cell products, including Tisagenlecleucel and Axicabtagene Ciloleucel have been approved by the USA FDA for the clinical use in case of pediatric acute lymphoblastic leukemia (ALL), adult diffuse large B-cell lymphoma subtypes (DLBCL), and axicabtagene ciloleucel for DLBCL (71). Despite the significant progress achieved in the adoptive cellular therapy (ACT) using CAR-T cells, toxicity is the main obstacle to the widespread use of engineered T cells in cancer treatment. Although CRS and CRES (CAR T cell-related encephalopathy syndrome) is the most common toxicities, other adverse effects such as ICANS (immune effector cell-associated neurotoxicity syndrome) should be considered after CAR-T cell infusion in clinical practice too [71].

Since the field of ACT using engineered T cells is still quite new, therefore, the management of its toxicities requires a lot of research and time. Certainly, CAR-T cell toxicity management will change considerably within the coming years as more data will be provided by the studies. By now, results of studies on the pathophysiology of CRS and neurotoxicity have shown that the early and peak levels of certain cytokines, patient disease burden, peak blood CAR T-cell levels, CAR T-cell dose, endothelial activation, and CAR design may play a role in CAR-T cell toxicities [72]. According to this data, different approaches are developed to overcome the toxicities of CAR-T cell therapy.

Systems using suicide genes, such as iCasp9 and tEGFR that are followed by the administration of the antibody or small dimerizer molecule agents to induce apoptosis in the transduced cells with the transgene, are under investigation for the toxicity management. Nevertheless, these systems affect the anti-malignancy activity of the therapy by the irreversible elimination of therapeutic CAR-T cells so that these systems may be more effective in case of life-threatening toxicity not controlled with immunosuppression or in the setting of ongoing long-term toxicities after malignancy remission. Another approach to overcome the toxicities

is by using endogenous switches such as synNotch and iCAR to intracellularly regulate CAR-T cells in a self-switch manner. In this method, the time and intensity of CAR-T cell activity cannot be controlled. Bispecific T cell engager and on-switch CAR system using exogenous small molecules are under evaluation too [36,72].

Altogether, toxicity management of the CAR-T cell therapy requires more research to eliminate shortcomings of the present approaches or introduce new methods. It is hoped that the development of the later generation CARs will increase the safety of cancer treatment using CAR-T cells and overcomes its present weaknesses.

Declaration of Competing Interest

The authors of this study were declared that there is not any conflicts of interest.

Acknowledgment

This work was approved under the supervision of Cancer Research Center, Cancer Institute of Iran, Tehran University of Medical Science, Tehran, Iran and were designed by Ehsan Moghanloo and Habibollah Mahmoodzadeh.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contribution

Study design: Ehsan Moghanloo, Hasan Mollanoori, Sahel Heidari and Habibollah Mahmoodzadeh.

Primary Search: Ehsan Moghanloo, Arefeh Ghafari Novin, Farzaneh Afshari, Sahel Heidari and Fatemeh Faraji.

Evaluation of the papers and extraction of data: Ehsan Moghanloo, Arefeh Ghafari Novin, Farzaneh Afshari, Farimah Hadjilooei and Farimah Hadjilooei.

Writing the draft: Ehsan Moghanloo, Farzaneh Afshari and Fatemeh Faraji.

Review the manuscript: Farimah Hadjilooei and Habibollah Mahmoodzadeh.

Edition and submit: Ehsan Moghanloo and Habibollah Mahmoodzadeh.

References

- [1] T. Otto, P. Sicsinski, Cell cycle proteins as promising targets in cancer therapy, *Nat. Rev. Cancer* 17 (2) (2017) 93–115 p.
- [2] M.A. Fischbach, J.A. Bluestone, W.A. Lim, Cell-based therapeutics: the next pillar of medicine, *Sci. Transl. Med.* 5 (179) (2013) 179 p.ps7.
- [3] D.N. Khalil, et al., The new era of cancer immunotherapy: manipulating T-cell activity to overcome malignancy, *Adv. Cancer Res.* 128 (2015) 1–68 p.
- [4] I. Mellman, G. Coukos, G. Dranoff, Cancer immunotherapy comes of age, *Nature* 480 (7378) (2011) 480–489 p.
- [5] Z. Zhao, et al., The application of CAR-T cell therapy in hematological malignancies: advantages and challenges, *Acta Pharm. Sin. B* 8 (4) (2018) 539–551 p.
- [6] M.S. Diamond, et al., Type I interferon is selectively required by dendritic cells for immune rejection of tumors, *J. Exp. Med.* 208 (10) (2011) 1989–2003 p.
- [7] S.L. Maude, et al., Chimeric antigen receptor T cells for sustained remissions in leukemia, *N. Engl. J. Med.* 371 (16) (2014) 1507–1517 p.
- [8] D.W. Lee, et al., T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial, *Lancet* 385 (9967) (2015) 517–528 p.
- [9] 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 (224) (2014) 224–225 p.
- [10] C.J. Turtle, et al., CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients, *J. Clin. Invest.* 126 (6) (2016) 2123–2138 p.
- [11] S.A. Grupp, et al., Chimeric antigen receptor-modified T cells for acute lymphoid leukemia, *N. Engl. J. Med.* 368 (16) (2013) 1509–1518 p.
- [12] D.L. Porter, et al., Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia, *Sci. Transl. Med.* 7 (303) (2015) 303 p.ra139.
- [13] D.L. Porter, et al., Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia, *N. Engl. J. Med.* 365 (8) (2011) 725–733 p.
- [14] J.N. Brudno, et al., Allogeneic T cells that express an anti-CD19 chimeric antigen receptor induce remissions of B-cell malignancies that progress after allogeneic hematopoietic stem-cell transplantation without causing graft-versus-host disease, *J. Clin. Oncol.* 34 (10) (2016) 1112–1121 p.
- [15] J.N. Kochenderfer, et al., Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor, *J. Clin. Oncol.* 33 (6) (2015) 540–549 p.
- [16] J. Maher, et al., Targeting of tumor-associated glycoforms of MUC1 with CAR T cells, *Immunity* 45 (5) (2016) 945–946 p.
- [17] A.D. Fesnak, C.H. June, B.L. Levine, Engineered T cells: the promise and challenges of cancer immunotherapy, *Nat. Rev. Cancer* 16 (9) (2016) 566–581 p.
- [18] T. Brocker, K. Karjalainen, Signals through T cell receptor-zeta chain alone are insufficient to prime resting T lymphocytes, *J. Exp. Med.* 181 (5) (1995) 1653–1659 p.
- [19] H.M. Finney, A.N. Akbar, A.D. Lawson, Activation of resting human primary T cells with chimeric receptors: costimulation from CD28, inducible costimulator, CD134, and CD137 in series with signals from the TCR zeta chain, *J. Immunol.* 172 (1) (2004) 104–113 p.
- [20] S. Di, Z. Li, Treatment of solid tumors with chimeric antigen receptor-engineered T cells: current status and future prospects, *Sci. China Life Sci.* 59 (4) (2016) 360–369 p.
- [21] 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 (1) (2014) 83–90 p.
- [22] M. Chmielewski, H. Abken, TRUCKs: the fourth generation of CARs, *Expert Opin. Biol. Ther.* 15 (8) (2015) 1145–1154 p.
- [23] P.J. Bugelski, et al., Monoclonal antibody-induced cytokine-release syndrome, *Expert Rev. Clin. Immunol.* 5 (5) (2009) 499–521 p.
- [24] J.C. Fitzgerald, et al., Cytokine release syndrome after chimeric antigen receptor T cell therapy for acute lymphoblastic leukemia, *Crit. Care Med.* 45 (2) (2017) e124–e131 p.
- [25] S.C. Howard, D.P. Jones, C.H. Pui, The tumor lysis syndrome, *N. Engl. J. Med.* 364 (19) (2011) 1844–1854 p.
- [26] K.A. Hay, et al., Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor-modified T-cell therapy, *Blood* 130 (21) (2017) 2295–2306 p.
- [27] X. Wang, et al., Phase 1 studies of central memory-derived CD19 CAR T-cell therapy following autologous HSCT in patients with B-cell NHL, *Blood* 127 (24) (2016) 2980–2990 p.
- [28] D.W. Lee, et al., Current concepts in the diagnosis and management of cytokine release syndrome, *Blood* 124 (2) (2014) 188–195 p.
- [29] M.V. Maus, et al., T cells expressing chimeric antigen receptors can cause anaphylaxis in humans, *Cancer Immunol. Res.* 1 (1) (2013) 26–31 p.
- [30] K.J. Curran, H.J. Pegram, R.J. Brentjens, Chimeric antigen receptors for T cell immunotherapy: current understanding and future directions, *J. Gene Med.* 14 (6) (2012) 405–415 p.
- [31] J. Scholler, et al., Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells, *Sci. Transl. Med.* 4 (132) (2012) 132–153 p.
- [32] E. Zhang, H. Xu, A new insight in chimeric antigen receptor-engineered T cells for cancer immunotherapy, *J. Hematol. Oncol.* 10 (1) (2017) 1 p.
- [33] T. Gargett, M.P. Brown, The inducible caspase-9 suicide gene system as a "safety switch" to limit on-target, off-tumor toxicities of chimeric antigen receptor T cells, *Front. Pharmacol.* 5 (2014) 235 p.
- [34] L. Gamboa, A.H. Zamat, G.A. Kwong, Synthetic immunity by remote control, *Theranostics* 10 (8) (2020) 3652–3667 p.
- [35] M. Casucci, A. Bondanza, Suicide gene therapy to increase the safety of chimeric antigen receptor-redirection T lymphocytes, *J. Cancer* 2 (2011) 378–382 p.
- [36] S. Yu, et al., Next generation chimeric antigen receptor T cells: safety strategies to overcome toxicity, *Mol. Cancer* 18 (1) (2019) 125 p.
- [37] F. Ciceri, et al., Infusion of suicide-gene-engineered donor lymphocytes after family haploidentical haemopoietic stem-cell transplantation for leukaemia (the TK007 trial): a non-randomised phase I-II study, *Lancet Oncol.* 10 (5) (2009) 489–500 p.
- [38] H.G. Caruso, A.B. Heimberger, L.J.N. Cooper, Steering CAR T cells to distinguish friend from foe, *Oncoimmunology* 8 (10) (2019) e1271857 p.
- [39] L.J.B. Brandt, et al., Emerging approaches for regulation and control of CAR T cells: a mini review, *Front. Immunol.* 11 (2020) 326 p.
- [40] B.S. Jones, et al., Improving the safety of cell therapy products by suicide gene transfer, *Front. Pharmacol.* 5 (2014) 254 p.
- [41] P.J. Paszkiewicz, et al., Targeted antibody-mediated depletion of murine CD19 CAR T cells permanently reverses B cell aplasia, *J. Clin. Invest.* 126 (11) (2016) 4262–4272 p.
- [42] R.L. Kao, et al., A cetuximab-mediated suicide system in chimeric antigen receptor-modified hematopoietic stem cells for cancer therapy, *Hum. Gene Ther.* 30 (4) (2019) 413–428 p.
- [43] K.T. Roybal, et al., Precision tumor recognition by T cells with combinatorial antigen-sensing circuits, *Cell* 164 (4) (2016) 770–779 p.
- [44] K.T. Roybal, et al., Engineering T cells with customized therapeutic response programs using synthetic notch receptors, *Cell* 167 (2) (2016) 419–432 p.e16.
- [45] S. Srivastava, et al., Logic-gated ROR1 chimeric antigen receptor expression rescues T cell-mediated toxicity to normal tissues and enables selective tumor targeting, *Cancer Cell* 35 (3) (2019) 489–503 p.e8.

- [46] V.D. Fedorov, M. Themeli, M. Sadelain, PD-1- and CTLA-4-based inhibitory chimeric antigen receptors (iCARs) divert off-target immunotherapy responses, *Sci. Transl. Med.* 5 (215) (2013) 215 p.ra172.
- [47] S. Srivastava, S.R. Riddell, Chimeric antigen receptor T cell therapy: challenges to bench-to-bedside efficacy, *J. Immunol.* 200 (2) (2018) 459–468 (Baltimore, Md.: 1950)p..
- [48] J.S. Ma, et al., Versatile strategy for controlling the specificity and activity of engineered T cells, *Proc. Natl. Acad. Sci. USA.* 113 (4) (2016) E450–E458 p.
- [49] M.S. Kim, et al., Redirection of genetically engineered CAR-T cells using bifunctional small molecules, *J. Am. Chem. Soc.* 137 (8) (2015) 2832–2835 p.
- [50] K. Tamada, et al., Redirecting gene-modified T cells toward various cancer types using tagged antibodies, *Clin. Cancer Res.* 18 (23) (2012) 6436–6445 p.
- [51] C.Y. Wu, et al., Remote control of therapeutic T cells through a small molecule-gated chimeric receptor, *Science* 350 (6258) (2015) aab4077 p.
- [52] A. Juillerat, et al., Design of chimeric antigen receptors with integrated controllable transient functions, *Sci. Rep.* 6 (1) (2016) 18950 p.
- [53] C.L. Bonifant, et al., Toxicity and management in CAR T-cell therapy, *Mol. Ther. Oncolytics* 3 (2016) 16011 p.
- [54] M. Stavrou, et al., A rapamycin-activated caspase 9-based suicide gene, *Mol. Ther.* 26 (5) (2018) 1266–1276 p.
- [55] K. Minagawa, M. Al-Obaidi, A. Di Stasi, Generation of suicide gene-modified chimeric antigen receptor-redireted t-cells for cancer immunotherapy, *Methods Mol. Biol.* 1895 (2019) 57–73 p.
- [56] A. Di Stasi, et al., Inducible apoptosis as a safety switch for adoptive cell therapy, *N. Engl. J. Med.* 365 (18) (2011) 1673–1683 p.
- [57] J.A. Hossain, et al., Cancer suicide gene therapy with TK.007, *Methods Mol. Biol.* 1895 (2019) 11–26 p.
- [58] M. Introna, et al., Genetic modification of human T cells with CD20: a strategy to purify and lyse transduced cells with anti-CD20 antibodies, *Hum. Gene Ther.* 11 (4) (2000) 611–620 p.
- [59] X. Wang, et al., A transgene-encoded cell surface polypeptide for selection, *in vivo* tracking, and ablation of engineered cells, *Blood* 118 (5) (2011) 1255–1263 p.
- [60] M. Ruella, et al., A cellular antidote to specifically deplete anti-CD19 chimeric antigen receptor-positive cells, *Blood* 135 (7) (2020) 505–509 p.
- [61] S. Koristka, et al., Anti-CAR-engineered T cells for epitope-based elimination of autologous CAR T cells, *Cancer Immunol. Immunother.* 68 (9) (2019) 1401–1415 p.
- [62] M. Ruella, et al., Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies, *J. Clin. Invest.* 126 (10) (2016) 3814–3826 p.
- [63] D. Schneider, et al., A tandem CD19/CD20 CAR lentiviral vector drives on-target and off-target antigen modulation in leukemia cell lines, *J. Immunother. Cancer* 5 (2017) 42 p.
- [64] J. Wei, et al., Target selection for CAR-T therapy, *J. Hematol. Oncol.* 12 (1) (2019) 62 p.
- [65] C.C. Kloss, et al., Combinatorial antigen recognition with balanced signaling promotes selective tumor eradication by engineered T cells, *Nat. Biotechnol.* 31 (1) (2013) 71–75 p.
- [66] E. Lanitis, et al., Chimeric antigen receptor T Cells with dissociated signaling domains exhibit focused antitumor activity with reduced potential for toxicity *in vivo*, *Cancer Immunol. Res.* 1 (1) (2013) 43–53 p.
- [67] X. Yuan, et al., Notch signaling: an emerging therapeutic target for cancer treatment, *Cancer Lett.* 369 (1) (2015) 20–27 p.
- [68] A. Huehls, T. Coupet, C. Sentman, Bispecific T-cell engagers for cancer immunotherapy, *Immunol. Cell Biol.* (2014) 93.
- [69] A. Weiss, D.R. Littman, Signal transduction by lymphocyte antigen receptors, *Cell* 76 (2) (1994) 263–274 p.
- [70] Y. Pang, et al., Advances on chimeric antigen receptor-modified T-cell therapy for oncotherapy, *Mol. Cancer* 17 (1) (2018) 91 p.
- [71] L. Yáñez, M. Sánchez-Escamilla, M.A. Perales, CAR T cell toxicity: current management and future directions, *Hemasphere* 3 (2) (2019) e186 p.
- [72] J.N. Brudno, J.N. Kochenderfer, Recent advances in CAR T-cell toxicity: mechanisms, manifestations and management, *Blood Rev.* 34 (2019) 45–55 p.