



Review Article

Synthetic immunology: T-cell engineering and adoptive immunotherapy

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ABSTRACT

During the past decades, the rapidly-evolving cancer is hard to be thoroughly eliminated even though the radiotherapy and chemotherapy do exhibit efficacy in some degree. However, a breakthrough appeared when the adoptive cancer therapy [1] was developed, especially T cells armed with chimeric antigen receptors (CARs) showed great potential in tumor clinical trials recently. CAR-T cells successfully elevated the efficiency and specificity of cytotoxicity. In this review, we will talk about the design of CAR and CAR-included combinatory therapeutic applications in the principles of systems and synthetic immunology.

1. Design principle of chimeric antigen receptor (CAR)

Chimeric antigen receptor (CAR) was originally conceptualized by Zelig Eshhar and his colleagues in 1989 [1,2]. The conception of CAR showed the feasibility of redirected T cell response can be activated through an engineered receptor, even with no immediate clinical successes. Later in 2008, Malcolm Brenner and colleagues at Baylor College of Medicine in Houston, TX declared the first success in clinical, based on a virus-specific cytotoxic T lymphocytes expressing neuroblastoma antigen-specific CAR [3]. Enormous achievements utilizing this newly-born approach have emerged since then, quite a few of which demonstrated the huge potential and even clinical efficacy of CAR-related cancer therapy, and researches of CAR-T cell therapy also enter the rapid developing period.

Briefly speaking, CAR is mainly comprised of an extracellular single-chain variable fragment (scFv) of an antibody and intracellular T cell signaling domains. And these two parts are linked with the extracellular hinge and transmembrane domain [4–6]. Extracellular scFv domain recognizes the tumor associated antigen (TAA), transmembrane domain transfers the extracellular signals inside, then the intracellular signaling domain converts the outer stimulus into T cell signals (Fig. 1). Such structure of CAR makes T cells specifically respond to tumor cells with the desired antigens.

1.1. Extracellular recognition domain

1.1.1. ScFv targeting TAAs

Introducing scFv into extracellular domain is determinative of CAR's specificity. Cancer cells, as a kind of abnormal tissue, are regarded as non-self components that should be eradicated. This clearance is based on immune system. Naturally, MHC class I/II molecules present epitopes to stimulate T cell receptors (TCRs). However, this binding affinity and specificity is not high enough [1,5]. Especially in some certain kinds of cancers where the expression of MHC I is downregulated, the efficiency in killing cancer is further limited [7,8]. This scFv-derived cell-cell interaction and activation ensure higher selectivity and affinity on target cells than the natural tumor immune response (Fig. 1).

The priority of engineering the extracellular scFv module of CARs is to select the proper candidate targets. The specificity of TAA determines the off-tumor rate to healthy tissue, while the binding affinity is related to the scavenging effects of tumor [5]. TAAs are usually membrane surface proteins which show higher expression level on tumor cells in compare to normal tissues. One of the promising targets, CD19, which is expressed on most of both normal and malignant B cells [5,9–11]. A-CD19 CAR-T cells are proved able to treat B cell malignancies, but also destroy healthy B cells. Although it would be ideal to choose TAAs that are specifically expressed on tumor cells, unfortunately, it remains extremely challenging to identify such distinct surface marker of tumor cells. In view of this, TAAs that leak to relatively dispensable tissues are more appropriate for safety desire.

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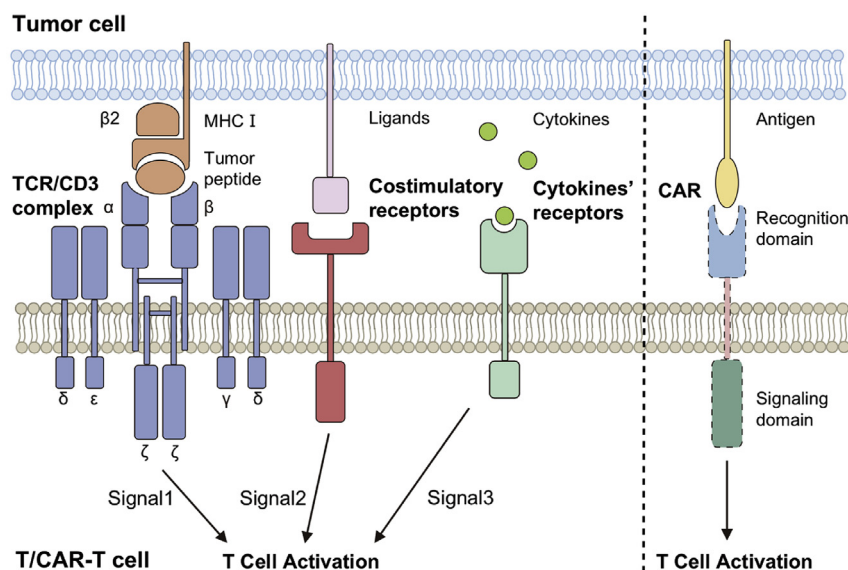


Fig. 1. Three-signal model in T cell activation and the design principle of CAR. Interaction between T cell receptor (TCR)/CD3 complex with tumor antigen peptide fragment presented by major histocompatibility complex (MHC) leads to phosphorylation of TCR/CD3 intracellular domain, which produces signal 1 to activate T cells. Meanwhile, some ligands such as B7H2, 4-1BBL, and OX40L on the antigen-presenting cells can be recognized by costimulatory receptors on T cells, like CD28, 4-1BB and OX40. This recognition helps the activation of T cell as signal 2. Cytokines are a broad category of small proteins secreted by many immune cells. Combination of cytokines and their receptors on T cells called as signal 3 can also enhance the T cells' activation. CARs are recombinant immune receptors that mimic the signal transduction of T cell activation and are independent of MHC.

Table 1
Targets in CAR-T cell therapy [4,9,15].

Cancer	Antigens targeted by CARs
Haematological malignancies	CD19 [16] CD20 [17] CD22 [18] CD30 [19] CD33 [20] CD38 [21] ROR1 [22] Lewis(Le)-T (LeY) [23] Interleukin-3 receptor α chain (CD123) [24] Ig κ light chain [25]
Non-small lung cancer (NSCLC)	Epidermal growth factor receptor (EGFR) [26]
Melanoma	HLA-A1/MAGE-A1 [27] Ganglioside GD2 [2,28]
Neuroblastoma	L1-cell adhesion molecule (CD171) [29]
Glioma	EGFRvIII [13]
Prostate cancer	Human prostate-specific membrane antigen (hPSMA) [30]
Mesotheliomas	Mesothelin [31] Fibroblast activation protein (FAP) [32]
Multiple myeloma (MM)	CD138 [33]
Osteosarcoma	Human epidermal growth factor receptor (HER2) [34] Interleukin-11 receptor α -chain (IL-11R α) [35]
Cervical carcinoma	CD44v7 and CD44v8 [36]
Gastrointestinal stromal tumor (GST)	KIT [37]
Ovarian cancer	Folate receptor- α (FR α) [38] NKG2D ligand [39]
Peritoneal ovarian tumors	MUC16 [40]
Glioblastoma	IL13R α 2 [41] HER2 and IL13R α 2 [42] Carboxy-anhydrase-IX (CAIX) [43]
Metastatic renal cell carcinoma (RCC)	
Pancreatic adenocarcinoma (PAC)	Her2/neu and CD24 [44]
Breast cancer	ErbB2 and MUC1 [45]
Liver metastases (LM)	Carcinoembryonic antigen (CEA) [46]
Universal	Biotin [47] FITC [48]
Tumor vasculature	VEGFR-2 [49]

As shown in the list (Table 1), many of them are special variants or modifications of surface proteins. For example, EGFRvIII, a strictly tumor-specific variant, is a novel target in treating glioblastoma [9,12].

Trials in EGFRvIII-specific CAR proved its anti-tumor efficacy as well as safety and feasibility. However, EGFRvIII may not be expressed throughout the tumor, only on about 30% cases of glioblastoma, which limits the application [7,13]. On the contrary, nonmutated EGFR, whose overexpression is also common in many tumor types, is doubtable in safety because of its ubiquitous expression pattern. Another “sweet” example targeting tumor specific modification is α -Tn(GalNAc1-O-Ser/Thr)-MUC1 CAR-T cells [14]. Tn is one of the most prevalent aberrant glycoforms found in cancer, and its expression is associated with quite a few malignant cancer types, including gastric cancer, leukemia and most adenocarcinomas. A-Tn-MUC1 CAR-T cells have been proved efficient in mouse model. Nowadays, it's also a novel and powerful method to employ sequencing in search of personalized TAAs. Undoubtedly, how to search for TAAs that keep the balance between efficiency and toxicity is a major problem for application of CAR-T cell therapy.

1.1.2. Multi-targeting CAR

One attractive improvement for CAR engineering is to develop a CAR that can target more than one TAA, and therefore can deal with various cancer cells in one single treatment. One solution is the tandem CAR, whose extracellular domain consists of two different scFvs. For example, a bispecific tandem CAR which joins α -HER2 and α -IL13R α 2 scFvs can be activated by either antigen, and it shows superadditive T cell activation when encountered with both antigens [50].

To further simplify CAR design and make CAR “off-the-shelf”, scientists brought up the concept of universal CAR [47,48,51]. Universal CARs could target alternative TAAs indirectly mediated by engineered adaptor molecules, such as engineered tumor-targeting antibodies conjugated with some molecules (e.g., fluorescein isothiocyanate (FITC)). Therefore, the scFv of such universal CAR is designed to recognize a bridging molecule (correspondingly, α -FITC CAR) (Fig. 2A). As a consequence, the activation of universal CAR-T cells to a given cancer marker is fully dependent by the designed adaptor molecules. In addition, as adaptors function in a dose-dependent way, universal CAR-T cells' activity is tunable and allows CAR-T cells practice much safer.

Similar to universal CAR design, Wong et al. developed a split, universal, and programmable (SUPRA) CAR system (composed of two separate parts, zipCAR and zipFv) [52]. The scFv module of ZipCAR is substituted by a leucine zipper peptide, while the zipFv is a scFv fused with the counterpart zipper peptide, therefore these two parts can bind to each other through leucine zipper pairs (Fig. 2B). This technique

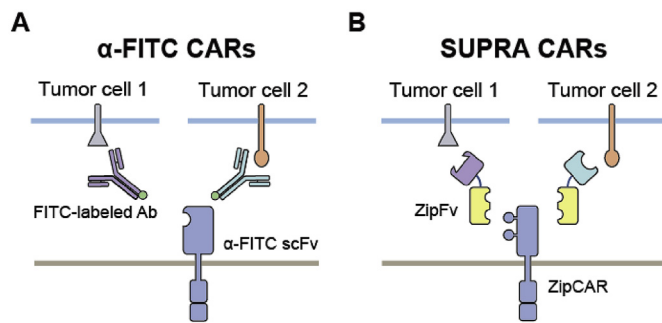


Fig. 2. Multi-targeting CARs: (A) α -FITC CARs: The extracellular domain of α -FITC CAR is the scFv which recognizes FITC. It can recognize many kinds of TAAs with the help of different antibodies modified with FITC. (B) SUPRA CARs: It's another design of two-component system based on leucine zipper. By changing the type of zipFv, zipCAR can indirectly interact with different TAAs.

allows a single CAR able to target various types of TAAs (only switching the scFv module in zipFv). And the T cell activation can also be tuned by dosage of zipFv molecules.

1.2. Hinge and transmembrane domain

Hinge, or the spacer, which exists between the extracellular scFv and the transmembrane(TM) domain is an important regulator for conforming the suitable structure when scFv of CAR interacts to antigens. The distance between scFv and cell membrane and the flexibility of hinge influence the T cell activation [53,54].

Transmembrane domain is usually derived from type I membrane proteins, such as CD3 ζ , CD4, CD8, or CD28 [55]. Transmembrane domain is the bridge and signal converter between outer space and the inner cell. It is pivotal for the function of CAR, as its conformational change serves as the initial signal for T cell activation. Scientists have demonstrated that some mutations of TM domain that abrogates the dimerization/aggregation of CAR will destroy CAR's capacity. We have observed that the TM domain has some relations with CAR's expression and reaction rate. Researches in TM and hinge are relatively fewer than those in the other parts. More information is needed to polish CAR's functional capacity.

1.3. Intracellular signaling domain

T cell activation relies on signals from several receptors. The most important is the first signal from T cell receptor (TCR)/CD3 complex, assisted by the second and third signal from costimulatory receptors and cytokines' receptors, respectively [56,57]. CAR mimics the function of TCR or together with the other receptors (Fig. 1). The basic structure of CAR's intracellular domain is from CD3 ζ , thus activating TCR downstream signals. Then costimulatory signals are implemented to improve the properties of CAR. Such improvement of intracellular modules is decisive in CAR's function.

1.3.1. The first generation CAR: signal from TCR/CD3 complex

The endodomain of the first-generation CAR only contains that of CD3 ζ (Fig. 3). Even limited in efficacy, the first generation α -GD2 CAR was reported successful in 2008 [1,3,58]: Three out of eleven pediatric patients with neuroblastoma showed a complete remission after treatment. This result proved the efficiency of the virus specific T cells with the first generation CARs, even the persistence in blood was not satisfactory.

1.3.2. The second and third generation CARs: signal from costimulatory receptors

Then came up the second and third generation CAR [59], which separately contains one or two intracellular domains of costimulatory

receptors, such as CD28 [60], 4-1BB (CD137) [61,62], ICOS [63], OX40 (CD134) [64], CD27 [65], etc (Fig. 3). Costimulatory modules effectively increase CAR-T cells' cell lytic ability, proliferation and other functions [66,67]. The third generation CAR has demonstrated enhanced cytokine secretion, T cell resistance and tumor inhibition in several experiments even though conflicts exist [55,68]. But the additional cosignaling domain increases the basal activity of CAR-T cell, thus decreasing the activation threshold. This augments the toxicity against normal tissue with low expression of TAA and restricts the third generation CAR-T's application [55,69].

Even different costimulatory domains can improve CAR-T cells' functions in some degree, but they vary in mechanisms and properties. For example, CD28 and 4-1BB, which are widely used in CAR and both able to augment cell proliferation, cytokine secretion and persistence, differ in many aspects [70]. CD28 [71,72], a member of immunoglobulin superfamily (IgSF), recruits PI3K, LCK, RAS and GRB2 by the basic amino acids and SH2/SH3 binding sites, then further activates AKT signal, recruits PKC θ , and induces Ca²⁺ influx, leading to the activation of NFAT, AP-1, NF- κ B nuclear signaling pathways. But 4-1BB and other members in TNF receptor superfamily (TNFRSF) work through different mechanisms [73,74]. 4-1BB activates MAPK, NF- κ B and other signaling pathways by recruiting TNF receptor-associated factor 1 (TRAF1), TRAF 2, TRAF3. Differences in mechanisms result in variations in properties between CD28- ζ and 4-1BB- ζ CAR-T cells [75]. CD28- ζ CAR functions more rapidly while 4-1BB CAR more sustained. CD28 signal leads to an effector memory T cell-like behavior. Prominent secretion of IL-2 and enhanced glycolysis determine the fast answer rate and the impaired long-time effects. In contrast, 4-1BB CAR is better in survival and long-lasting proliferation due to preference in Tcm differentiation, mitochondrial biogenesis, and fatty acid oxidative metabolism. Due to these characteristics, patients treated with 4-1 BB- ζ CAR-T cells have a better clinical efficacy than CD28 [76]. Better understanding in mechanisms and properties of these costimulating domains will help design and utilize CARs to meet with different situations.

Scientists are still trying to remold the signaling domain in order to delicately control T cell activities. For example, one mutation in CD28 PYAP motif that breaks Lck binding moiety is induced to CD28-CD3 ζ CAR, leading to abrogated IL-2 production without impairing IFN- γ secretion, proliferation and cytotoxicity of cytotoxic T lymphocytes (CTL) compared with normal one [77]. This modification disrupts the IL-2 based sustain of Treg cell, which improves the net efficacy against tumor, especially in cancer types with heavy infiltration of Tregs. However, modifications of the signaling domain are insufficient, and most of them are still far away from clinical application. Widely-used traditional CARs always directly take use of natural T cell receptors' complete endodomains. This simple design is unable to freely regulate T cell downstream signals and cell activities, resulting in the fact that available CAR-T cells cannot always satisfy varied clinical demands. We are trying to modulate T cell signals with strategies like feedback loops, hoping to delicately control cell fate and activities.

1.3.3. The next generation CAR: signal from cytokine receptors

For further development, the third signal for T cell activation from cytokines is also integrated. CAR-T cells directly secreting cytokines like IL-12, which is one kind of armored CAR-T cells, showed elevated anti-tumor efficacy [78–80] (Fig. 5A). There are many complex immunosuppressive factors, such as inhibitory cytokines and receptors, myeloid derived suppressor cells (MDSCs), and tumor associated macrophages (TAMs) in tumor microenvironment. Pro-inflammatory cytokines, like IL-12 can help relieve the suppression by working against these inhibitory factors. IL-12 has been proved able to enhance the cytotoxic capability of CD8⁺ cells, recruit macrophages, and reprogram MDSC. However, IL-12 is only produced by DCs, macrophages and neutrophils, not by T cells. Therefore, people construct CAR-T cells that constitutively secrete IL-12. This kind of CAR-T cells show better

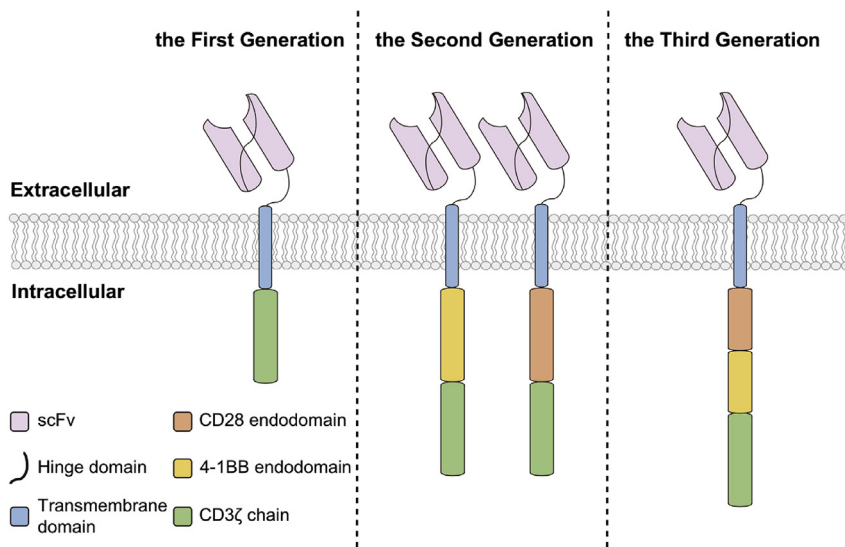


Fig. 3. The evolution of CAR designs. The first generation CAR contains a scFv, a hinge region, a transmembrane region and the endodomain of CD3 ζ chain. In contrast with the first generation CAR, one intracellular domain of costimulatory receptors, such as CD28 or 4-1BB, is added into the second generation CAR, and the third generation CAR combines two kinds of costimulatory signals like CD28 and 4-1BB.

proliferation, decreased apoptosis and increased cytotoxicity in the tumor immunosuppressive microenvironment. Besides, the intracellular domains of cytokine receptors are inserted into CAR's structure. Mark D Minden and Naoto Hirano's research group developed a novel CAR on the basis of traditional CD28-z CAR, which incorporated truncated cytoplasmic domain of IL-2 receptor β and STAT3-binding motif inside the CD28-z CAR [81]. As the new CAR strengthens the activation of JAK kinase, STAT3 and STAT5 signals, it promotes T cell proliferation and prevents terminal differentiation.

2. Safety and efficacy are the major concerns in CAR-T clinical trials

2.1. Safety concern and strategies

Due to the fact that TAAs are not only expressed on tumor cells, normal tissues with certain TAAs are also attacked by CAR-T cells. This on-target but off-tumor side-effect is one of the most difficult problems to be solved [55,69]. Essentially speaking, this problem is because cancer cells are still autogenous and hard to be distinctly separated from normal tissue, and similar problems also exist in other treatments such as radiotherapy and chemotherapy. Therefore, searching for more specific TAAs and scFvs is the fundamental solution. Another problem in safety is T cell over activation and huge secretion of cytokines, which is called cytokine storm. Such large amounts of cytokines are harmful to the whole body, leading to cytokine release syndrome (CRS) with symptoms like fever, edema, hypotension and even death. The commonly used correspondent clinical treatment is using blockade of cytokines, especially IL-6, to decrease the damage caused by side-effects [1,7,82].

Systems and synthetic biology helps a lot not only in construction but also in the utilizing mode of CAR. One important direction is controlling CAR-T cells [83]. James Onuffer and Wendell A. Lim's group developed an on-switch CAR that works in the way of AND-GATE [84] (Fig. 4A). It is comprised of two parts: The major component of Part I is the extracellular domain of CAR, and Part II is the signaling module that has the immunoreceptor tyrosine-based activation motifs (ITAMs) of CD3 ζ . These two parts are separately fused with FKBP and FRB, which can be triggered to heterodimerize by the small molecule rapalog. Only when adding rapalog, these two parts of CAR can join together to form a complete CAR that can transform the outer stimulus to the T cell activity.

In 2016, Wendell's group achieved another kind of AND-GATE design based on synthetic Notch (synNotch) receptor [85,86] (Fig. 4B).

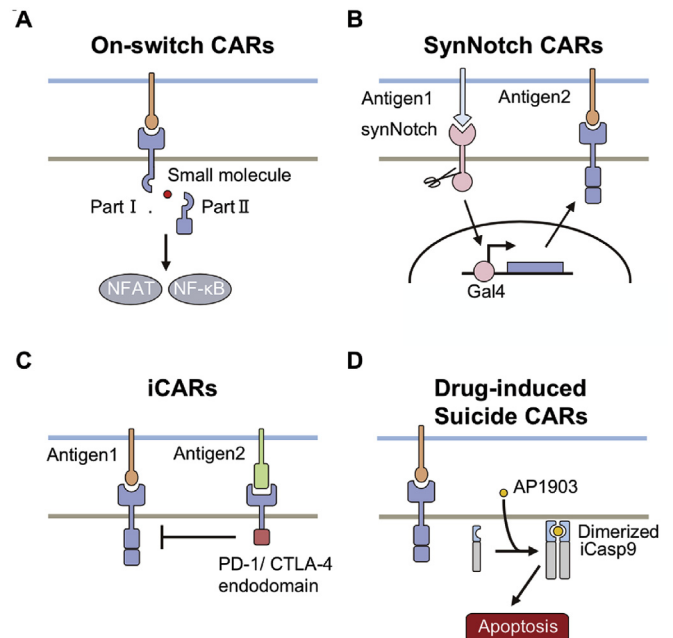


Fig. 4. Strategies to improve safety of CARs. (A) On-switch CARs: A CAR is split into two parts which form an AND-Gate. Only when adding small molecules, these two parts join together and transform the signal from antigen. (B) SynNotch CARs: Activation of synNotch induces expression of CARs. Only when there are both antigens corresponding to synNotch and CAR, T cells immune response can be activated. (C) Inhibitory CARs (iCARs): T cells cannot be activated when iCARs react with their antigen expressed on normal tissue even though CARs are stimulated at the same time. (D) Drug-induced suicide CARs: A small molecular named AP1903 leads to the dimerization of modified caspase9, and induces CAR-T cells apoptosis.

Like natural Notch receptor, synNotch receptor contains an extracellular domain to detect ligand on the surface of target cells. The recognition between synNotch receptor and ligand leads to cleavage of the receptor at Notch core domain. Intracellular transcriptional activator module is released subsequently to activate the expression of downstream genes. In this design, the first TAA detected by synNotch receptor initiates CAR expression, and the second TAA recognized by CAR activates T cells. Only if encountering tumor cells that express both kinds of TAAs, T cell can be activated.

Another strategy to regulate T cell activity is to incorporate

repressive signaling motif in the CAR design (i.e., Inhibitory CAR, iCAR), instead of active motif. iCAR can act as a NOT gate to prevent CAR-T cells' attacking normal tissues [87] (Fig. 4C). More specifically, a repressive motif from coinhibitory signal, such as PD-1 and CTLA4, was used as the intracellular part of iCAR, while the extracellular scFv recognizes epitopes that express on normal tissue but not on tumors. iCAR can inhibit T cell activities when it recognizes its antigen, and the inhibition is reversible. The introduction of iCAR is beneficial in decreasing on-target but off-tumor impairment of normal tissue.

To lessen the harm, transient CAR-T cells are taken into use. For example, transforming mRNA of CARs into T cells instead of permanently inserting DNA of CAR into genome can shorten the expression period of CAR [88]. Drug-induced suicide CAR-T cells are also promising in controlling the lifetime [89] (Fig. 4D). For example, iCasp9 cell-suicide system uses a small molecule called AP1903 to induce cell apoptosis. The inducible Caspase9 is based on the fusion of human caspase 9 to a modified human FK-binding protein (FKBP12), thus it can conditionally dimerize only when binding with AP1903. Consequently, the dimerized iCasp9 triggers cell apoptosis to kill detrimental T cells.

2.2. Efficacy limitations and improvements

As for efficacy, low persistence is one of the limitations [9,55]. Portions of stimulated T cells are going to exhaust, especially when over activated. Activation-induced cell death is averse to long-term functions. We prefer CARs with a lower rate of relapse that can augment cell proliferation and Tcm differentiating tendency besides of cytolytic ability.

The greatest challenge is in treating solid tumor [90]. There are quite a few inhibitory factors that prevent T cells from attacking tumor cells in the microenvironment. T cell intrinsic inhibitory receptors, such as PD-1 and CTLA-4, greatly limit T cell functions as tumor cells highly express their ligands. Many inhibitory cytokines, such as IL-4, IL-6, LIF, IL-10 and TGF- β have also been reported in solid tumor [78,91]. For example, IL-4, which suppresses Th1 cell function, plays negative roles in CAR-T cell infiltration and activation. Besides, there are many kinds of regulatory cells such as myeloid-derived suppressor cells (MDSCs) and T regulatory cells (T_{reg} cells) that can also lead to immune suppression in several ways [78,92,93]. These suppressive factors make it difficult for T cells to infiltrate and function inside tumor, thus leading to CAR-T cells' poor performance in treating solid tumor.

To ameliorate the situation, strategies targeting inhibitory factors are employed together with CAR-T cell therapy. As CAR is just one kind of armor that enhances the killing ability, regulation of the whole tumor microenvironment also needs to be considered. One of the most famous example is immunity checkpoint blockade antibodies, such as α -PD-1, α -PD-L1 and α -CTLA4, which show great potency in promoting tumor regression [94–96] (Fig. 5B). Synthetically engineered receptors also help to prevent the inhibitory factors. For instance, chimeric PD-1, whose intracellular domain is replaced by that of CD28, competitively inhibits repressive PD-1 signals and add costimulatory effects to T cell [97] (Fig. 5C). Another chimeric receptor comprised of IL-4R α extracellular domain and IL-7R α intracellular domain works in similar

principle, which inverts the normally inhibitory effects of Th1 in the presence of IL-4 [98].

Besides passively resisting inhibitory effects, scientists also turn to chemokines in order to drive T cells to migrate to tumor cells. CAR-T cells coexpressing CXCR2, the receptor of tumor-derived chemokine CXCL1, show enhanced migration towards tumor sites and elimination of tumor [99]. Apart from that, CAR-T cells secreting CCL19 can attract T cells and DCs, which improve tumor infiltration rate and anti-tumor ability to some extent [100].

3. The future of CAR

CAR is not limited as a powerful weapon to kill tumor, but to kill a much broad disease-causing abnormal cells, such as pathogen-infected cells and harmful immune cells in autoimmunity. As further application in clinical trials, CAR-T cells should be strengthened with both efficacy and controllability. Even though CAR-T cell-based therapeutics offer people great opportunities in combating cancer, cure rate is still very limited and tumor relapse is not rare. As for solid tumor, there are few successes reported. How to promote T cells infiltration and persistency in tumor microenvironment has been a great challenge in future. Apparently, constructing a CAR that can block the inhibitory signals itself is relatively dangerous and tends to be out of control, which is similar to a car without brakes. Apart from inhibitory factors, poor persistence of CAR-T cells caused by exhaustion and activation-induced cell death is one of the essential limitations for complete remission of tumor. The fact that BB- ζ CAR is preferred to CD28- ζ CAR in clinical trials is due to BB- ζ CAR-T cells' long-term effects in T cell persistence [9,75]. However, higher cytotoxicity of tumor is always accompanied with stronger harm to healthy tissue. Conflict between efficacy and safety in CAR-T cell therapy is also a great challenge, which requires CAR-T cells to be more controllable in temporal, spatial and functional dimensions.

As mentioned above, people have developed several strategies in regulating CAR-T cells' activity. Choosing specific TAAs and logic gate in distinguishing tumor and normal tissue helps lessen on-target but off tumor side effects. But regulation of CAR-T cells' functional strength and characteristics is insufficient. Efficiency in killing cancer is related to, but can be separated from other T cells' properties such as proliferation, differentiation tendency, and cytokine release preference. In principle, CAR-T cells should be used pertinently towards different cases. Unfortunately, we don't have enough choices to meet with varied requirements, even for the basic demand to balance efficiency and safety. One reason is that we still lack clear understanding of the receptors and their signals in T cells, and simply adding intracellular domains of these receptors into CAR also limit the controllable degree.

CAR structure design should be more delicate. Synthetic biology is not just overlaying elements from different sources, but making a functional artificial molecule with protein engineering principles. In this regard, optimization of structure needs not only proper elements like scFvs and signaling domains, but also the rational design of CAR's conformation and configuration. Actually, how to utilize these elements properly should be taken into consideration, such as their combinations, orders and transformations. Differences in construction of CARs

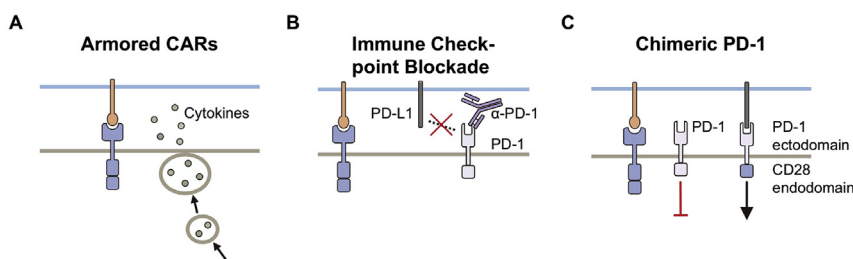


Fig. 5. Strategies to against tumor inhibitory micro-environment. (A) Armored CARs: Compared with traditional CAR-T cells, armored CAR-T cells are optimized to constitutively or inducibly secrete cytokines such as IL-12 or express costimulatory ligands like 4-1BBL [79], which strengthens the anti-tumor efficiency. (B) Immune checkpoint blockade: Utilizing antibodies to block immune-suppressive signals. (C) Chimeric PD-1: Chimeric PD-1 is a modified PD-1 whose intracellular domain is replaced by that of CD28. This receptor can recognize PD-L1 but induce a costimulatory signal.

influence the expression level, strength and specificity of recognition, and even T cell reaction rate, so systematically comparison is needed to design functional optimized CARs.

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