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Engineering Next-Generation CAR-T Cells: Overcoming Tumor Hypoxia and Metabolism

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Abstract

T cells engineered to express chimeric antigen receptors (CARs) have shown remarkable success in treating B-cell malignancies, reflected by multiple US Food and Drug Administration–approved CAR-T cell products currently on the market. However, various obstacles have thus far limited the use of approved products and constrained the efficacy of CAR-T cell therapy against solid tumors. Overcoming these obstacles will necessitate multidimensional CAR-T cell engineering approaches and better understanding of the intricate tumor microenvironment (TME). Key challenges include treatment-related toxicity, antigen escape and heterogeneity, and the highly immunosuppressive profile of the TME. Notably, the hypoxic and nutrient-deprived nature of the TME severely attenuates CAR-T cell fitness and efficacy, highlighting the need for more sophisticated engineering strategies. In this review, we examine recent advances in protein- and cell-engineering strategies to improve CAR-T cell safety and efficacy, with an emphasis on overcoming immunosuppression induced by tumor metabolism and hypoxia.

INTRODUCTION

Chimeric antigen receptors (CARs) are synthetic receptors that allow T cells to recognize specified antigens and initiate antigen-specific immune responses. The adoptive transfer of CAR-T cells has been evaluated in various contexts, including treatment for viral infections and autoimmune diseases (1, 2), but cancer therapy remains the dominant clinical application. In particular, CAR-T cell therapy has shown remarkable clinical efficacy in treating various B-cell malignancies (3–7), becoming the first genetically modified cell therapy to receive US Food and Drug Administration (FDA) approval (**Table 1**). However, broadening of CAR-T cell therapy outlook to solid tumors, which comprise most cancers, has been challenging in part owing to the highly immunosuppressive and heterogeneous nature of the solid tumor microenvironment (TME). Additionally, challenges such as loss of CAR-targeted tumor antigen (antigen escape) and on-target, off-tumor toxicities further emphasize the need for more sophisticated engineering approaches to address these limitations and expand the applicability of this therapeutic platform.

In this review, we assess the current status of the CAR-T cell therapy field along with prospective strategies to engineer next-generation CAR-T cell therapies for improved efficacy, safety, and applicability to overcome the aforementioned obstacles. We provide an overview of protein-engineering strategies employed to design and optimize the CAR molecule, cell-engineering strategies to maximize T-cell function, and synthetic biology and biomolecular engineering strategies to address toxicities associated with CAR-T cell therapy. Finally, we provide a detailed discussion on challenges posed by the hypoxic and nutrient-deprived nature of the TME that fuels immunosuppression and present promising strategies to overcome these challenges.

ENGINEERING THE CAR PROTEIN

CAR Protein Components

CARs are transmembrane proteins that transduce an extracellular antigen-binding event into an intracellular signaling cascade, resulting in downstream T-cell activation and subsequent effector functions such as cytokine production, T-cell proliferation, and target-cell killing. The CAR protein structure includes four major components: an extracellular antigen-binding domain, extracellular spacer domain, transmembrane domain, and intracellular signaling domain (**Figure 1*a***). Each of these components has distinct functions and effects on the resulting CAR-T cell product, and the modularity of the CAR architecture allows for optimization of various constituent domains to achieve the desired T-cell phenotype and function.

Table 1 Currently Food and Drug Administration–approved CAR-T cell therapies

Therapy	Trade name	Year approved	Antigen target	Indications
Tisagenlecleucel	Kymriah	2017	CD19	■ B-cell precursor acute lymphoblastic leukemia ■ Large B-cell lymphoma
Axicabtagene ciloleucel	Yescarta	2017	CD19	■ Large B-cell lymphoma ■ Follicular lymphoma
Brexucabtagene autoleucel	Tecartus	2020	CD19	■ Mantle cell lymphoma
Lisocabtagene maraleucel	Breyanzi	2021	CD19	■ Large B-cell lymphoma
Idecabtagene vicleucel	Abecma	2021	BCMA	■ Multiple myeloma
Ciltacabtagene autoleucel	Carvykti	2022	BCMA	■ Multiple myeloma

Abbreviations: BCMA, B-cell maturation antigen; CAR, chimeric antigen receptor.

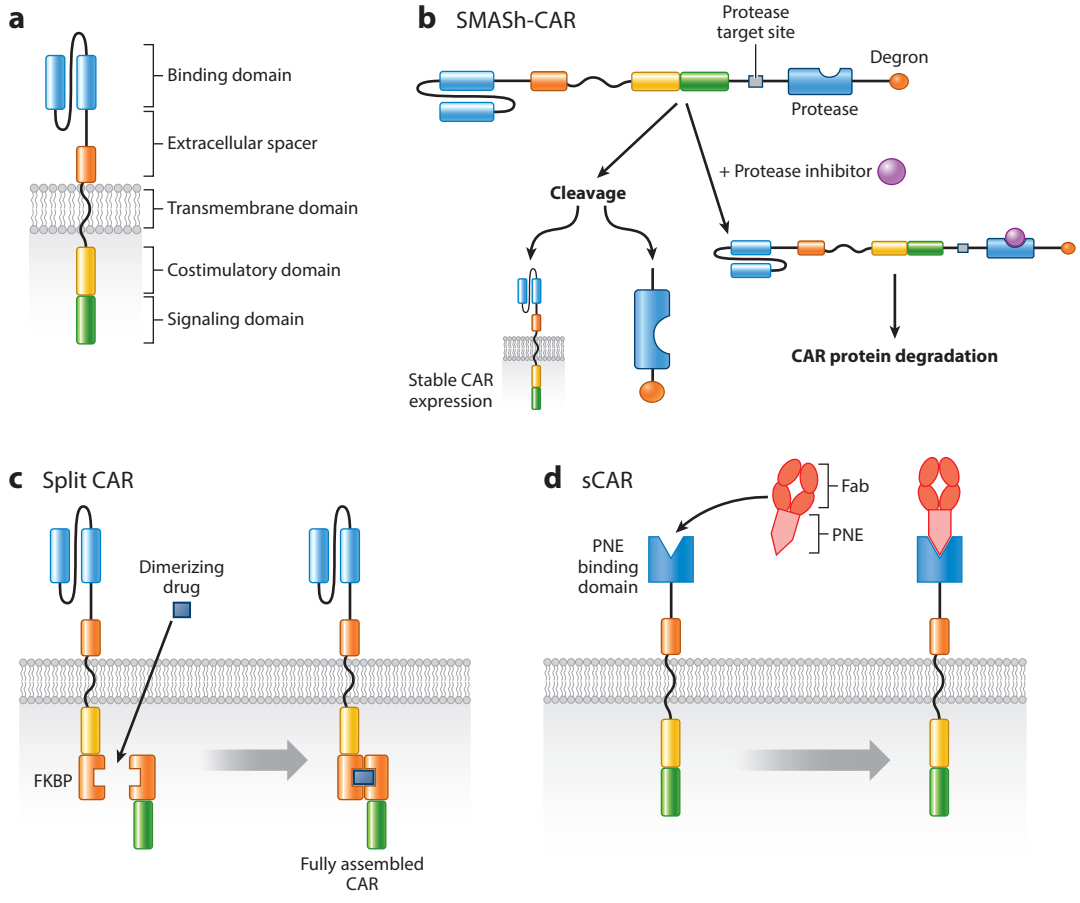

Figure 1

CAR structure and protein engineering strategies to improve safety. (*a*) CARs comprise an extracellular antigen-binding domain, extracellular spacer, transmembrane domain, intracellular domain typically consisting of one or two costimulatory domains (most commonly CD28 or 4–1BB), and signaling/activating domain (typically CD3ζ). (*b*) A protease-based ON/OFF switch allows control over CAR protein expression with protease inhibitors. In the absence of the protease inhibitor, a protease fused to the CAR cleaves a target site in *cis* to remove a degron and stabilize the CAR protein, resulting in a default ON state. Presence of the protease inhibitor prevents degron removal, leading to CAR protein degradation and an OFF state for the system. (*c*) The CAR protein can be split into two domains, each with partial function. Dimerizing drugs are required to assemble a fully functional CAR protein and allow control over CAR-T cell activity. (*d*) sCARs that bind a bi-orthogonal tag, such as PNE, can be directed to tumor antigens through the addition of a PNE-tagged Fab (protein switch) specific for a tumor antigen. CAR-T cell activity and specificity can be controlled and redirected by using different protein switches. Abbreviations: CAR, chimeric antigen receptor; Fab, antigen-binding fragment; FKBP, FK506-binding protein; PNE, peptide neoepitope; sCAR, switchable CAR; SMASh, small molecule–assisted shutoff.

The antigen-binding moiety is the extracellular, N-terminal portion of the CAR protein that confers programmed antigen-recognition ability to the CAR-T cell. Single-chain variable fragments (scFvs), composed of the variable heavy and light chains of monoclonal antibodies separated by a flexible linker, are most commonly used as the antigen-binding domain to target proteins expressed on the surface of cancer cells. In addition to scFvs, alternative antigen-binding domains, such as nanobodies, Fc receptor fragments, peptides, and endogenous receptor/ligand-based motifs, have also been used to construct CARs (8–10). Unlike T-cell receptors (TCRs),

CARs can directly recognize surface proteins without requiring antigen presentation by the major histocompatibility complex (MHC). MHC-independent antigen recognition allows CAR-T cells to sidestep MHC downregulation as a major tumor-defense mechanism, but it simultaneously limits the target repertoire to antigens that are naturally surface bound. Of note, CARs can also be engineered to target MHC-presented antigens (11, 12), and engineered TCRs targeting both intracellular and extracellular antigens in a MHC-dependent manner have also been developed (13).

The extracellular spacer and transmembrane domains bridge the antigen-binding domain to the intracellular signaling domain. The spacer region is a peptide fragment that provides conformational freedom to facilitate efficient CAR binding to the target antigen. The optimal spacer length and composition depend on the spatial proximity of the target antigen to its cell surface: Epitopes distal to the target-cell surface (and thus proximal to the T-cell surface) are better targeted using CARs containing a short extracellular spacer, and vice versa (14, 15). The transmembrane domain anchors the CAR protein to the T-cell surface and has been associated with CAR function and stability (16, 17). Furthermore, both the spacer and transmembrane domains have been implicated in modulating CAR-T cell cytokine secretion and activation-induced cell death (18).

The intracellular domain typically consists of an activating domain and one or more costimulatory domains. CD3ζ is the most widely employed activating domain, and first-generation CARs include only CD3ζ without costimulatory domains. However, early clinical studies revealed that first-generation CAR-T cells failed to elicit potent antitumor immunity (19, 20). Thus, second- and third-generation CARs were developed that incorporated either one or two costimulatory domains, respectively, to enhance CAR-T cell cytotoxicity, cytokine production, proliferation, and in vivo persistence (21, 22). Costimulatory domains derived from either CD28 or 4-1BB (CD137) are employed most commonly, and all currently FDA-approved CAR-T cell therapies use one of these domains. The choice of costimulatory domain can significantly affect the resulting CAR-T cell phenotype, metabolic profile, and antigen-independent tonic signaling, thus prompting research into other potential domains, such as CD40, MyD88, OX40, CD27, and inducible T-cell costimulator (23). More recently, CAR-T cells that secrete transgenic cytokines, termed fourth-generation or armored CAR-T cells, have been developed. For example, Chmielewski & Abken (24) have developed such a CAR, termed TRUCK (T cells redirected for universal cytokine-mediated killing), that elicits the secretion of the proinflammatory IL-12 cytokine to stimulate an innate immune response within the immunosuppressive tumor stroma.

Accumulating clinical and preclinical data indicate that the specific sequence, structure, and combination of different CAR domains can significantly influence the therapeutic efficacy of resulting CAR-T cells. In-depth analyses of these modular components and protein engineering efforts are available in several excellent reviews (25, 26). Given that CARs can incorporate nonhuman sequences (e.g., murine scFvs) and introduce novel junctions between domains taken from endogenous proteins, immunogenicity and subsequent rejection of CAR-T cells are a potential concern. Anti-CAR humoral immune responses have been observed in patients receiving tisagenlecleucel or axicabtagene ciloleucel, although these were not associated with worse clinical responses (27, 28). Anti–CAR-transgene immune responses mounted by cytotoxic T cells have also been observed in the clinic (29, 30). Humanization of scFv domains may reduce potential CAR immunogenicity, although fully human CARs can still induce endogenous immune responses due to the presence of anti-idiotypic antibodies. Further assessments of potential CAR-related immunogenicity and impacts on treatment outcome can be found in several reviews (31, 32). Conventional CAR-T cells, including all currently approved products, express a single transgenic construct—i.e.,

a CAR molecule that targets one specific antigen. However, safety concerns and efforts to increase efficacy and broaden CAR applicability have motivated the engineering of more sophisticated CAR designs, which are discussed below.

Implementing Control Switches to Improve Safety

Unlike traditional cancer treatments, such as antibodies and small-molecule drugs, adoptive T-cell therapy can be considered a living drug, with the ability to amplify, differentiate, persist, and circulate within the body. As such, T cell–based therapies can dynamically respond to their microenvironment to mount complex immune responses against specific cancer targets. However, the complexity of this treatment paradigm concurrently poses unique safety challenges that have been observed in the clinical setting (33). Commonly observed toxicities include

1. Tissue and organ damage caused by CAR interactions with antigens expressed on nonmalignant cells. These toxicities can be off-target (i.e., caused by CAR cross-recognition of an antigen that is not the target antigen), or on-target, off-tumor (i.e., when the target antigen is expressed on both cancerous and healthy tissues).
2. Systematic toxicities associated with excessive CAR-T cell activation and subsequent systemic release of high levels of cytokines, resulting in cytokine release syndrome.
3. Neurotoxicity associated with CAR-T cell infiltration into and/or elevated cytokine levels in the central nervous system.

One strategy to manage CAR-T cell toxicity is to implement a control switch that can be used to control CAR expression. Conventional CAR-T cells constitutively express the CAR protein, but this can be undesirable if on-target, off-tumor toxicity is expected. ON/OFF switches, which can be induced externally or self-regulated, provide a way to control CAR expression and T-cell activity. For example, CARs can be fused to destabilizing domains, which sets the CAR in a default inactive OFF state through rapid protein degradation. Such CARs can be turned ON by using small-molecule drugs to disable the destabilizing domains, thus stabilizing the CAR protein (34). Conversely, protease-based SMASh CARs (small molecule–assisted shutoff CARs) enable a default ON state by fusing the CAR to a protease target site followed by the cognate protease and a degron domain that induces CAR degradation (35) (**Figure 1*b***). In the absence of external input signals, the protease cleaves the target site, thus removing the degron and stabilizing the CAR protein. In contrast, administration of a small-molecule protease inhibitor prevents degron removal and turns OFF the CAR through protein degradation. These strategies allow temporal control over CAR expression, and thus control over CAR-T cell activation and antitumor activity. Likewise, spatial control over CAR expression can be achieved by, for example, engineering an oxygen-sensitive switch. Oxygen-sensitive domains of the hypoxia-inducible factor (HIF)-1α have been fused to the intracellular signaling domain of the CAR protein such that HIF degradation under normoxic conditions inactivates the CAR, but hypoxic conditions, hallmarks of many solid tumors, stabilize CAR surface expression (36).

The CAR protein can also be split into two nonfunctional domains such that dimerizing drugs are necessary to assemble a functional CAR protein. This split-receptor design expresses the extracellular antigen-binding and intracellular signaling domains as separate polypeptides (**Figure 1*c***). Both polypeptides contain a heterodimerization domain, such as FK506-binding protein (FKBP), that conditionally assembles in response to a dimerizing drug (37). Thus, CAR assembly can be chemically induced to control CAR-T cell activation and activity. As an alternative approach, switchable CARs have been engineered to bind a peptide neoepitope rather than a tumor antigen,

and target-cell recognition is facilitated by the addition of a peptide-neoepitope-tagged soluble antigen-binding fragment (protein switch) (**Figure 1*d***). In this system, one can in principle control the intensity of the T-cell response by regulating the timing and amount of protein switch added, as well as change the antigen specificity of the switchable CAR-T cell by changing the identity of the protein switch.

Combinatorial Antigen Sensing Using Logic-Gated CARs

Ideal tumor antigens are those that are expressed exclusively by tumor cells and not on healthy tissues. However, owing to a lack of these ideal tumor-specific antigens, most antigen targets are tumor-associated antigens (TAAs) that are overexpressed on tumor cells but also expressed in healthy tissues, albeit at lower levels, thus resulting in on-target off-tumor toxicity. A strategy to improve specificity and alleviate such toxicity is by using AND-gate logic, which requires the recognition of multiple TAAs for subsequent CAR-T cell activation. The synthetic Notch (synNotch) system is one such AND-gate system in which binding of antigen A by the synNotch receptor triggers downstream activation of a transcription factor that drives expression of a CAR specific for antigen B (38) (**Figure 2*a***). synNotch CAR-T cells exhibit improved specificity and reduced systemic toxicity in various murine tumor models (38–41). However, temporal delays between synNotch signaling and subsequent CAR expression allow for the possibility that the T cell could be triggered by one target cell expressing antigen A but attack a separate target cell expressing antigen B, thus losing the specificity advantage conferred by AND-gate computation. Indeed, the synNotch system cannot prevent on-target, off-tumor toxicity if the healthy tissue that express CAR-targeted antigen is colocalized with tumor cells (39).

Alternatively, AND-gate can be accomplished by separately expressing a CD3ζ-containing CAR and a costimulatory domain–containing chimeric costimulatory receptor (CCR), such that full T-cell activation is accomplished only when both the CAR and the CCR's cognate antigens are present on the target cell (42). CARs designed to execute AND-NOT logic using an inhibitory CAR (iCAR) have also been designed to help prevent off-target toxicity (**Figure 2*b***). iCARs target antigens present on healthy tissue and incorporate an inhibitory signaling domain, such as those derived from programmed cell death protein 1 (PD-1) or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), to dampen the T-cell response when the healthy antigen target is coexpressed by the target cell. CAR-T cells coexpressing both a CD19 CAR and an iCAR incorporating the intracellular domain of PD-1 or CTLA-4 have been shown to exert cytotoxicity against malignant B cells but spare healthy B cells both in vitro and in vivo (43, 44).

Whereas AND and AND-NOT logic gates can improve the safety of CAR-T cell products, they do not address other challenges, such as antigen escape. In fact, strategies that increase targeting specificity often concurrently increase the likelihood of tumor escape. Clinical trials with CD19 CAR-T cells have shown remarkable efficacy, achieving complete response rates up to 90%, yet relapse with CD19-negative tumors has been observed in up to 25% of responding patients (45–49). OR-gate CARs have been developed to circumvent this evasion mechanism by simultaneously targeting two antigens, such that binding of either antigen can activate the CAR-T cell (**Figure 2*c***). Tandem bispecific CARs achieve this by linking two scFv domains, separated by a linker, in series. Although these CARs require careful optimization of scFv orientation, scFv positioning, and linker length, they have demonstrated reduced tumor relapse and superior antitumor efficacy compared to single-input CARs. Notably, bispecific CARs targeting CD19/CD20 and CD19/CD22 are promising candidates in treating B-cell malignancies known to be susceptible to loss of the CD19 antigen (50, 51), and several ongoing clinical trials have shown promising early results (NCT04007029, NCT04700319, NCT03241940, and NCT03233854) (52–54).

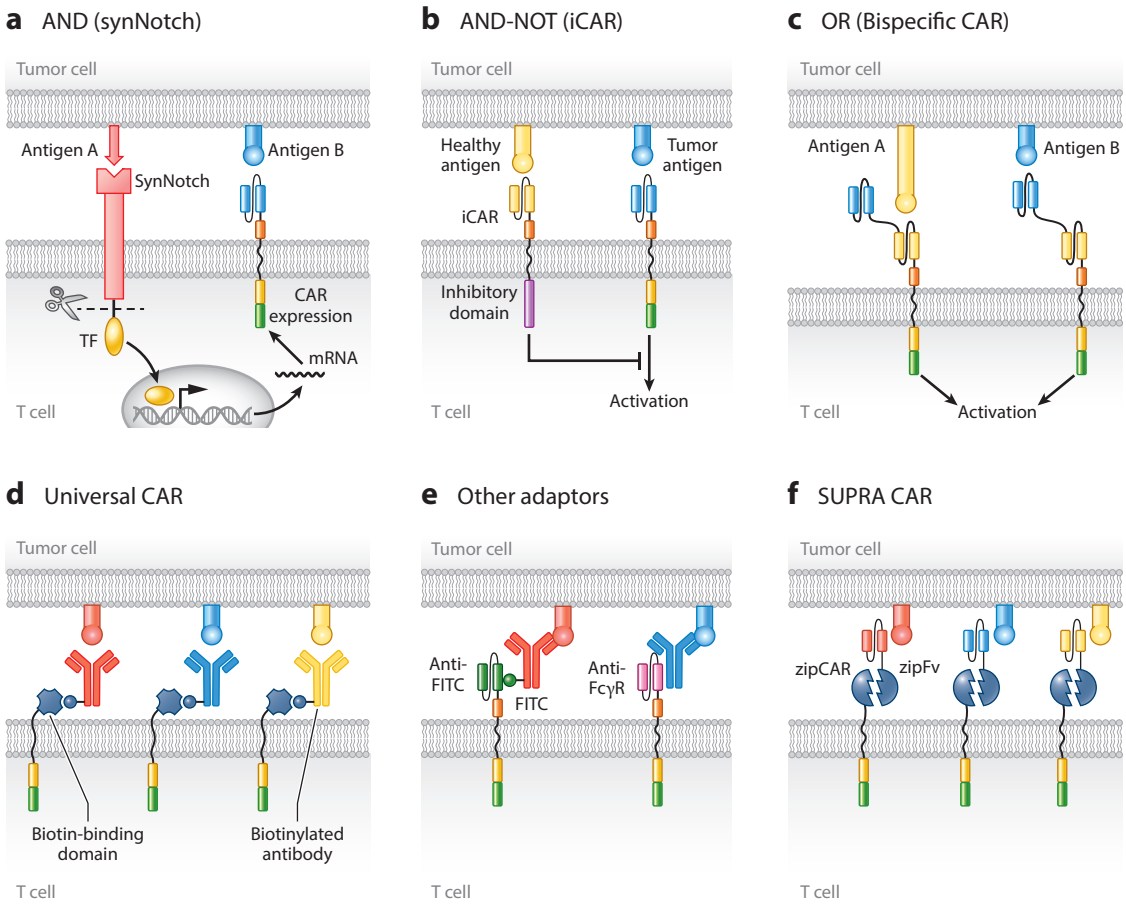

Figure 2

Combinatorial antigen sensing and adaptor-dependent engineering strategies to improve the safety, efficacy, and programmability of CAR-T cells. (*a*) The synNotch (AND logic) system requires recognition of multiple TAAs for CAR-T cell activation. Upon binding of antigen A, the synNotch receptor undergoes conformational changes leading to the release of a TF that turns on expression of a CAR specific for antigen B. (*b*) The iCAR (AND-NOT logic) system requires recognition of a TAA and the absence of a healthy-tissue antigen for adequate CAR-T cell activation. CAR-T cell response is dampened when the target cell coexpresses a healthy antigen targeted by the iCAR. (*c*) Tandem bispecific CARs (OR logic) include two extracellular antigen-binding domains such that binding of either antigen can activate the CAR-T cell. (*d*) Universal adaptor receptors consisting of an extracellular biotin-binding domain can target biotinylated antibodies with various TAA specificities. Different antibodies or antigen-binding adaptor molecules can be administered to redirect CAR-T cell specificity, and temporal control over CAR-T cell activity can be achieved by adjusting the timing of administration. (*e*) CARs targeting FITC and FcγRs represent additional adaptor systems to redirect CAR-T cell specificity based on the specificity of the administered adaptor protein (e.g., antibodies). (*f*) SUPRA CARs offer controllability over CAR-T cell specificity and activity based on leucine-zipper dimerization of the zipCAR and exogenously administered zipFv adaptor molecules. Abbreviations: CAR, chimeric antigen receptor; FcγR, Fc-gamma receptor; FITC, fluorescein isothiocyanate; iCAR, inhibitory CAR; mRNA, messenger RNA; SUPRA, split, universal, and programmable; synNotch, synthetic Notch receptor; TAA, tumor-associated antigen; TF, transcription factor; zipCAR, leucine-zipper-based universal receptor; zipFv, tumor-targeting scFv adaptor molecule.

CARpooling (co-administration of two CAR-T cell populations) and dual CARs (co-expression of two CAR proteins in the same T cell) are additional OR-gate strategies investigated in preclinical models, but tandem bispecific CARs have shown superior antitumor efficacy in most head-to-head comparisons (55–58).

Adaptor-Dependent CAR Designs to Expand Versatility

Traditional single-input CAR designs necessitate designing of new CAR proteins to target different antigens. Although OR-gate CARs can improve CAR versatility by targeting two antigens, the number of antigen specificities that can be hardwired into a single CAR is inherently limited. To further expand CAR-T cell versatility, universal adaptor receptors consisting of a biotin-binding domain fused to an intracellular T-cell signaling domain have been developed (59) (**Figure 2*d***). In principle, any biotinylated antibody or antigen-binding adaptor molecule can be administered to redirect CAR-T cell specificity and permit sequential or simultaneous targeting of various antigens. Furthermore, CAR-T cell activity can be controlled by the administration of such biotinylated antibodies, and this strategy works as a dual control switch. A similar approach uses a CAR that binds fluorescein isothiocyanate (FITC) and various FITC-tagged antigen-specific adaptor proteins, such as antibodies, to redirect CAR-T cell cytotoxicity (60) (**Figure 2*e***). Likewise, CARs that bind Fc-gamma receptors allow the tuning of CAR specificity by administrating different therapeutic antibodies targeting distinct antigens (61) (**Figure 2*e***). Yet another strategy uses the split, universal, and programmable CAR system in which T cells express a universal receptor (zipCAR) that contains a leucin zipper as its extracellular domain. The zipCAR alone has no antigen-targeting capability, but it can reconstitute a functional CAR upon leucine zipper–mediated dimerization with a tumor-targeting scFv (zipFv) (62) (**Figure 2*f***). Similar to the previous strategies, various scFvs can be implemented in the zipFv design to enable simultaneous targeting of multiple antigens, control over CAR-T cell activity, and logic-based antigen recognition. The use of universal CAR receptors in conjunction with adaptor molecules conferring antigen specificity is a promising approach in simultaneously enhancing CAR-T cell safety and versatility. However, addition of extra components to the CAR design requires additional CAR protein engineering and design optimization to achieve desired CAR-T cell efficacy and functionality. Use of external adaptor proteins will also necessitate optimization of dosing, binding kinetics, and specificity along with understanding of half-life, biodistribution, and pharmacokinetics for optimal CAR-T cell activation and effector functions. Therefore, although undeniably a promising approach, translation of such a complex CAR-T cell design to the clinic remains a challenge to be addressed.

ENGINEERING THE CAR-EXPRESSING T CELL

Beyond engineering strategies on the CAR protein itself, the availability of genetic engineering strategies permits rewiring of various endogenous T-cell pathways at the cellular level for additional control over the activity and functionality of the final CAR-T cell product. Here, we focus on how these gene-editing technologies can be employed to generate safer and more efficacious CAR-T cells. Together with CAR protein engineering strategies, these approaches represent promising methods to generate next-generation CAR-T cell therapies to overcome current roadblocks facing the field.

Engineering T-Cell Safety Mechanisms

The previous section outlined several strategies to control CAR-T cell function through transient and extrinsic regulation of CAR protein availability. An alternative approach to regulating CAR-T cell activity is to eliminate the CAR-expressing T cells themselves upon detection of signs of toxicity. For example, the inducible caspase 9 (iCasp9) suicide gene encodes caspase 9 fused to FKBP12-F36V, a protein that can form homodimers upon binding to the small molecule AP1903. Caspase 9 triggers the apoptosis cascade upon dimerization, eliminating up to 90% of iCasp9-expressing T cells within 30 min of AP1903 administration in human patients (63)

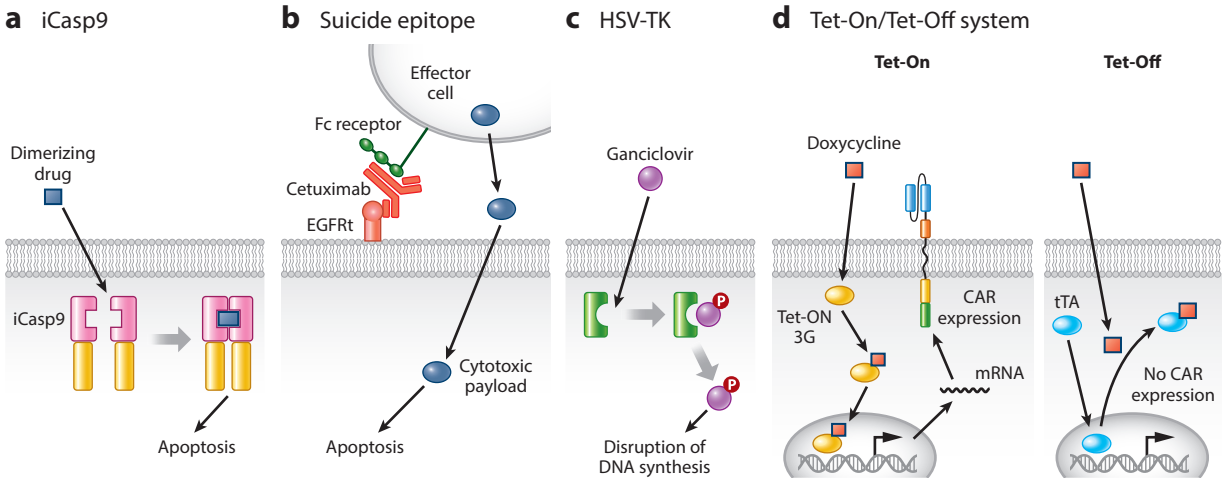

Figure 3

Regulating T-cell presence and CAR expression to improve safety. (*a*) The iCasp9 suicide genes allow rapid elimination of CAR-expressing cells. Administration of dimerizing drugs activates iCasp9 and triggers downstream signaling to induce apoptosis. (*b*) Epitope tags, such as EGFRt, can be coexpressed on the CAR-T cell as a suicide tag. Administration of antibodies, such as cetuximab, specific for the epitope tag triggers endogenous ADCC and CDC machineries to induce CAR-T cell apoptosis. (*c*) CAR-T cells expressing HSV-TK can convert ganciclovir, a synthetic analog of 2′-deoxyguanosine, into a cytotoxic molecule that disrupts DNA synthesis to eliminate CAR-T cells. (*d*) Inducible promoters can be used to control CAR expression using small-molecule drugs at the transcriptional level. In the Tet-On or Tet-Off system, tetracycline or doxycycline can be administered to control CAR gene transcription and thus CAR expression. Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; CAR, chimeric antigen receptor; CDC, complement-dependent cytotoxicity; EGFRt, truncated epidermal growth factor receptor; HSV-TK, herpes simplex virus thymidine kinase; iCasp9, inducible caspase 9; mRNA, messenger RNA; tTA, tetracycline transactivator.

(**Figure 3*a***). Alternatively, epitope tags, such as truncated epidermal growth factor (EGFRt), can be expressed on T cells as a suicide tag (**Figure 3*b***). Administration of therapeutic antibodies, such as the FDA-approved, EGFRt-targeting cetuximab, can induce T-cell apoptosis through endogenous antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity (64). The combination of a CD20 epitope tag and rituximab monoclonal antibody has also been applied to facilitate T-cell deletion (65). An alternative strategy uses herpes simplex virus thymidine kinase as the suicide gene to enable ganciclovir-mediated CAR-T cell elimination (66) (**Figure 3*c***).

However, unlike control switches, suicide genes result in T-cell death and potential termination of the CAR-T cell therapy. To enable more flexible control, noncytotoxic and reversible systems have been developed to ameliorate toxicity without eliminating the CAR-T cells themselves. For example, the CAR gene can be placed under inducible promoters, such as the well-established Tet-On and Tet-Off systems that allow the transcription of the CAR transgene to be controlled via small-molecule drugs such as doxycycline (67, 68) (**Figure 3*d***). Additionally, transient CAR expression using mRNA electroporation, rather than viral integration of the CAR transgene, can reduce off-target toxicities by limiting CAR-T cell persistence and activity in vivo (69), even though this method does not allow for explicit control of the timing or intensity of CAR expression. Production of transient CAR-T cells using mRNA electroporation is feasible at clinical scale, and mesothelin-targeting CAR-T cells generated in this manner have demonstrated antitumor responses in clinical trials without obvious evidence of off-target toxicity (NCT01355965, NCT01897415) (70). However, the durability of response for patients treated with transient CAR-T cells remains to be fully explored.

Targeted CAR Transgene Delivery

CAR-T cell manufacturing protocols typically use viral vectors to integrate the CAR gene into the T-cell genome. Although viral vectors offer robust transgene integration into various host cells and long-term expression, this strategy results in unpredictable integration sites and variable CAR transgene copy numbers, leading to unavoidable variabilities in CAR-T cell product quality. Certain retroviruses have the tendency to preferentially integrate near proto-oncogenes, which has caused severe adverse events, including development of leukemia, in gene therapy trials (71–73). However, no evidence of oncogenic transformation of CAR-T cells caused by viral transduction has been reported to date (74–76). Recent advances in gene-editing techniques, such as clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9), transcription activator-like effector nucleases, and zinc-finger nucleases, have enabled site-specific insertion of the CAR transgene to achieve uniform expression (77). Of note, Eyquem et al. (78) demonstrated that CRISPR/Cas9-mediated insertion of a CD19 CAR transgene into the TCRα constant (*TRAC*) locus results in CAR-T cells with enhanced in vivo antitumor efficacy and reduced propensity for exhaustion compared to their retrovirally transduced counterpart, although evidence from subsequent studies suggests that the benefit of integration into the *TRAC* locus may depend on the specific CAR construct used (58, 79). T cells have also been genetically edited at loci such as *CCR5*, *CD40L*, and *AAVS1* to generate functionally superior T cells in various therapeutic contexts beyond cancer (80–82). Detailed discussions of gene-editing applications in cell engineering can be found in several reviews (83–85).

Modulation of T-Cell Intrinsic Pathways to Enhance CAR-T Cell Function

Gene-editing technologies can also be used to modify endogenous T-cell pathways to abrogate or rewire negative signals and potentiate stimulatory signals to improve CAR-T cell fitness. Tumor cells often express ligands to inhibitory immune checkpoint receptors expressed on T cells, such as PD-1 and CTLA-4. The advent of immune checkpoint blockade (ICB) therapies using antibodies targeting these various inhibitory receptors and their ligands has significantly advanced immunotherapy and cancer treatment (86, 87). With more than 3,000 active clinical trials investigating these therapies, many of which assess combination therapy with CAR-T cells, modulation of T-cell activity through these T-cell modulatory axes is a proven strategy (86, 88). Rather than exogenously manipulating these pathways, gene-editing technologies allow intrinsic rewiring of these negative regulators. For example, CRISPR-Cas9 has been used to generate PD-1-knockout CD19 CAR-T cells with improved in vivo antitumor efficacy against a PD-1$^+$ xenograft leukemia model (89). This strategy has been translated to the clinic as the first CRISPR/Cas9-edited cell therapy evaluated in the United States. In this trial, PD-1 and endogenous TCRαβ were eliminated by CRISPR-Cas9 in autologous T cells engineered to express an HLA-A*0201-restricted TCR targeting New York esophageal squamous cell carcinoma 1 (NY-ESO-1) (NCT03399448). Results from the trial have confirmed the safety and practicability of these CRISPR-edited autologous T-cell products, paving the way for additional PD-1-knockout T cells to enter the clinic (NCT02867345, NCT03545815, NCT03706326) (90). CAR-T cells engineered to secrete anti-PD-1 molecules have also been developed (91). Likewise, transforming growth factor-β (TGF-β) is a potent immunosuppressive cytokine often overexpressed in the TME, and genetically engineered T cells expressing a dominant-negative TGF-β receptor II as a decoy receptor could abrogate TGF-β signaling for enhanced persistence and antitumor efficacy in a syngeneic melanoma model (92, 93). Chang and colleagues (94, 95) have further developed a TGF-β-responsive CAR-T cell that rewires TGF-β signaling to a potent stimulatory signal. CRISPR/Cas9 can also knock out

multiple targets simultaneously: T cells with triple knockouts of *TRAC*, *B2M*, and *PD-1*, as well as *TRAC*, *B2M*, and *FAS*, have been reported in preclinical models (96, 97).

In addition to ablating inhibitory signals, overexpression of stimulatory signals has been performed to enhance T-cell function. For example, PSMA-targeting CAR-T cells constitutively expressing CD80 and 4–1BB ligand (4–1BBL) can deliver costimulatory signals to each other in *cis* (autocostimulation) and to neighboring bystander tumor-infiltrating lymphocytes in *trans* (transcostimulation) to enhance antitumor immunity against mice with systemic prostate tumors (98). Furthermore, nonconventional costimulatory molecules, such as the Toll-like receptor adaptor molecule MyD88 and tumor necrosis factor family member CD40, enhance in vivo persistence of CD19 CAR-T cells in xenograft models of lymphoma and leukemia (99). As an alternative means to enhance T-cell persistence, constitutively active IL-7 receptors have been overexpressed in GD2-targeting CAR-T cells to promote T-cell survival, withstanding multiple rounds of tumor challenge, and prolonged antitumor efficacy in multiple tumor models (100). Similarly, CD20 CAR-T cells engineered to express IL-7 and chemokine ligand 19 have been developed with enhanced proliferative abilities and recruitment of T cells and dendritic cells to tumor tissue (101).

The ideal CAR-T cell therapy would combine strong efficacy, a robust safety profile, and long-term in vivo persistence. In practice, strategies devised to maximize one of these desirable traits often necessitate a trade-off in the other criteria. For example, many strategies to suppress off-tumor toxicity involve ablation of the CAR-T cell (e.g., suicide genes) or termination of CAR expression, thus compromising treatment durability. However, strategies to promote T-cell efficacy and durability can simultaneously increase the risk of toxicity. Thus, careful balancing of specificity, safety, and long-term persistence is required, and combinatorial approaches involving both CAR protein engineering and T-cell genetic engineering will most likely be necessary to yield superior CAR-T cell products.

ENGINEERING CAR-T CELLS TO OVERCOME THE HYPOXIC AND METABOLICALLY UNFAVORABLE TUMOR ENVIRONMENT

The hypoxic and metabolically challenging nature of the TME is an important driver of T-cell suppression and the immunosuppressive tumor profile. Hypoxia is a well-established hallmark of solid tumors, with far-reaching impacts on tumor biology and the establishment of a protumor niche that is frequently associated with poor survival in various cancer patients (102). Hypoxia-driven metabolic reprogramming of tumor and immune cells drives an immunosuppressive TME characterized by dysfunctional effector immune cells and protumor cell populations that limit immune-cell function via myriad mechanisms (**Figure 4*a***). Excessive nutrient and oxygen uptake by metabolically hyperactive tumor cells fuels a nutrient-deprived environment that favors tumor survival, expansion, and metastases (**Figure 4*b***). At the same time, effective antitumor responses by CAR-T cell therapies require robust T-cell expansion, differentiation, cytokine production, and cytolytic function, all of which are metabolically demanding tasks that are hindered in a hypoxic and nutrient-deprived environment (103, 104). Thus, overcoming this hypoxic and metabolically challenging immunosuppressive tumor environment is imperative to the development of effective next-generation CAR-T cell therapies against solid tumors.

Overcoming Tumor Hypoxia

Within the TME, oxygen demand quickly exceeds oxygen supply owing to the proliferation of cancer cells. Hypoxia-induced transcriptional remodeling in cancer cells induces the upregulation of proangiogenic molecules, such as vascular endothelial growth factors (VEGFs), leading to the growth of irregular, low-integrity vessels that reduce blood flow and hamper oxygen diffusion

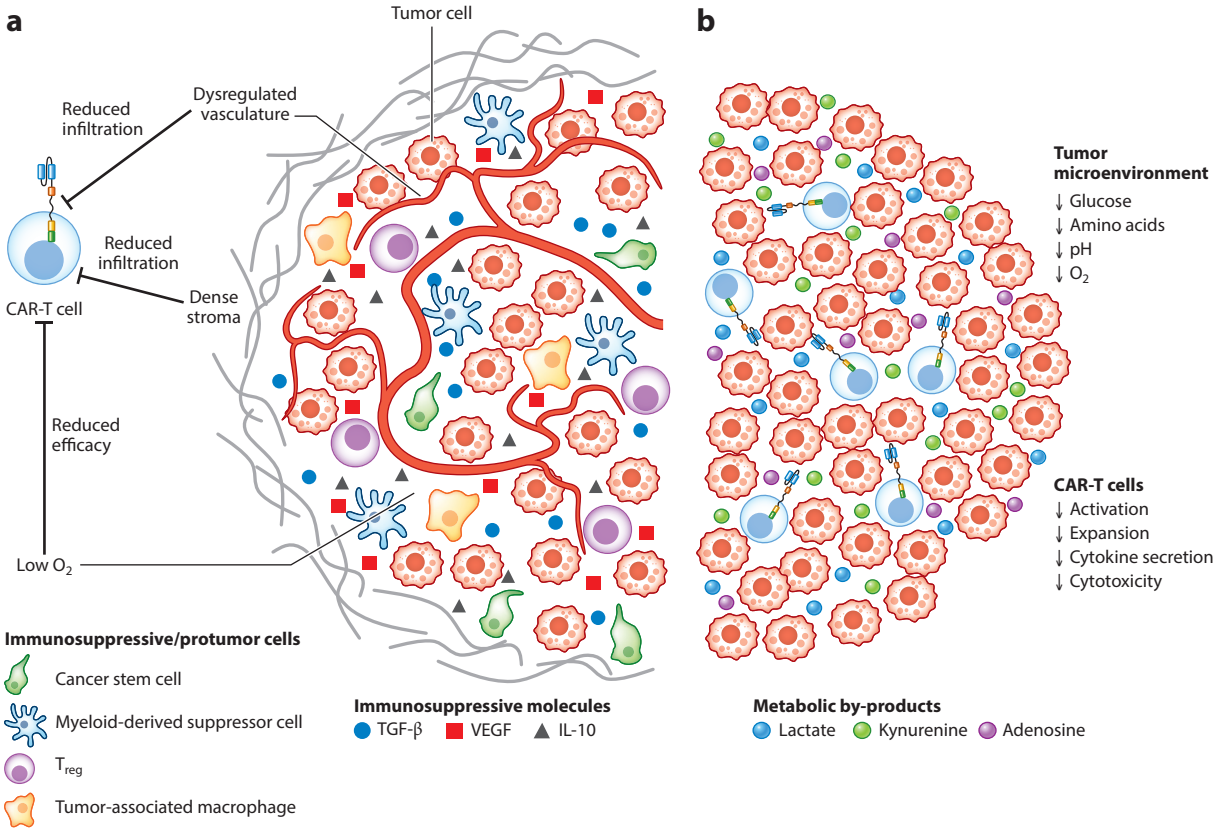

Figure 4

The hypoxic and nutrient-deprived TME. (*a*) Hypoxia-induced transcriptional remodeling in cancer cells induces the upregulation of proangiogenic molecules, such as VEGFs, leading to the growth of irregular, low-integrity vessels that reduce perfusion and hamper oxygen diffusion within the tumor tissue, further exacerbating hypoxia. The dysregulated vasculature with mismatched chemokine receptors and a lack of adhesion molecules limit T-cell infiltration into the tumor. Hypoxia-induced CAFs, comprising most of the tumor stroma, create a dense ECM physical barrier to further limit T-cell infiltration. Additionally, hypoxia can directly impair differentiation, expansion, and cytokine secretion by T cells. Furthermore, hypoxia can rewire and recruit immunosuppressive cell types, and immunosuppressive cytokines produced by these cell types dampen antitumor immune responses. (*b*) Metabolically hyperactive tumor cells deplete oxygen and nutrients within the TME necessary for robust T-cell functionality. Excessive lactate secretion, owing to the hyperactive aerobic glycolysis by tumor cells, drives TME acidification and T-cell anergy. Other metabolic by-products, such as kynurenine and adenosine, secreted into the TME further attenuate T-cell function. Abbreviations: CAF, cancer-associated fibroblast; CAR, chimeric antigen receptor; ECM, extracellular matrix; TGF-β, transforming growth factor-β; T_{reg}, regulatory T cell; VEGF, vascular endothelial growth factor.

within the tumor tissue, further exacerbating hypoxia (105). HIFs are master transcriptional regulators of hypoxic signaling stabilized by lack of oxygen and are frequently overexpressed in tumor cells (106). HIF-1, one of the members of the HIF family, regulates more than 100 genes, many of which are implicated in the selection of more aggressive, metastatic, and chemo- and radiation-resistant tumors, leading to worsened prognosis (107–109). Designing CAR-T cell treatment strategies to directly overcome tumor hypoxia is a difficult task owing to the multifaceted origin of hypoxia and lack of tumor-specific or -associated molecular targets of hypoxia. Thus, to date, engineering efforts have aimed mainly to overcome immunosuppressive aspects of the TME downstream of tumor hypoxia rather than targeting hypoxia itself, and these strategies have been

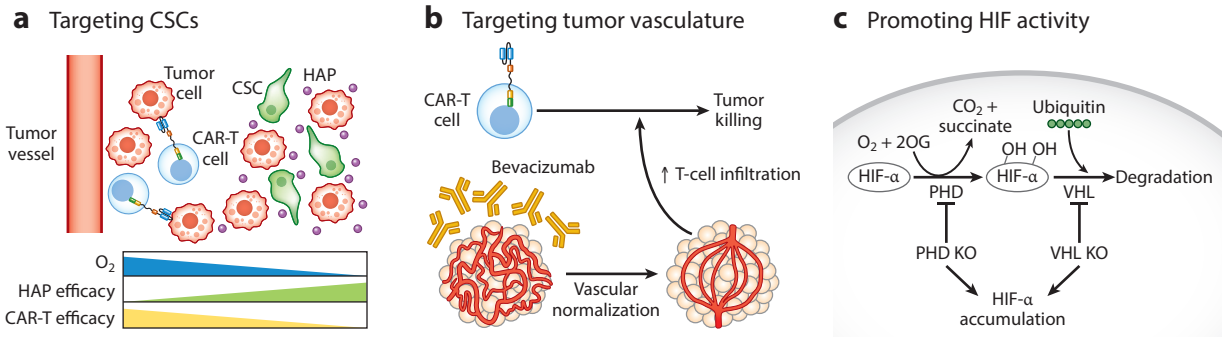

Figure 5

Strategies to overcome tumor hypoxia. (*a*) HAPs can selectively kill tumor cells and CSCs in hypoxic regions of the tumor. Combination therapy with CAR-T cells and HAPs may be promising in eliminating cancer cells in both non-hypoxic and hypoxic tumor regions. (*b*) Antiangiogenic therapies, such as the VEGF-targeting antibody bevacizumab, can induce vascular normalization to transiently restore normal vascular morphology and phenotype within the tumor. Vascular normalization can improve T-cell infiltration into the tumor and antitumor efficacy of CAR-T cells. (*c*) HIF-α subunits are hydroxylated by PHD and become susceptible to ubiquitination by the VHL protein in normoxic conditions, resulting in proteasomal degradation. Genetically engineered T cells with PHD or VHL knocked out have improved HIF-α accumulation and improved fitness and effector functions. Abbreviations: CAR, chimeric antigen receptor; CSC, cancer stem cell; HAP, hypoxia-activated prodrug; HIF, hypoxia-inducible factor; KO, knockout; PHD, prolyl-hydroxylase; VEGF, vascular endothelial growth factor; VHL, Von Hippel–Lindau.

reviewed extensively elsewhere (110, 111). Here, we focus on (*a*) how hypoxia-targeting drugs may be synergistic with adoptive T-cell transfer and (*b*) how CAR-T cell responses to hypoxia can be rewired.

HIF upregulation has been shown to support cancer stem cell (CSC) proliferation and survival in hypoxic environments (112). These CSCs are quiescent, stem-cell-like, and less immunogenic cells that can facilitate immune evasion by secreting immunosuppressive cytokines, recruiting immunosuppressive cells, and downregulating antigens and MHC-I/MHC-II important for endogenous immune recognition and response (113–115). Hypoxia-activated prodrugs (HAPs) can selectively kill tumor cells within the hypoxic regions of the TME, thereby eliminating CSCs (116). TH-302 (evofosfamide) is a nonlymphotoxic HAP that exhibits synergistic antitumor efficacy when combined with ICB therapies. In a preclinical model of prostate cancer, TH-302 and ICB combination therapy resulted in enhanced T-cell infiltration, increased granzyme B and proinflammatory cytokine production, and an attenuated immunosuppressive myeloid stroma (117). TH-302 can further kill both quiescent and actively proliferating cells, stem-like cells, and possibly chemotherapy-resistant cells (118). This evidence suggests a potential combinatorial strategy involving adoptive cell therapy (ACT) and HAPs for improved hypoxia-responsive CAR-T cell treatments (**Figure 5*a***).

Targeting the aberrant tumor vasculature and its associated VEGF pathways offers additional methods to alleviate the hypoxic TME. This vasculature has emerged as a prominent physical barrier that limits T-cell infiltration and contributes to immunosuppression by maintaining the TME's hypoxia, acidosis, and high interstitial fluid pressure (119). Antiangiogenic therapies offer synergistic potential with tumor-targeting therapy by normalizing the tumor vasculature and facilitating transport into the tumor mass (105, 120) (**Figure 5*b***). As an example, Shrimali et al. (121) demonstrated that combining ACT with anti-VEGF therapy increased T-cell infiltration and improved antitumor efficacy in syngeneic murine melanoma models and showed that multiple low doses of anti-VEGF antibody resulted in maximal ACT efficacy. Various studies have suggested that high doses of antiangiogenic drugs, such as the anti-VEGF antibody bevacizumab,

could rapidly decrease blood perfusion and exacerbate hypoxia. In contrast, low, normalizing doses improve perfusion and may enhance immune-cell infiltration to the tumor (122, 123). Optimization of dosing schemes may vary depending on patients or disease status, and better understanding of vessel-normalization mechanisms is needed. Nonetheless, approaches to combine immunotherapy with vascular normalization hold immense promise for improving CAR-T cell therapies.

In addition to altering the TME through antiangiogenic therapy, one could also precondition CAR-T cells in a low-oxygen environment ex vivo, to prepare them for encountering hypoxic environments in vivo. Gropper et al. (124) reported that hypoxic cytotoxic T lymphocytes (CTLs) cultured under 1% oxygen conditions acquire a transcriptomic signature indicative of enhanced glycolytic metabolism. Furthermore, hypoxic CTLs exhibit slower proliferation as compared to the normoxic CTLs cultured in 20% oxygen, acquire a more effector-memory phenotype during in vitro activation and expansion, and display improved in vitro and in vivo tumor rejection (124, 125). Similarly, $CD4^+$ T-cell activation and expansion under hypoxic conditions can enhance the production of cytokines including interferon gamma (126). Accumulating transcriptomic and phenotypic analyses suggest metabolic adaptations triggered by hypoxic priming as an explanation for the salutary effects observed, and more in-depth studies are required to fully elucidate the underlying mechanisms. The observations that intentionally exposing T cells to hypoxia ex vivo could lead to improved T-cell function but that hypoxic TME inhibits T-cell activity point to the highly context-dependent and not yet fully understood nature of hypoxia's effects on T cells. In fact, numerous efforts have been made to leverage the potentially positive effect that HIF-mediated hypoxic response may have on the therapeutic efficacy of ACT.

HIFs are heterodimers composed of alpha and beta subunits. In the presence of oxygen, HIF-α subunits are hydroxylated by prolyl-hydroxylases and subsequently ubiquitinated by the Von Hippel–Lindau (VHL) protein, resulting in proteasomal degradation and impaired HIF signaling (127). Factor-inhibiting HIF serves as an additional oxygen-sensitive regulator of HIF by hydroxylating a conserved asparagine residue in HIF-α subunits to inhibit HIF transcriptional activity (128). Consequently, deletion of *Vhl* or genes encoding the three prolyl-hydroxylase isoforms in T cells has been shown to increase intracellular HIF accumulation, which was associated with increased cytotoxic differentiation, delayed terminal differentiation, and improved tumor rejection in syngeneic murine models (129, 130) (**Figure 5*c***). *Vhl* deletion and elevated HIF activity in $CD8^+$ T cells were also associated with augmented glycolytic metabolism, resulting in improved activation and effector functions (129). Ectopic expression of HIF elements has also been explored as a method to enhance HIF signaling and antitumor T-cell responses. Veliça et al. (131) report that ectopic expression of HIF-2α in $CD8^+$ T cells drove broad transcriptional changes, including enhanced expression of chemokines associated with T-cell trafficking (e.g., CXCR4), costimulatory molecules (e.g., 4–1BB and OX40), and cytotoxic proteins (e.g., perforin and granzyme B). A mutated form of ectopic HIF-2α that inhibits factor-inhibiting HIF association with HIF-2α gives rise to an even more effective transgenic T-cell product with improved antitumor responses against a xenograft lymphoma model (131). Despite these results, conflicting evidence on the roles of HIF subunits in T-cell function and inconclusive mechanistic explanations to the observed transcriptional and phenotypic changes highlight the need for additional investigations into the HIF pathways before clinically relevant strategies can take form.

Rewiring T-Cell Metabolism to Overcome the Metabolically Unfavorable TME

An emerging hallmark of cancer is the metabolic reprogramming required to sustain energy metabolism essential to their uncontrolled cellular proliferation and growth (132). Central to this metabolic switch is the Warburg effect: a phenomenon in which cancer cells generate energy primarily through aerobic glycolysis instead of the more energetically efficient mitochondrial

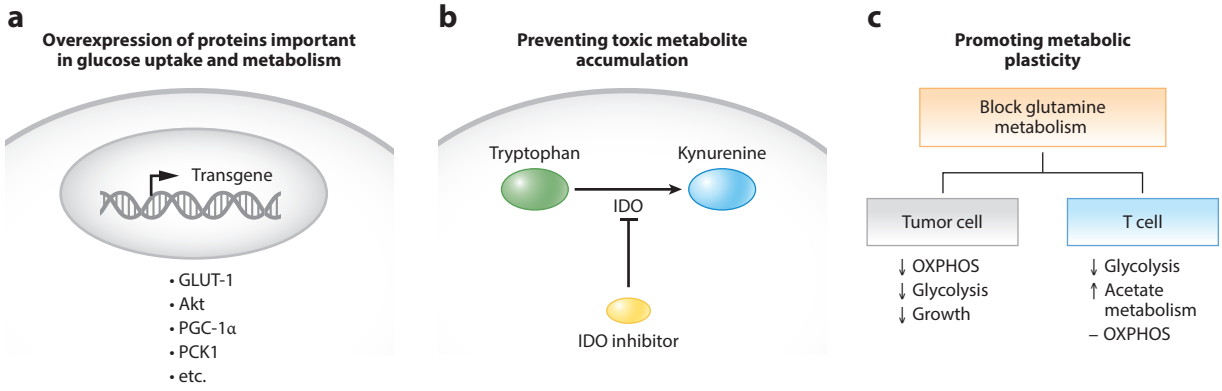

Figure 6

Strategies to overcome the nutrient-deficient TME. (*a*) Transgenic overexpression of proteins important in glucose metabolism can enhance glucose uptake and energy metabolism within T cells, thus allowing T cells to more efficiently scavenge and use scarce glucose within the TME to drive effector functions. (*b*) Amino-acid metabolism can be rewired by using small-molecule inhibitors (e.g., IDO inhibitors) to prevent the accumulation of metabolites (e.g., kynurenine) known to suppress T-cell proliferation and cytotoxic function. (*c*) Blockage of glutamine metabolism can take advantage of T-cell-intrinsic metabolic plasticity to enable selective augmentation or maintenance of T-cell metabolism while negatively regulating tumor metabolism. Abbreviations: GLUT-1, glucose transporter 1; IDO, indoleamine 2,3-dioxygenase; OXPHOS, oxidative phosphorylation; PCK1, phosphoenolpyruvate carboxykinase 1; PGC-1α, proliferator-activated receptor gamma coactivator 1-alpha; TME, tumor microenvironment.

oxidative phosphorylation (OXPHOS) (133). Tumor cells' high glycolytic activity generates a nutrient-deficient TME, in which tumors outcompete immune cells in satisfying their metabolic needs. Given the critical role of metabolism in both tumor and T-cell biology, there is growing interest in metabolic strategies to improve the antitumor efficacy of CAR-T cell therapy. Here, we focus on how desirable T-cell phenotypes can be achieved via manipulation of the three main pillars of T-cell metabolism: glucose metabolism, amino-acid metabolism, and lipid-/fatty-acid metabolism.

Glucose metabolism is one of the main ways in which cells extract energy, and T-cell activation, proliferation, and effector function/differentiation all hinge on efficient glucose metabolism. Importantly, glucose usage via glycolysis versus OXPHOS is a marker of T-cell fate, with long-lived memory T cells relying more on OXPHOS and effector T cells relying more on glycolysis to support their highly anabolic metabolic signature (104). Therefore, glucose-metabolism pathways offer multiple nodes by which T-cell biology and function could be modulated (**Figure 6*a***). Upon T-cell activation, phosphoinositide 3-kinase (PI3K)/Akt signaling is engaged, resulting in downstream mTOR (mammalian target of rapamycin) signaling and upregulation of glucose transporter 1, thus promoting glucose uptake (134). Transgenic and constitutive overexpression of glucose transporter 1 and myristoylated Akt proteins synergistically increases glucose uptake and enhances T-cell activation (135). Furthermore, the AMP-activated protein kinase (AMPK) inhibits mTOR activity to dampen anabolism and promote energy metabolism during metabolically stressful conditions. Accordingly, AMPKα1-knockout T cells have reduced mitochondrial bioenergetics, and AMPKα1 has been shown to be important in creating durable antitumor T-cell responses in vivo (136). Likewise, overexpression of proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) can favor mitochondrial biogenesis and the formation of central memory T cells for improved mitochondrial activity and robust responses to tumor rechallenges (137). Glycolytic activity of T cells can be similarly augmented by overexpressing phosphoenolpyruvate carboxykinase 1, which increases phosphoenolpyruvate production to sustain NFAT (nuclear factor of activated T cells) signaling and effector functions (138).

Interestingly, the expression of a CAR molecule can also alter glucose metabolism in engineered T cells, and the specific CAR protein sequence alters the metabolic impact of CAR expression (139). Kawalekar et al. (140) reported that anti-CD19 and mesothelin CARs containing the 4–1BB costimulatory domain promoted the differentiation of central memory T cells with enhanced respiratory capacity, mitochondrial biogenesis, and associated oxidative metabolism. In contrast, the CD28 domain promoted the differentiation of effector memory T cells with enhanced glycolytic metabolism, albeit with reduced oxidative metabolism (140). Ex vivo CAR-T cell expansion presents unique opportunities for metabolic reprogramming as well. Low-dose IL-2 environments during CAR-T cell expansion have been associated with reduced terminal differentiation and enhanced generation of memory phenotypes with increased potential for persistence and antitumor functions (141, 142). Similarly, culturing T cells with IL-15 has been shown to improve mitochondrial fitness and prevent terminal differentiation (143). Direct manipulation of nutrient availability during ex vivo expansion can also be used to enhance antitumor efficacy of ACT products. For example, Sukumar et al. (144) reported that inhibition of glycolysis via glucose deprivation preferentially shifts T-cell metabolism toward mitochondrial OXPHOS, resulting in more memory-like T-cell phenotypes with enhanced tumor-homing and tumor-suppressive abilities.

In addition to glucose metabolism, targeting amino-acid metabolism can be equally effective in rewiring T-cell metabolism for improved effector functions. Kynurenine, a metabolite generated from indoleamine 2,3-dioxygenase (IDO)-mediated catabolism of tryptophan, directly suppresses the proliferative and cytolytic ability of effector T cells, while simultaneously inducing regulatory T cell (T_{reg}) differentiation (145, 146). IDO inhibitors have been shown to positively modulate T-cell proliferation and interferon gamma secretion and relieve tryptophan-mediated suppression of cytotoxic T cells (147, 148) (**Figure 6*b***). Hence, direct modulation of this metabolic network by CAR-T cells remains an interesting possibility. Arginine is often severely depleted within the TME, and ex vivo T-cell expansion with arginine-enriched media has been shown to cause a shift from glycolysis to OXPHOS in unison with differentiation of central-memory T cells with enhanced survival and antitumor activity (149). Cancer cells and surrounding protumorigenic cell populations rely on arginase for arginine catabolism; thus, arginase inhibitors are under clinical evaluation and could be a strategy used prior to adoptive transfer of CAR-T cells to increase the arginine availability within the TME (150).

Glutamine is similarly depleted within the TME, and glutamine antagonists—notably DON (6-diazo-5-oxo-L-norleucine)—can rescue intratumoral glutamine levels and are associated with improved antitumor efficacy of T cells (150, 151). Moreover, Leone et al. (152) have demonstrated that a prodrug form of DON, JHU083, can inhibit glutamine metabolism to effectively shut down glycolysis and OXPHOS to blunt tumor growth. JHU083 also inhibits glycolysis in endogenous T cells, but interestingly, JHU083-treated T cells exhibit substantially greater metabolic plasticity and respond to glycolysis inhibition by increasing mitochondrial capacity and upregulating acetate metabolism to continually fuel OXPHOS, resulting in differentiation into long-lived memory-like phenotypes. Next-generation CAR-T cell therapies that selectively exploit such differences in metabolic plasticity may be an exciting area for future engineering efforts (**Figure 6*c***). Moreover, metabolically rewiring CAR-T cells based on the metabolic phenotype and plasticity of the target tumor may become important in engineering effective CAR-T cell therapies against solid tumors.

Targeting lipid, specifically fatty acid, metabolism is another area of interest, as cancer cells upregulate fatty acid synthesis and the accumulation of lipid-associated metabolites have broad implications on the immunosuppressive TME. On the one hand, immunosuppressive T_{reg}s rely heavily on fatty-acid oxidation (FAO) owing to high expression of AMPK compared to other T-cell subsets, and lipid accumulation within the TME can favor T_{reg} survival and differentiation (153). On the other hand, FAO is critical for memory T-cell generation, and evidence suggests

that sustained FAO after immune activation is necessary to develop long-term protection against the malignancy (104). As such, modulation of fatty-acid metabolism is likely to have significant impacts on the antitumor efficacy of T-cell therapies, but it must be done in a well-calibrated manner to achieve the desired outcome. FAO can be targeted via the PI3K/Akt pathway, similar to glucose metabolism, and pharmaceutical inhibition of Akt during ex vivo T-cell expansion has been shown to promote FAO metabolism and memory T-cell differentiation with enhanced persistence and cytokine secretion (154, 155). Pharmaceutical agonists of PGC-1α/peroxisome proliferator-activated receptor complexes can also improve CTL FAO and mitochondrial fitness. This strategy has been shown to block CTL apoptosis and enhance mitochondrial activity, allowing for better CTL survival and tumor rejection when combined with ICB therapies, which could be promising in combination with CAR-T cell therapies (156).

CONCLUSIONS

By using CAR-T cells with redirected cytotoxicity against malignant cells, ACT has reinvigorated the field of cancer immunotherapy and has already demonstrated remarkable efficacy against B-cell malignancies. Despite these clinical successes, CAR-T cell therapy has had limited success against solid tumors, which comprise the vast majority of cancers. This review discussed various protein, cell, and metabolic engineering approaches to minimize toxicity, increase antitumor efficacy, and overcome challenges such as hypoxia and high metabolic activity that severely limit the fitness and efficacy of CAR-T cells within the TME. Multidimensional approaches that bridge biomolecular engineering with immunology fundamentals will be required to develop next-generation CAR-T cell therapies that are safe and effective against a wide range of diseases still awaiting effective treatment options.

Despite recent advances in CAR-T cell engineering, much is still unknown about the complex metabolic network within T cells, metabolic interactions within the TME, and the multifaceted effects of hypoxia. Advancements in single-cell analytical technologies and high-dimensional proteomic and metabolic phenotyping have enabled better understanding of the intricate interplay among different cell types within the TME, and further investigation will facilitate rational engineering of CAR-T cells that can better sustain metabolic needs and resist hypoxia-mediated immunosuppressive mechanisms within the TME. Altogether, a holistic approach encompassing multifaceted CAR-T cell engineering and exploration of the complex interactions within the TME will drive synergistic advancements in the fight against solid tumors.

DISCLOSURE STATEMENT

Y.Y.C. reports patents, consulting activities, and equity holdings related to CAR-T cell technologies.

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