The Emerging Role of Engineering Immune Cells in Cancer Treatments

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Abstract. Cell-based immunotherapy has become one of the forefronts of cancer treatments and led to significant clinical success in multiple refractory/recurrent hematological malignancies. Compared with other conventional treatment approaches, engineered immune cells are considered "living factories" that are able to continually produce anti-tumor factors and have the potential to mediate long-lasting therapeutic benefits following a single application. The intrinsic ability to expand and respond in portion to needs encompasses this technology a greater and more transformative potential to enable a more effective anti-tumor response with less off-target toxicity. Nevertheless, there are still some significant barriers to successfully applying cell-based therapy to treat solid tumors. Five main challenges include restricted trafficking and infiltration, antigen escape and heterogeneity, suboptimal persistence, immunosuppressive tumor microenvironment (TME), and potentially severe side effects and immune-related toxicities. The technological advancement of various biomolecular tools and genetic engineering strategies provides exciting opportunities to address these limitations. In addition, combination therapy that incorporates other treatment modalities within the treatment regimen of cell-based strategy also creates therapeutic synergies that can greatly improve the clinical success of the therapy. This review introduces current observed challenges in treating cancers, with an emphasis on solid malignancies, and discusses some potential engineering solutions that have shown promising results in recent preclinical studies.

Keywords: Chimeric antigen receptor, solid tumor, engineered immune cells, cell-based immunotherapy.

1. Introduction

Cancer is a disease characterized by the uncontrolled proliferation and expansion of abnormal cells. It is the second leading contributor to the disease burden worldwide, resulting in 23.6 million cancer incidences and 10 million deaths in 2019 alone [1]. By 2035, it is estimated that one-quarter of the global population will be directly affected by cancers. Despite the pandemic, cancer mortality has shown a trend of decline in 2020, contributing to a 33% overall reduction since 1991 [1]. The gradual decline reflects the technological advances in cancer treatments. Immunotherapy has significantly revolutionized the field of cancer management and treatments in the past decades. Unlike traditional cancer treatments such as radiotherapy and chemotherapy, immunotherapy represents an innovative paradigm in dynamically modulating the immune system and immune microenvironment to favor anti-tumor responses. Various methods have been developed, including targeted antibodies, immunomodulators, cancer vaccines, oncolytic virus therapy, and adoptive cell transfers, to exploit the intrinsic characteristics of different immune components and potentially eliminate cancers in a specific, adaptive, and durable fashion. In particular, adoptive cell transfers that are involved the use of either native or engineered immune cells have shown great promise in treating or even curing certain types of cancers, such as those with hematological lineages, including lymphomas, some forms of leukemia, and several myelomas. In addition, advancement in molecular and synthetic biology and innovation in different genetic engineering approaches further augment the potential of utilizing engineered immune cells for treating cancers by improving their potency, safety, and applicability. Introduction of multiple concurrent genetic circuits (accessory genes) to confer additional functions and genetic modifications to modulate the immune cell signaling pathways to ensure effective and safer killing actions are currently emerging as the main trend for improving cell-based immunotherapy. Many of the genetic engineering approaches have been focused on

improvement based on conventional chimeric antigen receptor (CAR), which is an artificial cell surface receptor that is antigen-specific and can redirect the engineered immune cells to carry out the direct killing of the target cell that is expressing the target antigen. It is generally comprised of four domains, including an antigen recognition domain, a hinge region, a transmembrane domain, and an intracellular signaling domain, and modification of target specificity and intracellular pathways that modulate effector functions of engineered immune cells have led to promising clinical outcomes in several clinical trials [2]. In this paper, multiple challenges in solid tumors will be further elaborated, and specific immune cell-based engineering approaches will be introduced and discussed in detail.

2. Solid Tumor Challenges

Despite the clinical success in treating hematological cancers and a few other solid tumors with limited efficacy, only a minority of terminally-ill patients with solid malignancies have experienced life-altering and durable responses and benefit from immune cell-based therapies. The tumor microenvironment (TME), especially in the case of solid tumors, represents a highly complex and dynamic collection of infiltrating and resident cells, blood vessels, and a variety of non-cellular components, including cytokines, chemokines, growth factors, and other signaling molecules, and extracellular matrix. While the accumulation of genetic or epigenetic aberrations caused by either intrinsic or extrinsic factors is thought to be primarily responsible for cancer initiation and formation, tumor progression is further perpetuated and advanced through the constant interactions between the neoplastic and adjacent stromal cells. The constant crosstalk, dominated by the tumor, contributes to the complexity and plasticity of TME, leading to the continuous evolution and conditioning of the local environment that can further support tumor survival, proliferation, and metastasis. An intertwining network of inflammatory mediators, growth factors, proteolytic enzymes, and particularly, both cytokines and chemokines form the basis of intercellular communication, along with other cellular and molecular mechanisms, such as the release of cancer exosomes, apoptotic bodies, and cell-free DNA (cfDNA), that are currently emerging [3].

Several challenges, including insufficient intratumoral infiltrations, poor cell survival and persistence, antigen escapes, immunosuppression, and potential on-target off-tumor toxicity, are still predominately hindering the therapeutic translation of immune cell-based therapy in treating solid cancers. One leading factor contributing to the roadblock is the inter- and intra-tumor heterogeneity that can lead to clinical outcomes being highly dependent on the types of cancer and individual genetic makeup. This genetic heterogeneity diversifies the cellular and metabolic pathways of solid tumors and can contribute to tumor resistance through a process called immunoediting. Immunoediting is a common mechanism that describes a dynamic interaction between the cancer cells and the immune system, which can constantly shape the immunogenicity of the cancer cells and ultimately lead to the potential emergence of immune-resistance mutants. In addition, the immune selections resulting from the immunoediting can also lead to antigen escape and downregulation, largely limiting the specificity and efficacy for recognizing solid tumor targets of many current immune cell-based approaches. In addition, multiple mechanisms, including negative feedback loops, induced apoptosis, checkpoints inhibition, and polarization and recruitment of immunosuppressive cells, have been utilized by solids cancers to immunologically suppress the cellular activities and anti-tumor responses of engineered immune cells. The on-target off-tumor of most of the current cell-based therapies also manifests as a significant challenge for impeding their general applications and dose-dependent efficacy in different cancer patients. Therefore, there is an urgent need to develop effective immunotherapeutic approaches that are safer, more reliable, and more durable.

3. Engineered Immune Cell Approaches

The highly heterogeneous and dynamic features of solid tumors complicate the regimen and outcomes of various conventional cancer treatments, and cell-based immunotherapy has been heavily

exploited in recent years. The concept of using a living cell as therapeutics that is capable of intelligent sensing, self-adapting, and specifically responsive is what makes this strategy ideal for treating cancers that are also continually evolving in response to the environment. Several engineering approaches have been currently devoted to improving the efficacy and specificity of immune-cells based therapies to be able to achieve a more durable response and lead to better overall survival of patients (Figure 1).

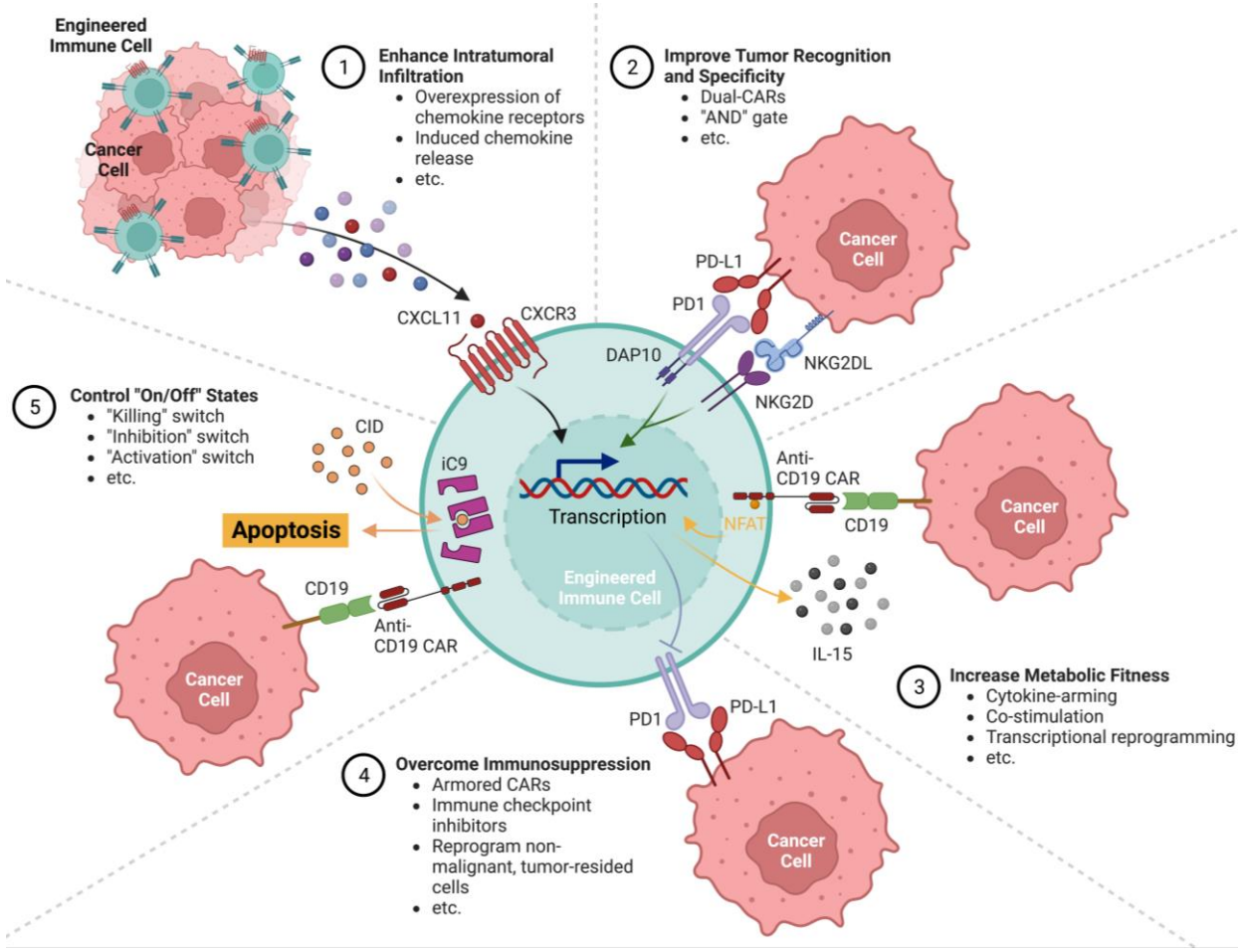

Figure 1. Examples of Engineering Approaches to Enhance Cell-based Immunotherapy

3.1. Strategies to Enhance Intratumoral Infiltration

Cytokines are small proteins (6-70 kDa) that can secrete by tumor, stromal, and immune cells to trigger various intracellular signals, including growth, proliferation, apoptosis, and differentiation, upon binding to their cognate receptors [4]. Chemokine is a subcategory of cytokine that mainly involves providing chemotactic signals to mediate the trafficking of immune cells into the tumor site. In the context of TME, the presence of cytokine and chemokines, collectively produced by tumor cells, stromal cells, and infiltrating immune cells, often facilitate invasion and metastasis of cancer cells yet limit the pro-inflammatory leukocyte extravasation to exert anti-tumor responses. Since chemokine dictates the types of migratory immune cells and their immune response to a great extent, its diverse profile, as a direct result of heterogeneous cellular composition, creates a significant obstacle for sufficient immune infiltration due to the mismatch or downregulation of migratory signaling elements or chemokines/chemokine receptors axes. A study using prostate specific membrane antigen (PSMA)-specific CAR-T cells to treat prostate cancer cells with ectopically expressed PSMA had shown less than 0.2% of the transferred T cells were successfully homed to the tumor, and the majority of the CAR-T cells were distributed into other distal organs, such as thyroid, salivary glands, stomach, and bladder [5]. As a result, engineered immune cells with transgenic

chemokines receptors provide a unique opportunity to reduce on-target off-tumor toxicity in the periphery through the enhanced directed-trafficking capacity.

For example, CD70-specific CAR-T cells modified with interleukin-8 (IL-8) chemokine receptors (CXCR1 or CXCR2) had shown enhanced migration and persistence in a glioblastoma xenograft model compared to the non-modified counterpart, resulting complete tumor regression and immunologic memory upon tumor rechallenges [6]. Similarly, co-expression of CXCR1 chemokine receptor on NKG2D-specific CAR-NKs enhanced their migration capacity *in vitro*, and infusion of NKG2D-CAR+ CXCR1+ NK cells prolonged the median survival rate by 20% as compared to the control without transgenic CXCR1 [7]. Alternatively, researchers from one study had demonstrated the feasibility of reprogramming the TME of glioblastoma (GBM), a highly aggressive brain tumor, using oncolytic adenovirus (oAd) with transgenic CXCL11 (a chemotaxis-stimulating factor). The engineered CXCL11-armed oAd exploits a unique opportunity for specific targeting by utilizing a specific promoter of human telomerase reverse transcriptase (hTERT), which is reactivated during carcinogenesis, limiting the virus proliferation in healthy tissue [8]. Intratumoral administration of CXCL11-armed oAd alters the unfavorable chemokine gradient within TME, increasing the CAR-T cell trafficking into the tumor as well as a higher level of infiltrated NK cells and M1-phenotype macrophages. The strategy of using CXCL11-armed oAd to enhance the chemotactic signal for directing CAR-T cell trafficking induced a better anti-tumor activity and enhanced survival in GL261 xenograft mouse models compared to either oAd or CAR-T mono-treatment alone [8]. As a result, directed trafficking of engineered immune cells by enhancing the intercellular communication through anti-tumor cytokine/chemokine signaling pathways presents a promising strategy to improve the therapeutic outcome; not only does it enhance penetration of the engineered immune cells into the tumor cores, but also it limits their non-specific dissemination in the non-target tissues where on-target off-tumor toxicity usually occur.

3.2. Strategies to Improve Tumor Recognition and Specificity

Another essential aspect of ensuring the clinical success and optimal patient prognosis of cell-based therapy is the selection of an “ideal” antigen that is homogeneously and highly expressed on the tumor but only minimally present or absent from healthy and normal tissue. Antigen escape and downregulation have been represented as one of the major limiting factors for effective therapeutic response. As reported from several clinical trials using CD19-specific CAR-T therapy in treating relapsed and/or refractory acute lymphoblastic leukemia (ALL) patients, although 70 to 90% of ALL patients have shown initial completed responses, 30 to 70% of patients who have a recurrent disease developed CD19-negative or partially negative relapses that are highly resistant to the original treatment [9]. Although utilization of specific tumor-associated antigens (TAAs) or neoantigens have been heavily exploited in recent years, such as mesothelin, MUC1, and PSMA, the risk of potential on-target off-tumor toxicity resulted from a lack of tumor antigen specificity due to the lower expression of the targeted antigen on other tissues is another major obstacle, limiting the therapeutic translation and clinical success. In addition, many other neoantigens described so far are intracellular origin, which can render certain cell-based therapy, such as CAR-T, unrecognizable of or inaccessible for the tumor targets [10]. Furthermore, compared to hematological malignancies, solid tumors are often characterized by a higher heterogeneity, and tumor cells with lower antigen density can escape immune cell-mediated effector cytotoxicity, further hindering the therapeutic effect of single-target immune cell therapy and contributing to tumor recurrence. Moreover, patient heterogenicity can further complicate the therapeutic regimen. As a result, several engineering approaches have been exploited to increase tumor recognition and specificity, including modulation of antigen-binding affinity, a combination of multiple antigen specificity, and localized activation restricted to tumor tissues.

For example, in one study, researchers have shown CAR-T with ICAM-1-specific binding to a micromolar range (~10 μM) exhibited a better therapeutic index *in vivo* compared to those in a nanomolar range (1-100 nM) [11]. Despite ICAM-1 is a cell adhesion molecule upregulated in several

carcinomas, it is constitutively expressed in other normal cell types, such as endothelial cells and immune cells [11]. Modification of the affinity of CARs is critical to ensure appropriate binding kinetics to the cognate ligand; not only can it prevent the hyperactivation that might lead to early T cell exhaustion, but also it can limit the potential, unbiased on-target off-tumor reactivity. Furthermore, as demonstrated in another study, a dual-CAR NK construct that combines an NKG2D receptor (targets NKG2DL that is commonly expressed on tumor cells) and a PD-1/DAP10 switch-receptor (composed of an inhibitory ligand binding domain and a stimulatory signaling domain) also present as a promising approach for enhanced selective killing of gastric tumors without noticeable toxic side effect on healthy cells [12]. As a result, the dual targeting strategy can vastly improve the therapeutic index of CAR-NK cells by limiting the potential side effects through enhanced specificity. In addition, the ability to temporally and spatially limit the immune cell activity to the tumor sites also enables a more target-specific therapy to further reduce the potential side effects and toxicity. A recent study has demonstrated CAR-T cells with a logic "AND" gate-like function (LINK CAR) can only be activated when simultaneously encountering two antigens through combined LAT- and SLP-76-mediated proximal intracellular signaling [13]. LINK CAR comprises dual targeting CARs that are separately linked to a LAT and an SLP76 molecule (signal-transducing adaptor proteins), of which phosphorylation of both molecules by ZAP-70 protein is required for T cell activation. Outperformed other recently developed systems, such as synNotch receptor, LINK CAR that is paired with LAT and SLP-76 is able to eliminate malignant cells only but spare the normal counterpart without evidence of off-target toxicity to a greater extent [13]. Therefore, enhanced tumor recognition and specificity through various engineered approaches can further augment the anti-tumor response exhibited by the engineered immune cell and, at the same time, reduce the potential toxicity that has been observed in current cell-based therapies.

3.3. Strategies to Increase Metabolic Fitness

The ability of engineered immune cells to survive, persist, and further expand in adequate numbers after infusion into patients is another important factor in dictating therapeutic outcomes and long-term remission. Functional activities of the immune cells can either be suppressed or reprogrammed to favor the tumor progression and metastasis, thus further hampering the anti-tumor responses. Diverse molecular, cellular, biochemical, and physical changes are stimulated and constantly modulated by the immunosuppressive TME, and the resulting heterogeneity is further diversified among different tumor types, actively contributing to tumor differentiation, dissemination, metastasis, and immune escape. Immune cell exhaustion is one of the major hallmarks, and the immune cells can no longer functional in response to cancer cells and cannot control disease progression or even contribute to the advancement. The persistent antigenic and inflammatory stimulation during chronic infections and cancer development is thought to be the primary cause of T-cells entering the state of exhaustion [14]. Although there is still an ongoing debate on the correlation between immunological exhaustion and general dysfunction as well as biological characteristics, distinct metabolic, transcriptional, and epigenetic changes are often observed in the case of exhausted immune cells [14]. For example, mitochondrial dysfunctions have been frequently observed in exhausted T cells after persistence stimulation, and mitochondrial stresses induced by the harsh TME conditions, such as hypoxia, can potentiate the altered differentiation, such as upregulation of transcriptional repressor BLIMP1 that can interact with a transcriptional coactivator PGC1α responsible for maintaining T cell bioenergetics, toward T cells exhaustion [15]. Furthermore, engineered immune cells like CAR-T and CAR-NK possess limited capacity to maintain activation and immune activity when recursively exposed to tumor cells. As a result, several strategies have been proposed to augment metabolic fitness and increase the survival and persistence of engineered immune cells, including transgenic expression of stimulatory cytokines, the addition of co-stimulatory signals, and the modulation of transcriptional factors.

TRUCK stands for T cell redirected for universal cytokine-mediated killing and represents an engineering strategy that was originally utilized in CAR-T cells to enhance the persistency and anti-

tumor activity by using a nuclear factor of activated T cell (NFAT) transcription factor to enable the expression of early immune response-related proteins and cytokines [16]. Similar to other TRUCK CAR-T constructs, CAR-NK utilized the TURCK strategy has also shown promising results. As shown in one study, a group of researchers has designed an iC9/CAR19/IL-15 CAR-NK cell that expresses an anti-CD19 CAR and can ectopically produce IL-15 along with a caspase-9 (iC9) suicide gene that can be pharmacologically induced to trigger cell apoptosis. As suggested by the result, the TRUCK CAR-NK cells exhibit an enhanced eradication of primary chronic lymphocytic leukemia (CLL) cells compared to non-engineered NK cells [17]. Moreover, providing an additional co-stimulatory signal can also enhance the intracellular signals that augment the functional behavior of engineered immune cells, leading to an improved anti-tumor response. As shown in one study, the researchers have shown incorporation of 41BB ligand onto CAR-T with a CAR-specificity against B7-H3, a transmembrane glycoprotein that is overexpressed in solid tumors, significantly enhanced the effector functions and had led to an improved long term survival rate for most treated mice compared to other similar CAR-T constructs [18]. Transcription reprogramming is one of the emerging approaches that has been shown to enhance engineered immune cell functions by increasing the proliferation rates and metabolic activities that regulate early T cell activation. For example, in one study, the deletion of *MED12* (mediator complex subunit 12) or *CCNC* (cyclin C), which are subunits of Mediator that is responsible for linking RNA polymerase II to enhancer-bound transcription factors that are shown to limit T-cell functions, in CAR-T cells exhibit a better killing capacity toward both leukemia and osteosarcoma tumor models [19]. In conclusion, strategies to improve T cell fitness, including survival, persistence, and activity, can serve as a promising alternative to overcome therapeutic limitations of engineered immune cells caused by several intrinsic factors, such as exhaustion, further improving their therapeutic translation in a clinical setting.

3.4. Strategies to Overcome Immunosuppression

The immunosuppressive characteristics of TME that are contributed by both cellular and soluble components significantly contribute to the roadblock of engineered immune cell-based approach in the current therapeutic landscape. Several major cellular components, including tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs), myeloid-derived suppressor cells (MDSCs), tumor-associated dendritic cells (tDCs), tumor-associated fibroblast (TAFs), and regulatory T cells (Tregs), can suppress the effector functions of engineered immune cells via direct cell-to-cell contacts or secretion of inhibitory mediators [20, 21]. In addition, tumor and other tumor-related cells in the TME can release immunomodulatory molecules, such as vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF-β), and pro-inflammatory cytokines like interleukin-10 (IL-10) and interleukin-27 (IL-27), to further augment the pro-tumor responses through several mechanisms [21]. These include induction of immune cell apoptosis, activation of immune checkpoints signaling, and recruitment of additional immunosuppressive cell types. Furthermore, hostile metabolic byproducts, hypoxia, and nutrition depletion can also lead to metabolic arrests or malfunctions of both native and engineered immune cells. For example, the presence of excessive lactic acid resulting from tumor cell metabolism has been shown to impair functions of immune cells like T and NK cells, hampering their ability to upregulate the NFAT signal and thus reducing the production of NFAT-regulated pro-inflammatory cytokines like interleukin-2 (IL-2) and interferon-gamma (IFN-γ), respectively [21].

Immune checkpoint upregulation is, perhaps, one of the most well-known immunosuppressive strategies utilized by cancer cells to suppress immune activity and circumvent the anti-tumor efficacy of most immune cells. Immune checkpoints consist of both inhibitory and stimulatory regulators that help maintain self-tolerance to prevent autoimmune attach and assist with immune responses. These pathways have often been hijacked by the tumor cells to prevent the activation of nascent anti-tumor responses, and some of the commonly hijacked pathways include PD-1, CTLA-4, LAG3, TIM3, TIGIT, and BTLA [22]. Therefore, strategies, including suppression of immune checkpoint pathways and reprogramming of non-malignant cells in the TME, that can counteract the immunosuppressive

microenvironment or signaling provide an additional route to augment the immunological response of the engineered immune cells. As shown in one study, researchers have demonstrated the potential of utilizing armored CAR-NK as another strategy, which involves genetic modification to suppress a specific immune checkpoint by exploiting the intrinsic function of an immune checkpoint switch receptor. Armored CAR-NK can increase the function and penetration capacity of the CAR-NKs by abrogating the immune inhibitory signals. As demonstrated by another subsequent study, genetically knockout of a cytokine-inducible Src homology 2–containing protein (CIS), which can suppress downstream cytokine signaling pathways, in iC9/CAR19/IL-15 CAR-NK cells exhibit an enhanced percent survival and even eradication of lymphoma across the treated xenograft mice model without observed systematic toxicity [23]. Similarly, PD-L1 is a potent immune checkpoint inhibitor that can prevent activation of immune effector functions when binding to the associated receptor PD-1 often expressed on immune cells. It is one of the most researched immune checkpoints and has led to several clinical-approved approaches in treating cancers. As a result, many attempts, such as immune checkpoint inhibitor co-administration, have been carried out to augment the therapeutic index of cell-based therapy. As shown in one study, CRISPR/Casa9-mediated disruption of PD-1/PD-L1 immune checkpoint signaling in EGFRvIII-specific CAR-T (EvCAR-T) cells have been shown to improve the anti-tumor activity against glioblastoma (GBM) [24]. Another promising approach that is also emerging is to reprogram non-malignant cells residing at the TME. Several cells, such as MDSCs, TAMs, and Tregs, that are present in the tumor stroma, have played a huge role in contributing to the cancer progression and metastasis by maintaining the immunosuppressive environment and creating tumor-associated ECM that can further limit immune cell infiltration. As a result, several studies have focused on reprogramming the non-malignant cells to exhibit anti-tumor behavior [16]. In one particular study, researchers have demonstrated the feasibility of selectively targeting M2 macrophages, the immunosuppressive subtype of TAMs, by recognizing folate receptor β (FRβ), which is upregulated in M2-polarized TAMs [25]. Folate-coated liposomes can specifically target the M2-polarized TAMs and deliver a BIM-S-containing plasmid (pBIM) that expresses a protein isoform of BCL-2-interacting mediator of cell, which can potently induce apoptosis in the transformed cells. Selective elimination of the M2-polarized TAMs has significantly slowed down the tumor growth in a lung cancer cell line A549 xenograft nude mouse model. Therefore, similar strategies in enhancing the anti-tumor effects of engineered immune cells through overcoming either cellular or molecular immunosuppressive pathways can improve the chance of therapeutic success in treating cancers.

3.5. Strategies to Control "On/Off" States

Strategies that are aimed to improve the immunologic functionality and anti-tumor activity, as described in previous sections, do not account for the potential tradeoff between the response and toxicities. Several critical adverse events have been associated with immune cell-based therapies, such as CAR- and TCR-T cells, including hemophagocytic lymph histiocytosis (HLH), macrophages activation syndrome (MAS), cytokine-release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), and possibly graft-versus-host-disease (GVHD), if allogeneic cell sources are used [26]. In particular, CRS leads to massive production of cytokines resulting from the over-activation of engineered immune cells, which can form an immunological, positive feedback loop for a constant pro-inflammatory response, while HLH and/or MAS result in dysregulated activation and accumulation of NK cells, CD8+ T-cells, and macrophages, leading to hem phagocytosis and organ-damaging inflammation due to the accumulated activated immune cells, and ICANS, the second most commonly noted adverse side effect, led to the elevation of cytokine in cerebrospinal fluid and disruption of the blood-brain barrier [27]. For example, in both phase I and phase II clinical trials of KTE-X19, a CD28-costimulated, anti-CD19 CAR-T cell, significant evaluation of CRS and ICANS adverse events have been observed. In particular, a grade ≥3 CRS and ICANS occurred in 31% and 38% of treated patients, respectively, in the phase I trial. Similarly, the grade ≥3 CRS and ICANS have also been observed in 15% and 31% of the treated patients,

respectively, in the case of phase II [27, 28]. Engineered immune cell-mediated toxicities can be divided into two categories, namely systemic production of pro-inflammatory cytokine resulting from the over-activation of infused cells and toxicities resulting from on-target, off-tumor effects. Despite the co-administration of immunosuppressants, such as corticosteroids and anti-IL-6 antibodies, which have shown effects in limiting the over-activation of immune responses, limited success has been shown in clinical translation. As a result, the ability to overcome the roadblock of induced toxicities through conditionally activated "switches" can significantly improve the therapeutic outcomes of the engineered immune cell-based therapy.

There are currently three main types of safety switches that have been utilized based on their conceptual functions in regulating the immune cell activity, including (1) "killing" switches that allow can induce apoptosis of functional immune cells, (2) "inhibition" switches that suppress anti-tumor responses upon addition of small molecule drugs, (3) "activation" switches that require co-administration of an additional molecule to activate activities of engineered immune cells [16]. The inducible caspase 9 (iC9) system is one of the earliest safety switches that have been used for regulating the immune response of engineered immune cells. The iC9 suicide gene consists of an intracellular portion of the caspase 9 protein fused to a drug-binding domain. The binding of a specific chemical inducer of dimerization (CID) can induce iC9 dimerization which induces a signaling cascade leading to apoptosis. In one study, not only have the researchers demonstrated the selective potency in eliminating iC9-transduced T cells upon administration of CID, but also, they have shown the remaining CAR-T after treatment can effectively control the tumor growth upon a tumor rechallenge with a low dose administration of CID [37]. Unlike the resulting apoptosis in the case of the "killing" switch, "inhibition" switches allow transiently suspension of the cellular functions without affecting the viability. In one study, a group of researchers constructed a CAR-T cell (STOP-switch CAR-T) that allows transient downregulation of its effector functions upon administration of a small molecule drug [30]. The STOP-switch CAR comprised of a recognition (antigen binding) and a signaling (T-cell activation) chains, linked via a computationally designed protein pair (CDH: chemically disruptable heterodimer), individually inserted in the endo-domains of the two separate chains. A comparable tumor inhibition capacity has been observed both *in vitro* and *in vivo* between the STOP-CAR and a second-generation CAR T cell. However, the ability of STOP-CARs to be specifically deactivated by a small-molecule drug in a dynamic fashion upon drug administration, which was reversed upon drug removal, offers the possibility to transiently tune down T-cell effector function, rather than a complete elimination exerted by a suicide switch. On the other, instead of inhibiting the anti-tumor responses, "activation" switches enable local and temporal activation of the engineered immune cells to regulate the immunological response. In one study, researchers utilized a lipocalin-mediated molecular ON-switch system to selectively activate the cytotoxicity of CAR-T cells upon administration of an oral drug, A1120 [31]. The On-switch system is comprised of two components, including a human retinol binding protein 4 (hRBP4), which can undergo conformational changes upon ligand binding, and an engineered hRBP4 binder. The binding affinity between the hRBP4 and hRBP4 binder can be induced to increase up to 550-fold when A1120 is applied. By separately attaching a hRBP4 binder, called RS3, to a second-generation CAR backbone and a hRBP4 to an anti-CD19 scFv fused with IgG1-Fc, the researchers have observed comparable cytotoxicity of the ON-switch CAR-T cells to the control group using unmodified anti-CD19 CAR-T cells in the presence of A1120, and no cytotoxicity cells have been observed in the absence of the drug. As a result, the safety switch approach provides a feasible alternative to regulate immune activity in a controlled and tumor-specific-activated manner to improve therapeutic outcomes.

4. Conclusion

Engineered immune cell-based therapy is a rapidly emerging class of immunotherapies that has been demonstrated as an effective approach to treating multiple hematological malignancies, and it is expected to continually impact the field of cancer treatment in the following years. The

heterogeneity of both solid tumors and the resided TME significantly impair the efficacy of engineered immune cells, and the dynamic interplays between the host immune system and cancer cells further provide a high degree of plasticity for cancer cells to better tolerate the mounted immune responses. Many recent effects have been focused on technological improvements for overcoming identified roadblocks that are limiting engineered immune cells in specifically and effectively targeting solid cancers in TME. Several developing strategies have shown promising clinical translations to improve the therapeutic index, including approaches to (1) enhance intratumoral infiltration to overcome ineffective accumulation, (2) improve tumor recognition and specificity to prevent on-target off-tumor toxicity, (3) increase metabolic fitness to maintain survival and persistence, (4) overcome immunosuppression, and (5) control "On/Off" states of the engineered immune cells at the tumor sites.

Besides the abovementioned strategies that have shed some new prospects on the design of next-generation engineered immune cells, other innovative genetic engineering approaches, such as different formats of Boolean gating and ectopic expression of transcription factors, are also on the horizon. In addition to the expensive, complicated, time-consuming ex vivo genetic manipulations, the development of *in situ* approach by using viral transduction and nanoparticle delivery to specifically target immune cells for modification is another intensive area of research and is presented as an alternative for improved long-term prognosis. Another strategy relies on the fact that engineered immune cells by themselves might not be able to achieve an optimal clinical outcome; instead, combination therapy is necessary to greatly improve the clinical success of the therapy. For example, the intrinsic nature of antibodies in recognizing particular antigens in high sensitivity and specificity and the ability to carry out numerous immune effectors through its Fc region provides an exciting opportunity to directly modulate the engineered immune cells to improve the anti-tumor activities. A recent study demonstrated a robust enhancement of anti-tumor cytotoxicity of an NKG2D-expressing CAR NK cell facilitated by a bispecific, tetravalent antibody that contained two NKG2D-binding and two HER2-specific single-chain fragment variable (scFv) domains in a syngeneic tumor, immunocompetent mice model and had successfully cured the majority of the animals, even with low or absent NKG2DL expression [32]. In addition, a therapy approach in combining both immune cell-based and oncolytic viral-based immunotherapies can potentially serve as an enhanced alternative. Not only does the oncolytic virus can directly inhibit solid tumors proliferation and survival but asl it can indirectly facilitate an anti-tumor immune response by modulating the tumor cells. In another study, researchers have demonstrated the potential of combining G47Δ, a third-generation oncolytic recombinant herpes simplex virus (HSV)-1 that has shown promising efficacy and safety for treating glioblastoma (GBM) in phase II clinical study, with podoplanin (PDPN) tumor antigen-specific scFv-based CAR-T cells to effectively inhibit the GBM tumor growth and significantly improve survival in a xenograft mice model [33]. As a result of promising preclinical achievements for emerging innovative genetic engineering strategies as well as combining with other treatment interventions, such as antibodies and oncolytic viruses, engineered immune cell-based therapy has a bright potential to cure various types of cancers.

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