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Review Article

Vaccine Boosting CAR-T Cell Therapy: Current and Future Strategies

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Immunotherapy for cancer treatment is growing at an unprecedented rate since the inception of chimeric antigen receptor T (CAR-T) cells. However, the efficacy of CAR-T cells against solid tumors is hampered by various issues, including "on-target, off-tumor toxicities," T cell exhaustion, and immunosuppressive tumor microenvironment. To overcome these limitations, recent advances focus on optimizing CAR-T cells using vaccines to develop more effective cell immunotherapies. Here, we summarize the most recent studies on how vaccine-based CAR-T therapies are advancing the response of cancer immunotherapy as well as the current state of their clinical and preclinical development. Finally, we share perspectives on how future studies can incorporate other strategies to augment the antitumor response of vaccine-assisted CAR-T cell therapy.

1. Introduction

The advent of chimeric antigen receptor T (CAR-T) cells has significantly improved the treatment of multiple tumors, especially hematological malignancies [1]. In particular, adaptive T cells destroy tumor cells by recognizing surface antigens through T cell receptors (TCR) [2]. In the same way, CAR-T is a patient-derived T cell that has been engineered with a chimeric antigen receptor (CAR) that can target a specific tumor-associated antigen (TAA) or tumor-specific antigen (TSA) present on the membrane of tumor cells [3]. The CAR represents a fusion of different crucial components that can recognize and initiate tumor lysis. It includes an extracellular domain, a transmembrane domain, an intracellular domain, and costimulatory domain. The extracellular domain is made up of an antibody arm, especially, a single chain variable fragment (scFv) linked together by a short hydrophobic linker which binds TAAs such as CD19. Once the surface antigen is recognized, a downstream signal transduction is initiated causing stimulation of the intracellular region, particularly TCR-derived CD3ζ with a costimulatory domain (CD28 or 41BB) [3]. Subsequently, the signalling cascades result in T cell activation and the killing of tumor cells. Previous clinical reports have shown promising antitumor activity of CAR-T cells targeting B cell malignancies [4, 5]. Currently, six CAR-T products have received FDA approval for the treatment of different types of cancer, including Kymriah and Yescarta, which are used for the treatment of leukemia and lymphoma, respectively, and many more products are currently under development [6]. This remarkable progress has not only advanced the clinical landscape towards the treatment of B cell malignancies but also presented the possibility of exploiting CAR-T cell therapy for the treatment of solid tumors. However, CAR-T therapy still faces substantial hurdles when targeting solid tumors [7]. Several reports have discussed possible mechanisms leading to poor treatment outcomes. These include the restrictive nature of T cells to infiltrate the tumor microenvironment, (TME), hyperglycosylation of the antigen, "on-target, off-tumor toxicity," and T cell exhaustion events [8, 9]. Due to these challenges, several approaches have emerged to treat solid tumors, including (1) the use

of dual CAR targets, (2) armored CARs, (3) selecting appropriate antigen targets, (4) suppressing inhibitory factors in TME, and (5) pretreatment of patients [10, 11]. However, only a few are being tested in clinical trials [12, 13].

Over the past years, the application of therapeutic vaccine in cancer immunotherapy has focused on using nucleic acid either naked or via a vehicle to stimulate the release of immune response against tumors. The vaccine is directed to precisely decorate the surfaces of antigen-presenting cells (APCs), especially dendritic cells (DCs) of the lymphoid organ. Upon T cell priming, APCs release tumor-associated cytokines which mediate T cell response against tumors [14]. Due to the immunotherapeutic benefit of the cancer vaccine as well as the quest to achieve robust CAR-T cell expansion and persistence in vivo, new approaches center on incorporating the vaccine technology into CAR-T cell therapies. Here, the vaccine is delivered to be efficiently expressed on the surface of APCs, as a cognate ligand for CAR-T cells [15]. The vaccine potentially induces the production of tumor-associated cytokines leading to CAR-T cell expansion and increases persistence. The vaccine does not only enhance CAR-T proliferation but also increase the therapeutic efficacy of CAR-T cells. This review discusses vaccine-based strategies in CAR-T cell therapy with an emphasis on the most recent approaches. The review also highlights the current state of their clinical and preclinical development.

2. Exhaustion in CAR-T Cell Therapy

Although CAR-T cells have been shown to be effective in B cell malignancies with a high response rate, relapse still remains a crucial challenge [16, 17]. Similarly, the clinical efficacy of CAR-T cells for the treatment of solid tumors is limited [18]. Reduced antitumor efficacy and the lack of a durable response have been linked to the immunosuppressive mechanism of the TME, including CAR-T cell exhaustion. Notably, CAR-T cells sometimes become exhausted when exposed to tumor antigen for a period of time resulting in loss of effector function and impair in vivo persistence. As a result, the cancer cell can then override the limited power of the CAR-T cell and continue to grow, leading to resistance and relapse in CAR-T cell therapy [19, 20]. According to clinical reports, a high rate of T cell exhaustions is a major cause of the poor performance of CAR-T cells in cancer immunotherapy [21]. Several mechanisms have been mentioned as possible causes of CAR-T exhaustion [22, 23]. Accordingly, scientists have applied several approaches to reverse the process of CAR-T cell exhaustion. However, most of these strategies have produced only a moderate therapeutic effect and are only based on (1) targeting checkpoint molecules such as programmed death ligand 1 (PD-L1) and regulatory proteins [24], (2) dual-specific Chimeric antigen receptor T [25], and (3) inhibition of relevant transcription factors such as TOX and Nr4a family proteins [26, 27].

Nevertheless, recent modification incorporates vaccine therapeutics to support CAR-T activity in vivo as well as to circumvent CAR-T cell exhaustion. In this new strategy, the nucleic acid vaccine is injected to stimulate the activity of CAR-T cells. Generally, researchers have represented this promising strategy in diverse ways. For example, different groups have developed CAR-T cells from virus-specific endogenous lymphocytes. They engineered the virus-derived CAR to recognize native TCR and thus provoke an immune response against solid tumors with enhanced efficacy [28]. Furthermore, researchers have generated CAR-T cells that express a secondary TCR, where the second TCR was specifically tailored to bind a target peptide encoded by the injected vaccine or the TCR can be redirected to target self-antigens [29]. The incredible progress made in these areas highlights the multiple ways in which CAR-T cells synergistically express CAR and a TCR in a specific manner. Alternatively, it suggests the possibility of harnessing the immunogenicity of viruses to increase the antitumor activity of CAR-T cells in cancer immunotherapy. It is worth mentioning that most of the strategies discussed above in this context are preclinical studies (Table 1).

3. Amph-Ligand CAR-T Cell Therapy

Since reliable CAR-T cell therapy requires that CAR-T cells expand, persist, and repeatedly kill cancer cells, recent advances focus on improving the in vivo durability of CAR-T cells. This approach recapitulates the natural way by which endogenous T cells response to tumors. The injected vaccine will travel into the lymph node microenvironment and upon interaction with antigen-presenting cells (APCs), especially DCs, trigger CAR-T cell expansion. In particular, the vaccine acts as a potential booster that mediates massive proliferation and enhances the antitumor activity of CAR-T cells [30]. In 2019, researchers at MIT adapted amph-ligand technology into CAR-T cell therapy, hence named amph-ligands CAR-T cell therapy. Here, they used amph-FITC vaccine where FITC is the cognate peptide for the CAR to target the lymph node. Inside the lymph node, amph-FITC was taken up by the plasma membrane of APCs. Bispecific CAR-T cells which can recognize FITC and tumor antigen are stimulated and proliferated relying on FITC presented by APC and target tumor cells by tumor antigen.

Preclinical evaluations in mice models that received the booster after CAR-T infusion showed a complete remission of 60% [30]. Furthermore, the amph-FITC vaccine doubled the number of CAR-T cells, indicating that it can prolong and improve the in vivo functionality of CAR-T cells. Because the amph-FITC lacks a delivery system that can protect the ligand against endonucleases, its clinical application may be limited. Nevertheless, the most recent approach is the use of chimeric antigen receptor plus mRNA vaccine (CARVac), and this novel technique has been extensively discussed in this review.

4. Dendritic Cell: A Potent Mediator for Antitumor Immunity

Since Steinman and Cohn identified DC in 1973, the field of immunotherapy has gained tremendous success [34]. DCs are the most potent APCs that play an important role in

Table 1: Preclinical and clinical studies with CAR-T cells utilizing a booster vaccine platform. (Clinical trials are registered at *clinicaltrials.gov*).

Strategy	Cancer type	CAR target	Vaccine used	Vaccine target	Status	Ref
CAR-T cell amplifying RNA vaccine	Relapsed or refractory advanced solid tumors	CLDN6	CLDN6-LPX	DCs	Clinical trials (NCT04503278)	[31]
Viral T cells modified with a CAR	Metastatic, sarcoma, and neuroblastoma	GD2	Live attenuated VZV vaccine	VZV specific T cells	Clinical trials (NCT01953900)	[28]
Amphiphile CAR-T ligands	Breast cancer Lung cancer	FITC	Amph-FITC	APCs	Preclinical	[30]
Bispecific CMV/CD1 9 T cells	Lymphoma	CD19	CMV vaccine	CMVpp65	Preclinical	[32]
CAR-T therapy plus DC vaccine	$WT1^{+}$/HLA-$A^{*}2402^{+}$ tumors	WT1 peptide	DCs loaded with WT1 antigen	WT1 peptide	Preclinical	[33]
ACTIV therapy	Melanoma Breast cancer Liver cancer	Her2	Live recombinant vaccinia virus	Melanocyte protein (gp100)	Preclinical	[29]

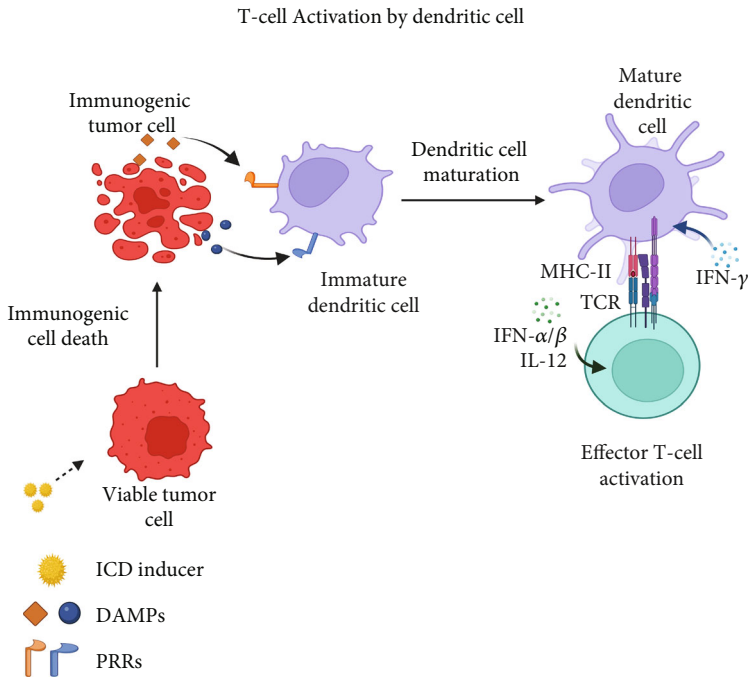

Figure 1: Dendritic cell activates T cell by cross-presenting exogenous tumor antigen.

naive T cell activation. Within lymph nodes, T cells that are actively looking for target cognate peptides can rapidly recognize antigens in the form of major histocompatibility molecules on the surface of DCs via TCRs. The interaction between T cells and DC results in T cell activation, expansion, and differentiation of T cells into effector and memory T cells [34]. In cancer biology, the recognition of TAAs present in the class I major histocompatibility complex by tumor-infiltrated dendritic cells (TIDC) plays a critical role in mediating T cell antitumor activity [35–37]. DCs can recognize TAAs, process them, and present them to native antigen-specific T cells to initiate tumor cytotoxicity (Figure 1). Based on this, several studies have directly engineered DC to effectively express tumor-specific antigens, thus inducing the differentiation of naïve T cells that can potentially kill tumor in antigen specificity manner [38–40]. More importantly, DCs can promote cellular immunity via the release of proinflammatory cytokines or immunostimulatory signals following TCR stimulation. Accordingly, IL 12 and type I interferons are the most common signals that are produced by DCs. Moreover, in a TME, DCs can secrete chemokines to potentially recruit T cells to the site of the dividing tumor. For example, DCs can produce the CXC chemokine ligand 9 (CXCL9) and

CXCL10, which will possibly attract CD8+ T cells into the TME [41–43].

Recent studies highlighted the clinical potency of engineered DCs for patients with advanced melanoma. In a trial, researchers targeted DCs via intravenously administered RNA-lipoplex (RNA-LPX) encoding four tumor antigens (NY-ESO-1, MAGE-A3, tyrosinase, and TPTE) (*ClinicalTrials.gov Identifier: NCT02410733*). The results showed a significant production of IFNα accompanied by strong antigen-specific T cell responses in all patients. The results strongly suggest that DCs can act as a potent mediator of antitumor effect by initiating strong secretion of tumor-associated cytokines [44]. However, emerging reports have shown that the inhibitory activity of the myeloid compartment can actively limit the DC-mediated production of proinflammatory cytokines, leading to impaired T cell function [45–47]. Additionally, others have utilized the ex vivo gene therapy approach to introduce tumor antigens into DCs and then inject systemically or locally into cancer patients, with the intention to induce the activation of antigen-specific T cells, DC vaccination [48–50]. And not only do DCs promote antitumor immunity, but it can also induce the release of immunological memory to control subsequent evading tumors [51]. In summary, DC vaccination has shown great success with the prospect to extensively ameliorate cancer with markedly low and no adverse effect.

5. CAR-T Technology of mRNA-Based Vaccines

The development of mRNA vaccines has widely illuminated the field of oncology. Generally, mRNA vaccines are made up of messenger RNA molecules that have been synthesized artificially using the bacteriophage RNA polymerase system, which can transcribe template DNA to mRNA inside a host cytoplasm, and finally, mRNA can be translated into functional proteins [52]. Many scientists have harnessed this molecule to develop exciting products for the treatment of both infectious and noninfectious diseases, and the most recent among these are the two FDA-approved mRNA vaccines for the treatment of COVID 19 [53]. In cancer immunotherapeutic, the mRNA vaccine can be administered to APCs to potentially trigger the antitumor activity of the adaptive immune system. Mechanically, DCs can uptake therapeutic mRNA and translate it into specific proteins that are efficiently expressed as surface antigens. Subsequently, T cells can bind to DC and illicit tumor-specific antibodies against cancer cells [54–56].

To further advance the clinical response of CAR-T cell therapy against solid tumors, a group of scientists from BioNTech have recently integrated mRNA-based vaccines into adoptive T cell therapy (ACT), aiming to extensively expand CAR-T cells, extend its life span, and double its antitumor activity [31]. Using its proprietary RNA-lipoplex (RNA-LPX) as a delivery system, the vaccine can be delivered into the lymphoid compartment, specifically tailored to DCs following CAR-T cell infusion (Figure 2, thus stimulating the expansion and persistence of adoptively transferred CAR-T cells in cancer patients. It is important to note that intravenous delivery of RNA-LPX to lymphoid organs holds great promise for cancer vaccines [38, 57]. In line with the above, the expression of the target mRNA vaccine on the surface of native DCs will allow cognate CAR-T cells to bind, activating CAR-T cells to expand and kill the tumor. This is a first-in-kind RNA vaccine platform that can be used to significantly enhance the in vivo proliferation and persistence of CAR-T cells, while empowering the functionality of CAR-T. Unlike the amph-ligand CAR-T technology which uses amphiphilic platform to transfer the ligand to DCs, mRNA-based vaccine CAR-T technology utilizes an RNA-lipoplex to deliver the ligand into DCs. A major advantage is that mRNA-based vaccines may overcome potential barriers such as the attack from extracellular endonucleases.

Moreover, it is worth noting that the chimeric antigen receptor and vaccine is targeted to claudin 6- (CLDN6-) and claudin 18 isoform 2- (CLDN18.2-) positive tumors including ovarian, testicular, endometrial, and gastric tumors. The anti-CLDN6 scFv was fused to the CD8α transmembrane domain followed by 4-1BB and CD3ζ intracellular domains (Figure 2). Notably, CLDN6 was identified among clinically relevant targets in cancer immunotherapy [58, 59]. In biology, CLDN6 is a member of the claudin (CLDN) family of genes involved in tight junction formation. In particular, it functions to regulate cellular permeability and defense, and it also plays a major role in cell proliferation and differentiation. Furthermore, CLDN6 is only expressed in fetal tissues, including the stomach, pancreas, lung, and kidney, but not in normal human normal tissue [59, 60]. In accordance with previous studies [61], this study has further confirmed that anti-CLDN6 does not cross react with any of the ten homologous classic CLDN family proteins. Due to the uniqueness of CLDN6-associated tumors and the lack of shared epitopes, targeting it will ultimately prevent the tumor-prone off-target effect and thus overcome the possibility of producing autoantibodies against self-antigens.

In a preclinical in vivo model study, mice that received CAR-CLDN6 followed by CLDN6-LPX demonstrated a remarkable increase in proliferating CLDN6-CAR-T cells, which was accompanied by complete tumor regression within 14 days of treatment. Furthermore, targeting CLDN18.2 and CD19 tumor antigens also demonstrated complete tumor regression, suggesting that the approach may have a wider application—not only in solid CAR-T cell therapy but also in liquid tumors. Nevertheless, the results shown a high percentage of proliferating CD4+ and CD8+ CAR-T cells indicating that the vaccine can allow formation of memory T cells that can persist in high densities and capable of producing response to tumor rechallenges.

In a phase 1/2 trial, patients with ovarian cancer showed an overall response and a control rate of 43% and 86%, respectively. Likewise, patients suffering from testicular cancer demonstrated a remarkable response at dose 2 [62]. According to the initial report on 16 September 2021, 9 patients had been treated with dose level 1 (DL1); however, a more recent update on 19 January 2022 indicated that 16 patients have gained complete remission, with treatment performed for part 1 (CLDN6 CAR-T cell only) and part 2

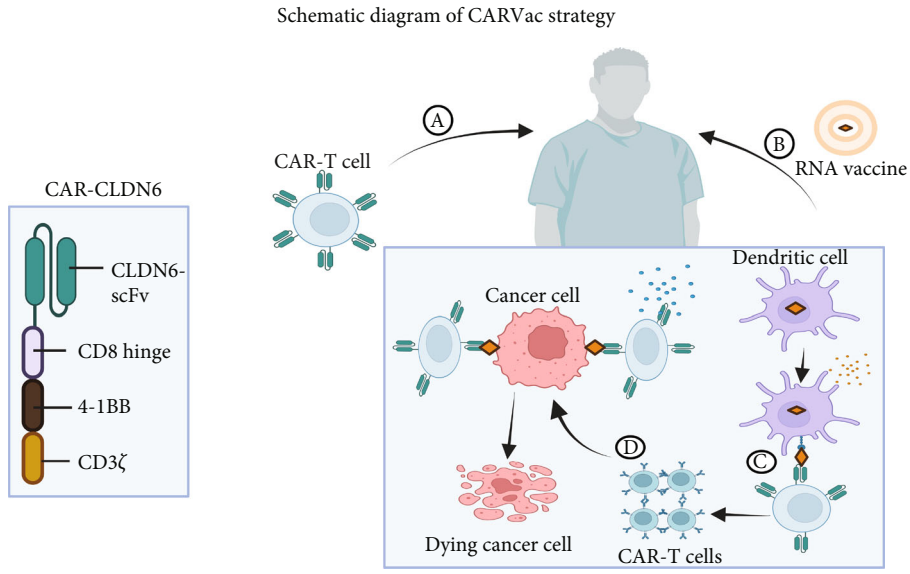

Figure 2: Targeting the lymph node with mRNA-based vaccine. (A) CAR-T cell expressing CAR-CLDN6 scFv is infused into cancer patients after lymphodepletion. (B) Lipid nanoparticle is loaded with immunostimulatory CLDN6 molecule and vaccinated to cancer patients following CAR-T infusion, where it traffics to lymph node. (C) Antigen-presenting cells especially dendritic cells within lymph nodes internalize the vaccine and efficiently display it as a surface ligand for CAR-T cells. (D) CAR-T cell interact with DC via CAR resulting in expansion and cytotoxicity.

(combination of CLDN6 CAR-T and CARVac) at dose level 2 (DL2). Comparably, antitumor efficacy was higher in the combined therapy with 5 of 10 patients showing a partial response than in the monotherapy where only 2 of 9 patients showed a partial response. Although some of the patients experienced adverse effects in both cases, it was transient and manageable.

6. Perspectives on Vaccine-Based CAR-T Therapy

The main rationale behind developing a vaccine for CAR-T cells in cancer immunotherapy is to expand CAR-T cells and increase their persistence in vivo, providing lasting treatment against relapsed/refractory solid cancers. While the restrictive nature of TME continues to be a major challenge for conventional CAR-T cell therapies, the addition of vaccine can potentially empower CAR-T cells and improve the antitumor efficacy. In the context of solid tumors, the frequency of infiltrating CAR-T cells drastically reduces because of the restrictive nature of the TME. Accordingly, CAR-T cells are unable to reach the center of the growing tumor to expel their cytotoxic agents, and even if the CAR-T cell finds its way into solid tumor lesions, it may not last long or sometimes become exhausted and cannot completely remove all cancer cells. Consequently, the remaining cancer cells continue to grow leading to post CAR-T relapses. Accumulating reports have shown that many cancer types come back after CAR-T treatment [63–65]. Therefore, we emphasize that the combination of booster vaccine will concomitantly expand and prolong the life span of CAR-T cells, making it possible to remove all tumors, including relapsed tumors, to achieve complete remission. Unlike previous applications where immunogenic viral-specific T cells were used, current approaches utilize humanized antigens and may not trigger an immune attack. A focal point of emphasis is that this platform not only boosts CAR-T cells but also allows the administration of a suboptimal therapeutic dose of CAR-T cells. Accordingly, it will greatly reduce the cost involved in production as well as the time used to amplify the CAR-T cells in vitro.

Although the mRNA vaccine CAR-T cell therapy can be used to treat relapsed/refractory cancers, it is important to note that there are two types of relapses in CAR-T immunotherapy [66, 67]: first, relapse that can result from poor persistence of CAR-T cells (positive relapse) and, second, those that occur due to the loss or downregulation of tumor antigen. For instance, in CD19-positive relapse, CD19 antigens can still be found on the surface of the tumor and can be recognized and killed by persistent CAR-T cells. But in the case of CD19-negative relapse, CD19 antigens are completely absent leading to tumor escape [67]. While good persistence of CAR-T cells via the vaccine approach can circumvent positive relapse, it may not be able to completely clear negative relapse tumors. Additionally, the generation of tumor escape variants due to genomic instability and natural selection of tumors can hamper CAR-T cells even if they persist longer [68, 69]. Consequently, the approach may be further enhanced by utilizing bivalent vaccine candidates. Bivalent vaccine boosters can allow targeting two tumor antigen variants simultaneously, and nonetheless, provoke broader immunity against other tumor variants. Here, CAR-T cells that recognize the two fused immunogenic vaccine molecules can differentiate into more than one specific lymphocyte and thus can target multiple tumors.

In immunology, immature DCs can rapidly uptake tumor antigens but may have reduced motility, whereas mature DCs have reduced ability to internalize antigens but may present antigens to T cells within lymph nodes in a vigorous manner. In some solid tumors, such as breast cancers, mature DCs have been shown to possess functional characteristics as immature DCs and therefore cannot strongly activate T cells in a robust manner in contrast to mature DCs [70]. As a result, DCs become unresponsive to T cells, allowing the tumor to progress. Furthermore, dysregulation of DCs can result in low antigen presentation ability and may hamper CAR-T cell activation [71]. On this account, it may be prudent to incorporate ex vivo DC therapy into ACT. Since DCs are capable of expressing specific tumor antigens through an antigenic modification of hiPSCs, these DCs can act as vaccines that can prime CAR-T cells in vivo to stimulate the production of immune memory cells. Nevertheless, cancer cell-based vaccines can be employed to stimulate CAR-T cells since tumor cells can be synthetically manipulated to produce immune activating cytokines and can trigger potent antitumor response [72]. More recently, Shah and colleagues repurposed living tumor cells that can migrate to the site of their counterpart tumors and release not only antitumor agents but also factors that can be recognized by the immune system, thus removing tumors as well as inducing long term immunity [73]. Based on this, we emphasize that engineered living tumor cells can be a suitable vaccine candidate for priming CAR-T cells to confer lasting antitumor immunity.

Moving on, to advance the translational potential of vaccine-based CAR-T therapy, it will be important to engineer vaccines that combine bispecific CAR-T cell engagers retargeted to DCs. In general, T cell or CAR-T cell specificity and binding to tumor-associated MHC molecule on the surface of DCs are a primary requirement leading to T cell activation, clonal expansion, and tumor cytotoxicity. However, increasing evidence has mentioned several inhibitory and immune tolerance mechanisms that can alter CAR-T cell interacting with DCs [74–76]. A bispecific T cell engager (BiTE) vaccine construct can be developed to mediate the interaction of CAR-T cells with DCs. In the current BiTE approach, T cells located at close proximity to BiTE-associated cancer cells are recruited to the CD33 binding arm of the BiTE molecule, leading to the formation of immunological synapses between T cells and tumor cells. Subsequently, T cells become activated and then secrete cytotoxic granules, which eventually result in the lysis of the targeted tumor cell [77]. In the same way, CAR-T cells that are inside lymph nodes are expected to be recruited or engaged via the BiTE vaccine to rapidly recognize and bind to DC.

7. Conclusion

The application of vaccine in CAR-T cell therapy can provide a means to improve the persistency of CAR-T cells and also overcome the inactivity rendered by the TME. Unlike liquid tumors, solid tumor complex organ-like structures contain not only growing tumor cells but also vasculature, ECM, and stromal cells. Besides, the TME can support the proliferation of tumor and restrict the efficacy of CAR-T cells. Before eliminating the tumor cells, CAR-T cells become exhausted and stop proliferation. However, vaccine can help to optimize CAR-T cells and provide more effective cell immunotherapies. Several nucleic acid-based therapeutic vaccines can help CAR-T cell expansion and persistence and increase their therapeutic efficacy in vivo. Besides, the selection of targeting cells such as dendritic cells also plays an important role in vaccine boosting CAR-T cell therapy. This review not only shows the several different designs of cancer vaccines but also suggests more robust strategies for vaccines boosting CAR-T cells design including bivalent vaccine candidates, BiTE vaccine constructs, and cancer cell-based vaccines.

Data Availability

This study did not perform any data.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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