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CAR T-cell therapy for lung cancer and malignant pleural mesothelioma

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Abstract

Immunotherapy is a promising field that harnesses the power of the immune system as a therapeutic agent for cancer treatment. Beneficial outcomes shown in patients with non-small cell lung cancer (NSCLC) and malignant pleural mesothelioma (MPM) with relatively higher tumor-infiltrating T cells, combined with impressive responses obtained in a cohort of patients with NSCLC following checkpoint blockade therapy, lays a strong foundation to promote effector immune responses in these patients. One such approach being investigated is administration of tumor antigen-targeted T cells with transduction of a chimeric antigen receptor (CAR). CARs are synthetic receptors that enhance T-cell antitumor effector function and have gained momentum to investigate in solid tumors based on recent successes of clinical trials treating patients with B-cell hematologic malignancies. This review summarizes target antigens for CAR T-cell therapy that are being investigated in preclinical studies as well as clinical trials for both NSCLC and MPM patients. We discuss the rationale for combination immunotherapies for NSCLC and MPM patients. Additionally, we have highlighted the challenges and strategies for overcoming the obstacles facing translation of CAR T-cell therapy to solid tumors.

Keywords

Adoptive T-cell therapy; adoptive cell therapy; chimeric antigen receptors; mesothelin

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INTRODUCTION

Thoracic malignancies represent a major health concern with lung cancer being one of the most common and deadliest cancers in the world. In the United States, there were over 240 000 new cases diagnosed and over 150 000 deaths within the past year alone [1].

Approximately 85% of these cases are non-small cell lung cancer (NSCLC) and the remaining 15% are small cell lung cancer (SCLC). Despite advances in surgical techniques and trimodality therapy with chemotherapy and radiation, the prognosis of lung cancer has not changed significantly over the past 3 decades, with 5-year survival rates at approximately 18% in the United States [1]. Malignant pleural mesothelioma (MPM) is a highly aggressive primary pleural disease that is commonly associated with previous asbestos exposure. Due to its locoregionally aggressive nature, curative treatment options remain limited and median survival at time of diagnosis is only 8 to 14 months [2]. Even with the introduction of targeted molecular therapies for patients with epidermal growth factor receptor (EGFR)-positive or anaplastic lymphoma kinase (ALK)-positive lung cancers, the improved outcomes are short-lived as the majority of these patients eventually experience disease recurrence [3, 4]. With advancements in our knowledge of tumor immunology and the growing field of immunotherapy, mounting evidence supports the prognostic value of identifying tumor-infiltrating immune cells in the solid tumor microenvironment. It is with great optimism that researchers turn to expanding the armamentarium of cancer treatment by harnessing patients' own immune defenses to join the fight against cancer.

The clinical impact of the immune microenvironment has been demonstrated in both NSCLC and SCLC. We have previously reported on the prognostic significance of tumor interleukin (IL)-12 receptor $\beta 2$ (IL-12R $\beta 2$), IL-7R, and stromal FoxP3/CD3 ratio as independent predictors of recurrence in patients with stage I lung adenocarcinoma (ADC) [5]. Similarly, we also found that tumor-infiltrating CD10⁺ neutrophil/CD20⁺ lymphocyte ratio was an independent prognostic factor in patients with squamous cell carcinoma [6]. In MPM patients who have undergone surgical resection, those who had a higher density of CD8⁺ tumor-infiltrating lymphocytes (TILs) in their tumors were correlated significantly with better survival ($p < 0.05$) [7]. Immunosuppressive factors, such as regulatory T cells (Tregs) and inhibitory cytokines can dampen the antitumor immune responses in favor of MPM tumor growth [8]. In an *in vivo* model of MPM, Hegmans et al. demonstrated that survival increases when FoxP3+CD4+CD25+ Tregs were depleted [8]. These findings stress the importance of understanding the dynamic flux between the protumorigenic and antitumorigenic components of the immune microenvironment. The interplay of these factors not only influences tumor growth, but also tumor aggressiveness and patient prognosis.

This has led to the development of novel immunotherapeutic strategies aimed at activating the host's immune system or overcoming components of the immunosuppressive tumor microenvironment. For solid tumors, such as NSCLC and MPM, these include biologic agents, cytokine therapy, antibody-mediated therapy, cancer vaccines, and adoptive cell therapy (ACT). Studies like the Phase II POPLAR trial (NCT01903993) and the Phase III KEYNOTE-024 trial (NCT02142738) have investigated the efficacy of checkpoint inhibition

using monoclonal antibodies targeting the programmed cell death protein 1 (PD-1)/programmed death ligand 1 (PD-L1) signaling pathway [9, 10]. Atezolizumab and pembrolizumab are novel immune checkpoint inhibitors that have been recently been approved by the Food and Drug Administration (FDA) to treat metastatic NSCLC in patients whose tumors overexpress PD-L1. These landmark studies have demonstrated survival benefits compared with chemotherapy and, not only reinforce the importance of understanding the central role of the immune system in tumor development, but also reveal that we have only begun to scratch the surface of the microcosmic world of cancer biology and immunology. This review focuses on a promising type of ACT using chimeric antigen receptor (CAR) T cells and summarizes target antigens that are being investigated in preclinical studies and clinical trials for both lung cancer and MPM. Furthermore, we discuss the rationale for combination immunotherapies and highlight challenges and strategies for overcoming the obstacles facing translation of CAR T-cell therapy to solid tumors.

EVOLUTION OF ADOPTIVE CELL THERAPY

The last few decades have seen resurgence in the field of immunotherapy, with rapid growth and development in the adoptive transfer of immune cells. As defined by the National Cancer Institute (NCI), ACT is a process by which tumor-reactive lymphocytes are collected from a patient, grown *ex vivo*, and then re-administered to the patient as therapy. Using this approach, reactive T cells are able to eradicate tumors by recognizing and targeting tumor-associated antigens (TAAs), thereby establishing tumor immunity. Early successes in melanoma patients have described the generation of tumor-reactive TILs by isolating autologous T cells from tumor biopsy specimens and rapidly expanding the most active TIL cultures screened for the greatest antitumor activity [11]. The combination of non-myeloablative lymphodepleting chemotherapy, followed by ACT, has led to an objective clinical response in 18 (51%; 3 complete responses and 15 partial responses) of 35 patients with refractory metastatic melanoma who were also heavily pretreated with IL-2 [12]. Rosenberg et al. similarly found that ACT with autologous TILs mediated complete response in 20 (22%) of 93 patients with metastatic melanoma, irrespective of prior treatment [13]. Despite these successes, the use of TILs still poses several challenges. Specifically relevant to NSCLC and MPM, these cells can be difficult to isolate and expand in such a way that allows them to retain their specificity and functionality [14]. Additionally, without preconditioned lymphodepleting chemotherapy, clinical response to TILs is often short lived [15, 16]. These challenges have led to the development of different strategies aimed at harnessing the antitumor efficacy of T cells.

The first strategy used genetically engineered T cells that targeted specific TAAs through a T-cell receptor (TCR). The α - and β -chain heterodimers of the TCR can be modified to target a specific antigen and this complex can be transduced retrovirally onto the T cells of healthy donors. While antigen specificity is imparted through these α - and β -chains, the activation signal is mediated through the CD3 complex of the TCR. A major limitation of TCRs is that they are restricted by the human leukocyte antigens (HLAs) of the major histocompatibility complex (MHC) presented on tumor cells. As a mechanism of evasion, these tumor cells escape recognition by downregulating their MHC class I expression,

thereby resulting in decreased T-cell-dependent tumor lysis [14]. In contrast to TCRs, CAR T-cell binding, with subsequent activation, occurs independently of MHC class. CARs can bind directly to an expanded range of cell-surface targets, including lipids, carbohydrates, and proteins [17]. First generation CARs contained a single signaling receptor that consisted of an extracellular domain that binds the tumor antigen and transduced the signal through a transmembrane complex that is fused to a CD3 ζ intracellular activating domain [18]. Upon stimulation, these CARs exhibited cytotoxic ability, but were limited in that activation did not trigger additional effector functions such as cytokine secretion or clonal proliferation. To overcome these limitations, second generation CARs were developed and aimed at facilitating antigen-specific binding by replacing the extracellular binding moiety with a single-chain variable fragment (scFv) connected to a co-stimulatory intracellular signaling domain in tandem with the CD3 ζ chain. Commonly incorporated co-stimulatory domains include CD28 and 4-1BB, and can lead to enhanced cytokine production, increased T-cell proliferation and persistence, and delayed apoptosis. More recently, third generation CARs have been developed that incorporate 2 co-stimulatory domains within their cytoplasmic tail [18]. Once constructed, CARs can be inserted into autologous T cells by way of viral (lentiviral or retroviral) or nonviral (transposon) gene transfer systems [19]. Transient CAR expression can also be achieved using methods, such as mRNA electroporation, that will be further discussed in the next section. Following transduction, these CAR T cells are expanded *ex vivo* in specialized gene transfer facilities and subsequently re-administered to the patient, either systemically or regionally, as a therapeutic intervention. The dramatic results seen in clinical trials using CD19-targeted CAR T-cell therapy for hematological malignancies, such as acute lymphoblastic leukemia and chronic lymphocytic leukemia, has re-energized the field of immunotherapy and prompted a rigorous search for candidate antigens to target in solid tumors [20–24].

TARGET ANTIGENS FOR CARs

ACT using CAR T cells is a growing and promising strategy that is being investigated actively for the treatment of solid tumors, including lung cancer and MPM. However, finding a suitable target antigen has been one of the greatest challenges in the development of CAR T-cell therapy for these cancers. With durable remissions seen in several CD19 CAR T-cell clinical trials for patients with refractory B-cell malignancies, translating this treatment strategy to solid tumors has become an area of active investigation. CD19 is a cell-surface antigen present physiologically on B cells and is expressed pathologically at high levels in B-cell lineage lymphomas and leukemias. Given its frequent and relative uniform expression on most malignant cells while being highly restricted in normal tissues, CD19 is as close to an ideal target that has been discovered in the field of CAR T-cell therapy. Similarly, an ideal target antigen for solid tumors should be highly and selectively expressed on cancer cells with minimal to no expression on normal tissue (see Figure 1). Candidate target antigens currently being investigated in clinical trials for lung cancer and MPM include overexpressed TAAs (carcinoembryonic antigen, CEA; disialoganglioside, GD2; glypican-3, GPC3; human epidermal growth factor receptor 2, HER2; mesothelin, MSLN; PD-1; receptor tyrosine-kinase-like orphan receptor, ROR1), novel gene products that arise from splice variants (epidermal growth factor receptor, EGFR) and abnormal glycosylation

(mucin 1, MUC1), and stromal elements associated with the tumor microenvironment (fibroblast activation protein, FAP; vascular endothelial growth factor receptor 2, VEGFR2; see Table 1).

Overexpressed differentiation antigens are attractive targets for CAR T-cell therapy because they are expressed at low levels on normal tissues, while being pathologically expressed at high levels on cancer cells. MSLN is one such cell-surface glycoprotein that is expressed at low levels on normal mesothelial cells of the pleura, pericardium, peritoneum, and tunica vaginalis, while it is overly expressed in the majority of MPM, lung, pancreatic, and ovarian carcinomas [17] [25]. MSLN is especially appealing since several preclinical and clinical studies found that it was involved in the malignant transformation of tumors and had a clear association with tumor aggressiveness, which led to local invasion and eventual metastasis [26–28]. Others and our group have demonstrated that MSLN expression is associated with an increased risk of recurrence with reduced overall survival in both early stage and advanced stage lung ADC [27, 29]. Observed dose-limiting toxicity of pleuritis following administration of the recombinant anti-MSLN immunotoxin SS1P, albeit uncommon, highlights the importance of understanding and minimizing the risk of on-target, off-tumor toxicity [30]. This has led to the development of different safety strategies that are being investigated in multiple Phase I clinical trials for MSLN-targeted CAR T-cell therapy (NCT02414269, NCT01583686, NCT02580747, NCT02159716, and NCT01355965). Based on the potent antitumor effects observed in preclinical studies using mRNA electroporation to engineer T cells that only transiently express the anti-MSLN CAR, a Phase I clinical trial at the University of Pennsylvania has been evaluating the safety and feasibility of this approach (NCT01355965) [31]. Of the 4 patients with MPM or metastatic pancreatic cancer that were treated with repeat doses of intravenous or intratumoral MSLN-targeted CAR T cells, moderate clinical responses were observed that demonstrated these transiently expressed CARs can induce epitope spreading and mediate antitumor immune responses without overt evidence of on-tumor, off-target toxicity against normal tissues [32]. Of note, the group did observe a serious anaphylactic reaction in one patient shortly following a third infusion of MSLN CAR T cells that was attributed to the immunogenicity of the murine SS1 scFv used in their CAR construct [33].

Our group has developed a fully human scFv targeting MSLN and investigated it in an orthotopic mouse model of MPM that faithfully mimicked human pleural disease [34] [35]. Our preclinical studies have demonstrated the potent and long-lasting antitumor efficacy of regionally delivered MSLN-targeted CAR T-cell therapy and served as the foundation of the rationale for opening a Phase I clinical trial of intrapleurally delivered MSLN-targeted CAR T cells in patients with primary or secondary pleural malignancies (NCT02414269). CEA is a fetal antigen normally produced in the colon during development, but displays minimal expression following birth. CEA levels rise in the presence of some cancers, including colorectal, lung, pancreatic, gastric and breast carcinomas, making it a useful tumor marker to monitor response to treatment. Preclinical evidence has established a link between pretreatment serum CEA levels with the presence of brain metastasis in patients with advanced NSCLC, thus providing a rationale for establishing a Phase I clinical trial that will evaluate the safety and maximum tolerated dose of anti-CEA CAR T cells in the treatment of CEA-positive lung, colorectal, gastric, breast, and pancreatic cancers (NCT02349724)

[36]. HER2 is another TAA and is 1 of 4 members of tyrosine kinase receptors that belong to the ERBB family. Based on the successes of humanized monoclonal antibodies that target HER2 in HER2-positive breast cancers, Morgan et al. developed a CAR fitted with both CD28 and 4-1BB co-stimulatory molecules that targets ERBB2 [37]. In a case report they discuss the sudden onset of pulmonary edema in a patient with colon cancer that metastasized to the lung and liver following a single intravenous dose of these cells [37]. Their experience reinforces the importance of understanding the basic role of these antigens as well as their baseline levels of expression in normal tissue, which, in normal lung cells, led to pulmonary toxicity and patient death. HER2 overexpression has also been found in patients with NSCLC and is associated with poor prognosis [38]. There are currently 3 Phase I and II clinical trials investigating the efficacy of HER2-targeted CAR T cells for HER2 positive solid tumors, including NSCLC (NCT02713984, NCT01935843, and NCT00889954). Preclinical studies, which includes ours, have demonstrated that free proteins in the serum, such as serum mesothelin related peptide (SMRP), can be useful in monitoring therapy response by indicating tumor regression or progression, but does not interfere with CAR T-cell activity [35] [28].

Tumor-associated antigens that arise from gene mutations and lead to novel peptide sequences are attractive CAR targets because their expression is limited to tumor cells. EGFR, also known as human epidermal receptor 1 (HER-1), is a transmembrane glycoprotein. EGFR mutation-positive NSCLC is emerging as an important biologic subtype of lung cancer, with an estimated prevalence of 10–15% in patients with non-squamous NSCLC [39]. Given the selective effectiveness seen with use of tyrosine-kinase inhibitors (TKIs) in prolonging progression-free survival, especially within specific mutant subtypes, it follows that further investigation and stratification of NSCLC patients, not only by histologic subtype but also by molecular profile, will prove invaluable for the future management of these patients [39]. With overexpression in epithelial-derived malignancies, such as NSCLC and its association with tumorigenesis and metastasis, EGFR has become a promising therapeutic target for adoptive T-cell therapy. Currently there is a single Phase I/II clinical trial in China that is investigating the safety and antitumor response of EGFR-targeted CAR T-cell therapy for the treatment of advanced EGFR-positive solid tumors, including lung, colorectal, ovarian, and pancreatic cancers (NCT01869166). There have been 11 patients with advanced relapsed/refractory NSCLC that have been enrolled in this study thus far with EGFR expression of at least 50%, as determined by immunohistochemistry [40]. There were 2 patients who achieved partial remission and 5 patients who had stable disease following an infusion of approximately 1×10^7 EGFR-targeted CAR T cells per kilogram. Mild skin toxicity (grade 1–2, 18.2%) and dyspnea (grade 1–2, 36.4%), which were graded according to the NCI's Common Terminology Criteria for Adverse Events (CTCAE), were the most commonly observed side effects following treatment, thus indicating that this therapy was safe and feasible for patients [40]. Interestingly, this study also demonstrated that where TKIs have failed to achieve clinical response, EGFR CAR T cells can reach a better outcome and may be more effective in the presence of secondary mutations.

Altered gene products resulting from modified post-translational modifications, such as those seen with the target antigen MUC1, are another group of potential candidates that can be targeted with CAR T-cell therapy. Overexpression of the tumor-selective glyco-epitopes

that arise from the abnormal glycosylation of this largely extracellular transmembrane glycoprotein is associated with tumor progression and poor prognosis [41]. With demonstrated preclinical evidence, there is an ongoing Phase I/II clinical trial in China that is recruiting patients with MUC1-positive advanced refractory solid tumors, such as NSCLC, hepatocellular, pancreatic, and triple-negative invasive breast carcinomas, for treatment with anti-MUC1 CAR T-cell therapy.

Solid tumor CAR targets arising from tumor-associated stroma, such as FAP and VEGFR2, are also appealing candidate antigens because of their expression not only on the surface of tumor cells but also by the cancer-associated fibroblasts (CAFs) of the tumor stroma. FAP is overexpressed in all 3 major MPM subtypes, including epitheloid, sarcomatoid, and biphasic [42]. Based on preclinical studies that have demonstrated antigen-specific functionality *in vitro* and FAP-targeted CAR T-cell antitumor efficacy *in vivo*, Schuberth et al. established the rationale for initiating a Phase I clinical trial to evaluate the safety of administering FAP-specific re-directed T cells to patients with MPM (NCT01722149). This group from the University of Zurich is investigating the safety of a fixed, single dose of 1×10^6 adoptively transferred, FAP-targeted CAR T cells administered directly into the pleural effusion of patients [43].

The critical component to success of any of these proposed candidate antigens will be determined by their safety profile and the degree of on-target, off-tumor toxicity. The risk of these feared complications has led to different strategies that attempt to address this concern. The University of Pennsylvania's clinical trial used autologous T cells electroporated with mRNA rather than lentivirus to transiently express the second generation MSLN CAR, thereby eliminating the safety concerns of prolonged expression of CARs (NCT01355965) [31]. Another approach to enhance the safety of these cellular therapies is to use suicide genes that get incorporated into the CAR construct. If an adverse event is suspected, the T cells can be eliminated following drug-induced activation of a suicide gene, such as the herpes simplex thymidine kinase (HSV-TK) gene, truncated human EGFR polypeptide (huEGFR), and inducible caspase-9 (iCasp9) [44–46]. In our ongoing clinical trial at Memorial Sloan Kettering Cancer Center (MSK), we administer MSLN CAR T cells fitted with the iCasp9 cell-suicide system to increase the safety profile of our ACT (NCT02414269). In an effort to enhance safety without resorting to the immunosuppressive effects of high-dose corticosteroid therapy or the irreversible methods of T-cell elimination by suicide genes, which subsequently limit the therapeutic efficacy of CAR T-cell therapy, Federov et al. at MSK's Center for Cell Engineering have designed an inhibitory chimeric antigen receptor (iCAR) with preclinical results that have demonstrated proof-of-concept [47]. Following surface antigen recognition, the iCAR signal travels through a transmembrane domain that fuses the scFv to the cytoplasmic machinery of inhibitory receptors, thereby specifically limiting those T cells that engage with the target antigen [47]. Cytotoxic T-lymphocyte associated protein 4 (CTLA-4) or PD-1-based iCARs offer a dynamic and self-regulating safety switch that can selectively limit cytokine secretion, cytotoxicity, and proliferation following the activation of both endogenous and CAR T cells in a temporary and reversible manner.

CHALLENGES FOR CARS TO OVERCOME SOLID TUMOR BARRIER

Following administration, tumor-infiltrating T cells encounter a hostile immunosuppressive terrain that they must overcome during their travel toward their target. The solid tumor microenvironment poses several challenges that limit the efficacy of ACT, including impaired trafficking to the tumor, penetration through the tumor stroma, and overcoming the immunosuppressive immune cells and inactivating effects of tumor-secreted factors. In an effort to enhance antitumor efficacy, several groups have developed different strategies that focus on optimizing and overcoming some of these hurdles.

The first barrier encountered by the infused CAR T-cell is the physical barrier of trafficking and homing to the tumor. In order to reach its target, there must be a successful pairing between adhesion molecules and chemokine receptors present on the T cells with those expressed and secreted by the tumor cells. Using a human MPM cell line *in vivo*, Moon et al. explored the efficacy of genetically enhancing a matched chemokine receptor (CCR2b) that is expressed on MSLN CAR T cells in response to measuring elevated levels of the corresponding tumor-secreted chemokine (CCL2) by the MPM cells [48]. This preclinical study has demonstrated that transduction of CCR2b onto MSLN CAR T cells was able to significantly enhance tumor localization, infiltration, and eradication. Another approach to enhance antitumor efficacy is to entirely bypass the systemic circulation that intravenously administered CAR T cells must traverse and regionally deliver the therapy instead. In an effort to overcome the inefficient T-cell tumor infiltration, our group evaluated intravenous versus regional administration of MSLN CAR T cells in a fully human mouse model of MPM [34]. We found that intrapleural delivery resulted in significantly enhanced antitumor efficacy and functional T-cell persistence as compared with systemic delivery. By dual tumor and T-cell bioluminescence imaging (BLI), we demonstrated that the increased efficacy following regional delivery is due to immediate antigen-activation of the majority of administered CAR T cells, as well as CD4⁺-dependent additive benefits for CD8⁺ CAR T-cell proliferation and persistence [34]. These positive results have established the rationale for our currently ongoing Phase I clinical trial that evaluates the safety of intrapleural administration of MSLN-targeted CAR T cells in patients with pleural-based malignancies (NCT02414269).

Upon arrival at the solid tumor, the T cells encounter a multitude of immunosuppressive immune cells and inhibitory factors within the tumor stroma that interfere with their effector function, which leads to inhibition and T-cell anergy. In the presence of suppressive cells, such as FoxP3⁺ expressing Tregs, myeloid-derived suppressor cells (MDSCs), CAFs, and tumor-associated macrophages (TAMs) that are known to secrete inhibitory molecules, including transforming growth factor beta (TGFβ), prostaglandin E2 (PGE2), FAP, and PD-L1, there has been an interest in exploring strategies to overcome these obstacles. Some preclinical studies have investigated the utility of incorporating vehicles such as heparanase, a matrix degrading enzyme, and genetically deleting diacylglycerol kinase, which is known to impair T-cell functionality (DGK) [49, 50]. Moon et al. found that MSLN CAR T cells undergo a tumor-induced hypofunction of cytolytic activity and gain the ability to secrete cytokines in the presence of human MPM flank tumors [51]. Failure to eliminate the tumor was in large part due to the progressive loss of function of T cells in the presence of the

immunosuppressive tumor microenvironment. This was partly attributed to upregulated intrinsic inhibitory effects of enzymes such as phosphatase SHP1 and DGK on the T cells. Blocking TGF β receptors, which is a well-known protumor cytokine that promotes tumor growth and metastasis while inhibiting the cytolytic function of T cells either systemically or by expressing a nonfunctional dominant-negative TGF β receptor, is an area that is actively being investigated as a way to preserve and enhance CAR T-cell effector functionality [52, 53].

The development of dual signaling second generation CARs, which combine activation with co-stimulatory signals, has led to a significant improvement in their functional persistence [54]. Schuberth and colleagues have demonstrated the potent effects of targeting FAP in MPM, which is expressed both by the tumor cells and in the tumor-associated stroma, with these second generation CARs [42]. With demonstrated functionality *in vitro* and *in vivo*, the University of Zurich group has translated their preclinical success using re-directed FAP-targeted CAR T cells into a Phase I clinical trial for patients with MPM (NCT01722149). However, these second generation CARs are still subject to multifactorial inhibition of their cytolytic and proliferative function that is partly mediated by the upregulation of intrinsic regulatory mechanisms and intracellular inhibitory receptors that interact with their cognate antigens on the tumor cells. CTLA-4 and PD-1 are receptors that engage following T-cell activation and act to blunt the effector response.

Our group has investigated the effect of PD-1/PD-L1 mediated T-cell exhaustion on MSLN CAR T cells using an orthotopic mouse model of MPM [55]. We have demonstrated that following repeated antigen exposure, both *in vitro* and *in vivo*, CAR T cells, although they persist, are exhausted and nonfunctional. This lack of functional persistence can be attributed to PD-1 upregulation on CAR T cells with corresponding upregulation of PD-L1 and PD-L2 on tumor cells that are exposed to effector cytokines. We have demonstrated that exhausted CAR T cells can be rescued by PD-1 checkpoint blockade; repeated administration of checkpoint blockade agents is required to sustain therapeutic efficacy. Even with a single, low dose of MSLN-targeted CAR T cells, mice survival is prolonged beyond 150 days [55]. To avoid multiple administrations of PD-1 blocking agents, we have developed T-cell intrinsic strategies. We discovered that by arming CAR-mediated co-stimulation with a PD-1 dominant negative receptor (DNR) we can enhance MSLN CAR T-cell functionality in the presence of tumor PD-L1 expression and prolong tumor-free survival [55]. PD-1 DNR *decoy receptor* binds to both PD-L1 and PD-L2. Liu et al. successfully demonstrated the utility of combining CAR T cells with a PD1CD28 chimeric switch-receptor to overcome the suppressive effects of this inhibitory regulator [56]. This switch-receptor fuses an extracellular PD-1 domain to a cytoplasmic CD28 domain, which stimulates the T-cell upon binding to the PD-L1 ligand, thereby activating, rather than inhibiting, T-cell effector function. The investigators tested the application of these cells using multiple solid tumor cell lines *in vivo*—including mesothelioma—derived from patient tumors and transduced to express mesothelin [56]. Administration of PD1CD28 resulted in significantly increased levels of T-cell infiltration, cytokine secretion, and lytic function with long-term statistical reductions in tumor volume [56]. These findings support the need for alternative therapeutic strategies that can overcome tumor-mediated inhibition of CAR T-cell effector functions.

COMBINATION STRATEGIES

Solid tumors are capable of developing multiple resistance mechanisms and combination therapies provide an attractive avenue to enhance immunotherapeutic attack from multiple directions. Although CARs have overcome the initial limitations of HLA restriction and MHC I downregulation first observed with TCRs, they are subject to novel methods of tumor escape. A concerning mechanism of relapse seen in CD19-targeted B-cell leukemia is loss of CD19 antigen expression by tumor cells. Following therapy, Ruella et al. observed this method of escape by demonstrating loss of CD19 expression in 60% of relapsed patients [57]. They concluded that dual therapy—in this case with CD19 and CD123—was a successful approach, not only to treat relapses in these patients, but as an initial therapeutic option to prevent antigen loss from the start [57]. It has also been known for over 3 decades that preconditioning the host with lymphodepleting agents, such as nonmyeloablative chemotherapy or total body irradiation, can enhance engraftment of adoptive T-cell transfer through elimination of endogenous lymphocytes, including immunosuppressive Tregs [58, 59]. Other promising strategies involve combining the recent successes of checkpoint inhibitors, such as CTLA-4 and PD-1, with ACT. CTLA-4, one of the first immune checkpoint receptors to be targeted using a monoclonal antibody, achieved early successes in melanoma patients and has been recently incorporated into first-line treatment with combination chemotherapy for extensive stage SCLC [60]. PD-1 blockade has demonstrated clinical activity as a single agent for solid tumors, such as NSCLC, melanoma, and renal cell carcinoma, and can serve to further enhance the clinical spectrum of activity of CAR T cells [61]. A Phase I clinical trial is currently recruiting patients with PD-1-positive lung, gastric, and liver cancers to receive PD-1-targeted CAR T-cell therapy (NCT02862028). A recently completed Phase Ib multicenter clinical trial has demonstrated antitumor efficacy with the combination of PD-L1 and CTLA-4 immune checkpoint inhibitors for patients with advanced squamous or non-squamous NSCLC [62]. Other promising strategies include combining CAR T-cell therapy with oncolytic viruses or whole-cell vaccines [63].

CONCLUSION

CAR T-cell therapy is a rapidly growing and promising immunotherapeutic approach to target solid tumors such as lung cancer and MPM. With an ever-evolving understanding of tumor biology and the delicate balance that exists between the protumor and proinflammatory elements of the dynamic immune system, it is more important than ever to cultivate and harness the body's own resources to fight disease. Within the expanding field of immunotherapy exists a perfect opportunity to accomplish this goal. The overwhelming successes seen in hematologic malignancies have prompted a robust investigation into better understanding barriers, such as T-cell trafficking and traversing a hostile tumor microenvironment, which can be overcome by fine-tuning the CAR construct, armoring it with additional defenses, and exploring combination therapies. The multitude of ongoing and newly established clinical trials attest to the excitement and rigorous search for a new treatment modality that is greatly needed for cancers, such as lung cancer and MPM, which, despite multimodality therapy with surgery, chemotherapy, and radiotherapy, continue to have a dismal prognosis.

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Abbreviations

ACT	adoptive cell therapy
ADC	adenocarcinoma
ALK	anaplastic lymphoma kinase
BLI	bioluminescence imaging
CAF	cancer-associated fibroblast
CAR	chimeric antigen receptor
CEA	carcinoembryonic antigen
CTLA-4	cytotoxic T-lymphocyte associated protein 4
CTCAE	Common Terminology Criteria for Adverse Events
DGK	diacylglycerol kinase
DNR	dominant negative receptor
EGFR	epidermal growth factor receptor
FAP	fibroblast activation protein
FDA	Food and Drug Administration
GD2	disialoganglioside
GPC3	glypican-3
HER2	human epidermal growth factor receptor 2
HLA	human leukocyte antigen
HSV-TK	herpes simplex thymidine kinase
huEGFR	human epidermal growth factor receptor
iCAR	inhibitory chimeric antigen receptor
iCasp9	inducible caspase-9
IL	interleukin
MDSC	myeloid-derived suppressor cell
MHC	major histocompatibility complex

MPM	malignant pleural mesothelioma
MSK	Memorial Sloan Kettering Cancer Center
MSLN	mesothelin
MUC1	mucin 1
NCI	National Cancer Institute
NSCLC	non-small cell lung cancer
PD-1	programmed cell death protein 1
PD-L1	programmed cell death-ligand 1
PGE2	prostaglandin E2
ROR1	receptor tyrosine-kinase-like orphan receptor
scFv	single-chain variable fragment
SCLC	small cell lung cancer
SMRP	serum mesothelin related peptide
TAA	tumor-associated antigen
TAM	tumor-associated macrophage
TCR	T-cell receptor
TGFβ	transforming growth factor beta
TIL	tumor-infiltrating lymphocyte
TKI	tyrosine-kinase inhibitor
Treg	regulatory T-cell
VEGFR2	vascular endothelial growth factor receptor 2

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Figure 1.

On-target off-tumor effects may be limited by targeting an antigen that is overexpressed on cancer cells relative to normal tissue.

Table 1

Current CAR T-cell Clinical Trials for Thoracic Malignancies on ClinicalTrials.gov

Target Antigen	Malignancies	Phase	NCT#	Location	Status
CEA	Breast, Colorectal, Gastric, Lung, Pancreatic	I	NCT02349724	China	Recruiting
EGFR	EGFR Positive Malignancies (Cholangiocarcinoma, Colorectal, Non-Small Cell Lung Cancer (NSCLC), Ovarian, Pancreatic, Renal)	I/II	NCT01869166	China	Recruiting
FAP	Mesothelioma	I	NCT01722149	Zurich	Recruiting
GD2	Solid tumors	I/II	NCT02992210	China	Recruiting
GPC3	Lung Squamous Cell Carcinoma	I	NCT02876978	China	Recruiting
HER2	Breast, Colorectal, Gastric, Glioma, Lung, Ovarian, Pancreatic	I/II	NCT02713984	China	Recruiting
Mesothelin	Breast, Lung, Malignant Pleural Disease, Mesothelioma, Metastases	I	NCT02414269	MSKCC	Recruiting
Mesothelin	Cervical, Lung, Mesothelioma, Ovarian, Pancreatic	I/II	NCT01583686	NCI	Recruiting
Mesothelin	Breast (Triple Negative), Endometrial, Mesothelioma, Ovarian, Pancreatic	I	NCT02580747	China	Recruiting
Mesothelin	Mesothelioma, Metastatic Pancreatic, Ovarian	I	NCT02159716	UPENN	Ongoing; NOT recruiting
Mesothelin	Mesothelioma	I	NCT01355965	UPENN	Ongoing; NOT recruiting
MUC1	Breast (Triple Negative), Hepatocellular, NSCLC, Pancreatic	I/II	NCT02587689	China	Recruiting
PD1	Gastric, Lung, Liver	I/II	NCT02862028	China	Recruiting
ROR1	Breast (Including Triple Negative), Leukemias (ALL, CLL, Mantle Cell), NSCLC; Estrogen, HER2/Neu, and Progesterone Receptor Negative	I	NCT02706392	NCI	Enrolling (by invitation only)
VEGFR2	Melanoma, Metastatic cancer, Renal	I/II	NCT01218867	NCI	Completed