Supercharging adoptive T cell therapy to overcome solid tumor–induced immunosuppression

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The development of new cancer immunotherapies including checkpoint blockade and chimeric antigen receptor (CAR) T cell therapy has revolutionized cancer treatment. CAR T cells have shown tremendous success in certain B cell malignancies, resulting in U.S. Food and Drug Administration (FDA) approval of this approach for certain types of leukemia and lymphoma. However, response rates against solid cancer have been less successful to date. Approaches to modulate the immunosuppressive tumor microenvironment including targeting checkpoint pathways, modulating metabolic pathways, and generating cytokine-producing T cells have led to considerable enhancement of adoptive T cell immunotherapy, first in preclinical models and now in patients. This review provides a discussion of the most recent strategies to enhance the efficacy of CAR T cell antitumor responses in solid cancers.

INTRODUCTION

Immunotherapy has provided substantial breakthroughs in the treatment of cancer in the last decade. One form of cancer immunotherapy is adoptive cell therapy (ACT), which is the infusion of autologous or non-autologous lymphocytes into patients after expansion ex vivo. More recently, ACT has been adapted to the utilization of genetically engineered T cells, providing either T cell receptor (TCR)– or chimeric antigen receptor (CAR)–based therapy. In particular, CAR T cell therapy has had tremendous success against B cell malignancies. However, CAR T cell efficacy in solid tumors has been limited to date, partly due to the immunosuppressive tumor microenvironment (TME) (1). This review will discuss the current state of ACT, challenges and promising strategies to augment ACT efficacy by means of targeting T cell checkpoints (Fig. 1), and the immunosuppressive TME (Fig. 2).

ADOPTIVE CELL THERAPY

Early studies investigating the use of ACT were performed using tumor-infiltrating lymphocytes (TILs). This approach has been shown to be effective for treating metastatic melanoma (2) and, to a more limited extent, other malignancies including glioma (3) and renal carcinoma (4). However, many cancers do not have a sufficient number of TILs with antitumor activity that can be collected for adoptive transfer and/or lack sufficiently immunogenic tumor (neo)antigens or major histocompatibility complex–I (MHC-I) expression required for an effective antitumor T cell response.

Building on TIL-based ACT, several creative strategies have been developed to broaden ACT application. This includes the introduction of genes encoding TCRs specific for tumor-associated antigens (TAAAs). With advances in sequencing and neoantigen prediction technology, the identification of TAAAs and tumor-specific neoantigens as targets for gene-engineered T cells is more feasible, allowing for isolation of neoantigen-specific TCRs and their expression in a bulk T cell population that can be subsequently used for ACT (5). Alternatively, T cells can be transduced with a CAR directed against a cell surface tumor antigen. A CAR consists of an antibody-derived single-chain variable fragment (scFv) designed to target a tumor antigen and is fused to T cell signaling domain(s). First-generation CARs contain the CD3ζ or FcRγ signaling domain, whereas second- and third-generation CARs contain one or two additional costimulatory domain(s), respectively, usually from a combination of CD28, CD70, 4-1BB, or OX40 (6). The impact of the signaling domains on CAR T cell function has been extensively reviewed elsewhere (7) and so will not be discussed here.

CAR T cell therapy has a number of advantages over conventional ACT. For example, unlike TCRs, CARs can recognize not only peptide antigens but also other antigens such as lipids and carbohydrates. Another major advantage of CARs is their recognition of antigens independently of MHC molecules. CAR T cells directed against CD19 have yielded remarkable clinical trial results in the treatment of certain types of B cell leukemia and lymphoma (8–11), as highlighted by the U.S. Food and Drug Administration (FDA) approval of two CAR T cell products, Kymriah and Yescarta. After this success, substantial effort has been invested in applying this approach to solid malignancies. Several antigens have been used as CAR T cell targets for solid tumors, including Her2, Lewis-Y, GD2, mesothelin, CD70, CD171, and carcinoembryonic antigen (CEA) (12). However, responses to CAR T cell therapy against solid cancers have been limited to date (1, 13). This discrepancy is thought to be partly due to the lack of CAR T cell trafficking to the tumor site, insufficient activation of the transferred T cells, and/or the increased immunosuppressive TME in established solid tumors (1). Attempts to overcome these issues have led to various innovative strategies involving combination of CAR T cells with adjuvant approaches aimed at promoting CAR T cell antitumor responses, persistence, and/or trafficking.

COMBINATION STRATEGIES TO ENHANCE ACT

Targeting immune checkpoints

T cells express various receptors on their surface that can either stimulate T cell activity or inhibit T cell responses and can be used by tumors to evade the immune system (14). PD-L1, for example, is...
overexpressed in a number of cancers, leading to impaired T cell antitumor responses via PD-L1-PD-1 interactions (14). Blocking this interaction systemically using α-PD-1 and/or α-PD-L1 monoclonal antibodies (mAbs) has shown remarkable success in the clinic (15). As a result, several α-PD-1/α-PD-L1 mAbs have been approved by the FDA, first for advanced melanoma and now for other cancer types. Similarly, α-CTLA-4 mAbs, which blocks the CTLA-4 inhibitory receptor expressed on T cells, preventing its ligation with CD80/CD86 on antigen-presenting cells (APCs), has also received FDA approval. Although these therapeutic mAbs have had remarkable success, as highlighted by the awarding of the 2018 Nobel Prize in physiology and medicine to Tasuku Honjo and James Allison (16), they are ineffective in patients with a low number of TILs (17), and therefore, administration of tumor-targeted engineered T cells makes an ideal partner for checkpoint inhibitors (Table 1). Several preclinical studies have shown that α-PD-1 mAb, either through systemic administration or via genetically engineered CAR T cells expressing α-PD-1 scFv, enhanced CAR T cell potency against solid tumors (18–20). The relevance of these immune checkpoints in the efficacy of CAR T cells in the clinic remains to be determined. However, biopsies from patients with glioma after CAR T cell therapy demonstrated increased expression of several markers associated with immunosuppression, including Foxp3 and PD-L1, highlighting the rationale for combination therapy (21). Recently, the combination of CD19-specific CAR T cells and α-PD-1 therapy was reported in a patient with refractory diffuse large B cell lymphoma (22) and demonstrated promising preliminary results. In this study, after failing to respond to CD19-specific CAR T cell therapy, the patient received α-PD-1 mAb (pembrolizumab) and had considerable clinical improvement, which was associated with a substantial expansion of CAR T cells and increased expression of granzyme B (22). This combination therapy has also been evaluated in the context of neuroblastoma involving administration of GD2-specific CAR T cells with pembrolizumab. Although pembrolizumab did not appear to enhance CAR T cell expansion in this small study, long-term follow-up revealed two of three patients treated with the combination therapy experienced complete remission, a phenomenon that was not observed in any of the patients treated with CAR T cells alone (23). Although there was no expansion of CAR T cells reported in the periphery, this study does not exclude the possibility that pembrolizumab may have increased and prolonged CAR T cell responses at the tumor site. Although this combination is promising, further testing is required to determine its broad therapeutic utility.

Successful preclinical alternative strategies to disrupt PD-1 signaling on CAR T cells include the expression of a dominant negative receptor (DNR) (24) or a switch receptor composed of a truncated extracellular PD-1 domain and a transmembrane-cytoplasmic CD28 domain (25). More recently, CRISPR-Cas9–mediated deletion of PD-1 has been demonstrated to improve CAR T cell antitumor responses (26). Together, although PD-1/PD-L1 interference is most commonly achieved by antibodies, approaches using PD-1 DNR, PD-1–CD28 switch receptor, or CRISPR-Cas9–mediated PD-1 disruption (Fig. 1) could provide an alternate route for PD-1 blockade, with the advantage of targeting only the adoptively transferred cells, hence reducing potential autoimmunity that has been observed in some cases after systemic administration of α-PD-1/PD-L1 blocking antibodies (15).

Although modulation of PD-1 signaling to enhance the efficacy of ACT has been extensively investigated, other immune checkpoints also represent promising candidates for augmenting the potency of T cell therapy. These include the adenosine receptor A2AR, CTLA-4, LAG-3, TIM-3, and indoleamine 2,3-dioxygenase (IDO). The adenosine receptor A2AR was found to be up-regulated after CAR T cell activation and limit antitumor efficacy in the solid tumor setting. In an in vivo model, A2AR blockade enhanced the capacity of α-PD-1 to enhance CAR T cell responses, indicating that this is a potential combination therapy that warrants further investigation (19). Similarly, inhibition of IDO and TIM-3 has been shown to enhance the activity of CAR T cells in preclinical models of hematological malignancy (27, 28). Although other immune checkpoints such as CTLA-4 and LAG-3 were successfully targeted using gene-editing technologies (29, 30), these studies did not reveal enhanced CAR T cell efficacy after targeting of these pathways. Therefore, the
mediated by CD8+ T cells. The use of an agonistic antibody to evoke anti-tumor responses in various preclinical models that appear to be primarily driven by the transcription of various genes involved in T cell survival, proliferation, and effector functions. The signaling pathway of CD27 is a T cell costimulatory receptor that supports T cell survival, and the production of various cytokines related to T helper cell (Th) type 1 and type 2 responses. CD27 is widely studied in preclinical models, and antibodies targeting these pathways are currently being tested in phase 1/2 clinical trials.

Using immune agonists to boost immune responses in ACT

An alternative approach to enhancing the efficacy of ACT is to use agonistic mAbs to activate costimulatory receptors. T cells express various costimulatory molecules that influence optimal T cell activation. One important costimulatory receptor family involved in immune activation is the tumor necrosis factor receptor (TNFR) superfamily. TNFR superfamily members such as CD137 (4-1BB), CD134 (OX40), CD40, and CD27 are potent costimulatory molecules that have been widely studied in preclinical models, and antibodies targeting these pathways are currently being tested in phase 1/2 clinical trials.

CD137 (4-1BB) signaling after binding of its ligand (4-1BBL), predominantly expressed on DCs, macrophages, and B cells, results in the transcription of various genes involved in T cell survival, proliferation, memory generation, and cytokine-secreting function (31). Anti–4-1BB agonistic antibodies have been shown to evoke antitumor responses in various preclinical models that appear to be primarily mediated by CD8+ T cells. The use of an agonistic α-4-1BB mAb has also been tested in combination with the adoptive transfer of transgenic T cells, resulting in improved antitumor efficacy (32). In the CAR T cell setting, several strategies to provide 4-1BB stimulation have been used. These include incorporation of the 4-1BB signaling domain into the CAR construct and overexpression of 4-1BB ligand (4-1BBL) on CAR T cells (33, 34), both of which have been tested in the clinic (35, 36). An alternative approach for providing 4-1BB costimulation involves the systemic administration of an α-4-1BB mAb in combination with CAR T cells, and this was recently shown to enhance CAR T cell effector function in mice (37). Notably, overexpression of 4-1BB and systemic α-4-1BB mAb administration have the distinct benefit of engaging not only the gene-modified CAR T cells but also other endogenous immune cells that express 4-1BB such as host T cells, NK cells, and DCs (31), potentially allowing for diversification of antitumor immune responses against target antigens beyond CAR specificity and overall increased therapeutic responses (34, 37). Together, these encouraging preclinical data have led to the development of two fully human α-4-1BB mAbs currently being tested in clinical trials. Initial results indicated that urelumab [immunoglobulin G4 (IgG4)] treatment was associated with liver toxicity in some cases. However, subsequent trials using either a distinct clone of α-4-1BB mAb (utomilumab; IgG2) or a lower dose of urelumab have shown an improved safety profile (38) and therefore could be used in conjunction with ACT.

OX40, a member of the TNFR superfamily, is a costimulatory receptor present on T cells, neutrophils, and NK cells. The engagement of OX40 by either its natural ligand OX40L that is expressed on APCs or agonistic α-OX40 antibodies induces T cell expansion, survival, and the production of various cytokines related to T helper cell (Th) type 1 and type 2 responses (39). Numerous preclinical studies have demonstrated that soluble OX40L or α-OX40 agonistic mAb promotes robust antitumor T cell immunity (40). Encouraging results have prompted multisite phase 1/2 clinical trials evaluating the safety and efficacy of α-OX40 mAbs in patients with metastatic and/or advanced solid tumors as single agents (identifiers: NCT02318394 and NCT02559024) or in combination with radiotherapy or other immunotherapy reagents (identifiers: NCT01862900, NCT02221960, and NCT02315066).

An agonistic α-OX40 mAb has been tested in combination with ACT using activated tumor-draining lymph node T cells and shown to be able to augment T cell antitumor responses in an in vivo model (41). More recently, in the context of CAR T cells, OX40 has been used as a signaling domain within the CAR construct, usually paired with the CD28 molecule in third-generation CARs, and has shown unique costimulatory activity by effectively suppressing IL-10 secretion (42). Stimulation of the OX40 receptor through the use of agonistic antibodies has yet to be tested in combination with CAR T cells, but will be an interesting alternative to direct OX40 incorporation as a signaling domain in the CAR construct.

CD27 is a T cell costimulatory receptor that supports T cell survival, proliferation, and effector functions. The signaling pathway of CD27 is primarily mediated by its ligand, CD70, which is expressed on B

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Table 1. Summary of ongoing clinical trials investigating the combination of ACT and immunomodulatory antibodies. CTL, cytotoxic T lymphocytes; DLBCL, diffuse large B cell lymphoma; NHL, non-Hodgkin’s lymphoma; N/A, not applicable.
activated TH cells leads to APC maturation (some cases, tumor cells. Stimulation of CD40 by its ligand CD40L on pressed on APCs and other cells, including nonimmune cells and, in therapeutic target to enhance antitumor immune responses. It is ex - in phase 1/2 clinical trials for patients with advanced malignancies ) and are currently being tested 44 agonistic antibodies targeting CD27 have been shown to enhance signaling molecule have been reported to date. On the other hand, preclinical data, no clinical data of CAR T cells containing the CD27 with CD28 and 4-1BB signaling domains (43). Despite emerging comparable tumor regression and T cell persistence to CAR T cells on CAR T cell activity in vitro and in vivo, and was shown to induce increased expression of IL-12 and IL-18 can lead to the stimulation of dendritic cells (DCs), resulting in further activation of T cells, whereas CCL19 can increase DC infiltration to the tumor site. Activated DCs then traffic to the lymph node and cross-present antigens to endogenous CD8 T cells, resulting in further activation of the host immune response.

**Fig. 2. Armored T cells genetically modified to express cytokines modulate the TME.** ACT of gene-engineered T cells improves therapeutic efficacy by modulating immune cells in the TME via secretion of cytokines and/or chemokines. Properly activated cells can traffic to and from the lymph node. Interleukin-18 (IL-18), IL-15, IL-12, IL-7, and CCL19 produc-duction by gene-engineered T cells increases cell proliferation, survival, activation, interferon-γ (IFN-γ) production, and memory stem cell phenotype of both engineered T cells through autostimulation and endogenous T cells. IL-18 and IL-12 can enhance natural killer (NK) cell number and activation, and polarize macrophages toward an M1 phenotype. Increased expression of IL-12 and IL-18 can lead to the stimulation of dendritic cells (DCs), resulting in further activation of T cells, whereas CCL19 can increase DC infiltration to the tumor site. Activated DCs then traffic to the lymph node and cross-present antigens to endogenous CD8 T cells, resulting in further activation of the host immune response.

cells, DCs, and activated T cells, resulting in the activation of the nuclear factor κB (NFκB) pathway (39). Incorporation of the CD27 signaling domain into CARs has been evaluated regarding its effect on CAR T cell activity in vitro and in vivo, and was shown to induce comparable tumor regression and T cell persistence to CAR T cells with CD28 and 4-1BB signaling domains (43). Despite emerging preclinical data, no clinical data of CAR T cells containing the CD27 signaling molecule have been reported to date. On the other hand, agonistic antibodies targeting CD27 have been shown to enhance antitumor immune responses (44) and are currently being tested in phase 1/2 clinical trials for patients with advanced malignancies (identifiers: NCT01460134, NCT03307746, and NCT02335918).

CD40 is a member of the TNFR superfamily that represents a therapeutic target to enhance antitumor immune responses. It is ex- pressed on APCs and other cells, including nonimmune cells and, in some cases, tumor cells. Stimulation of CD40 by its ligand CD40L on activated T cells leads to APC maturation (45), a key requirement for the induction of an effective adaptive immune response. The CD40:CD40L axis was shown to be required for effective ACT through modulation of intratumoral myeloid populations (46), and furthermore, an agonistic α-CD40 mAb has also been shown to boost antitumor responses in adoptive transfer models (47). In the context of CAR T cells, a different strategy to provide CD40 stimulation has been tested by engineering CAR T cells to express CD40L (48). This study used CD19-specific CAR T cells and reported that CAR T cells expressing CD40L exhibited enhanced cytotoxicity to CD40-expressing tumors in vitro. Treatment using these CD40L-expressing CAR T cells in vivo resulted in extended survival of mice transplanted with human B cell lymphoma cells compared to conventional CAR T cells (48). Evidence from these studies suggests that an important mechanism mediated by CD40 stimulation is the production of numerous immune-stimulating cytokines after activation of APCs, particularly DCs and macrophages (46, 47). The change in the tumor cytokine milieu toward a more immune-stimulating environment as a result of CD40 engagement is an important factor for enhancing adoptively transferred T cell activity. Agonistic α-CD40 mAbs have been developed by a number of pharmaceutical companies and are currently undergoing clinical trials in multiple types of solid cancer (identifiers: NCT02482168, NCT02379741, and NCT02588443). The combination of a CD40 agonist with other immunotherapies as discussed above in the context of ACT including CAR T cell therapy is warranted.

**Targeting the cytokine and/or chemokine milieu to enhance ACT**

An alternative approach to alleviate tumor-mediated immunosuppression is to modulate the cytokine microenvironment (Fig. 2). Modifi-cation of cytokine pathways to counteract the immunosuppressive TME has been explored in an attempt to increase the permissiveness of tumor killing by adoptively transferred T cells. Efforts into inves-tigating the potential use of this cytokine modulation approach have resulted in the generation of “armored” T cells, which are T cells engineered to overexpress pro-inflammatory cytokines. For example, insertion of a gene encoding for IL-12 into either TCR- or CAR-engineered T cells has resulted in improved efficacy in several tumor models in vivo, in part attributed to the intrinsic resistance to regulatory T cell (Treg)–mediated inhibition acquired by IL-12–secreting T cells (49, 50). In addition, ACT using IL-18–expressing CAR T cells has been shown to enhance antitumor responses in vivo through enhanced T cell proliferation and intratumoral infiltration (51, 52). A recent preclinical study comparing the activity of IL-12- and IL-18-secreting T cells found that IL-18–secreting T cells did not cause the
severe edema-like toxicity associated with elevated levels of pro-inflammatory cytokines, a toxicity that was associated with IL-12-secreting T cell treatment (53). Engineering T cells to express IL-15 has also been shown to enhance antitumor responses, associated with promotion of a T cell stem cell memory subset and augmented persistence of the adoptively transferred T cells (54, 55).

Another strategy to enhance CAR T cell efficacy is to engineer CAR T cells to be refractory to immunosuppressive cytokines present within the TME. For example, CAR T cells engineered to express a DNR to transforming growth factor-β (TGF-β) demonstrated augmented antitumor activity (56), and subsequently, clinical trials testing these “TGF-β-resistant” CAR T cells have commenced (identifiers: NCT03089203, NCT00889954, and NCT02065362). Alternatively, the TGF-β pathway can be targeted pharmacologically using TGF-β inhibitors, and such reagents have been shown to enhance antitumor immunity in preclinical models (57). Notably, TGF-β is associated with the “immune-exclusion” phenotype, which is associated with the lack of T cell penetration into the tumor center (58), and thus, targeting this axis has the potential to render solid tumors more susceptible to ACT treatment.

Enhancing trafficking of T cells to solid tumor sites

One contributing factor to the lack of CAR T cell efficacy in the solid cancer setting to date is the low efficiency of trafficking to the tumor site. This contrasts to the situation in hematological malignancies where CAR T cells encounter tumor cells in the blood immediately after infusion. An alternative approach that is feasible in some tumor types is the localized delivery of CAR T cells. For example, intracranial injections of CAR T cells were shown to elicit a successful clinical response in a patient with glioblastoma (59), a tumor type that is historically refractory to CAR T cell therapy. In many cancer types, however, or in the case of metastatic disease, localized delivery is not feasible, and therefore, attempts have been made to optimize the trafficking of adoptively transferred T cells.

T cells often express chemokine receptors that do not match the tumor chemokine signature, thus resulting in inefficient trafficking to the tumor (60). An attractive approach to overcome this problem is to match CAR T cell chemokine receptors to the tumor chemokine signature. For example, engineering T cells to express CXCR2 has been shown to enhance their migration toward various CXCL1-expressing tumor cells (61). A similar approach has also been tested using CCR2b- and CCR4-expressing CAR T cells (62, 63). The co-expression of cytokines and chemokines has also been investigated in the context of CAR T cells expressing IL-7 and CCL19, which showed superior antitumor activity in vivo compared to conventional CAR T cells. Analysis from this study reported enhanced infiltration of both T cells and DCs into tumor tissues (64).

Targeting T cell metabolism in CAR T cell therapy

Modification of T cell metabolism pathways offers yet another alternative strategy to counteract the immunosuppressive TME. The availability of nutrients and substrates in the TME is known to have a substantial influence on T cell function (65–67). For example, tumor cells can impose glucose restriction on T cells that then dampens their function by constraining the expression of EZH2, a methyltransferase consequently affecting T cell glycolysis (68). A recent study found that overabundance of potassium in the TME can decrease T cell nutrient uptake, hence restricting T cell effector programming while preserving the T cell stem-like state (69). Further, emerging evidence suggests that T cell function and differentiation are regulated by metabolism-driven processes, highlighting the potential of manipulating metabolic parameters as a way of optimizing ACT (70–72). Given that T cell persistence, expansion, and differentiation play crucial roles in determining the efficacy of adoptively transferred T cells (73, 74), tailoring culture conditions based on T cell metabolic requirements might represent a potential strategy to generate desirable characteristics of T cells for ACT.

One approach of modifying T cell metabolism that has been demonstrated to enhance the antitumor response is through the inhibition of glycolytic metabolism. Work by Sukumar et al. (73) showed that cells exhibiting high-glucose intake had reduced potential to become long-lived memory T cells compared to those with low glucose uptake. Accordingly, the use of a glycolysis inhibitor, 2-deoxyglucose, was able to increase the memory cell pool and promote enhanced antitumor function. Another study demonstrated improved T cell antitumor response by metabolically reprogramming T cells to enhance the production of phosphoenolpyruvate, a metabolite important for sustaining T cell effector functions (75).

Another way to increase the capacity of T cells to persist and replicate in the context of ACT is by promotion of mitochondrial biogenesis or oxidative phosphorylation (OXPHOS). In the context of mouse and human cancers, tumor-infiltrating T cells exhibit reduced mitochondrial mass and function and progressively lose an important transcriptional coactivator, PGC-1α, which regulates genes involved in mitochondrial biogenesis. A study reported that tumor-specific T cells could be reprogrammed by overexpression of PGC-1α to meet the metabolic needs of effector T cells, consequently rescuing their effector function and improving the efficacy of transgenic T cells in an ACT setting (76). PGC-1α overexpression has not been examined in the context of CAR T cells; however, recently, a study reported that mitochondrial biogenesis in CAR T cells can be augmented via inclusion of the 4-1BB costimulatory domain in the CAR receptor, indicating that these processes are relevant in the setting of CAR T cells (77). One strategy to increase OXPHOS in activated T cells is to provision l-arginine supplementation. In a mouse model, the combination of adoptively transferred T cells with l-arginine was shown to enhance antitumor activity (78).

Although reprogramming of immune metabolism is still in its early stages, intense research on this topic is ongoing and holds great potential. Furthermore, in addition to glycolysis and mitochondrial biogenesis, other metabolic targets such as fatty acid synthesis/oxidation, glutaminolysis, and amino acid transports seem to play important roles in T cell regulation and therefore warrant exploration in ACT (79).

Selecting T cell subsets for use in CAR T cell therapy

Therapeutic responses in patients after transfer of gene-engineered T cells have been implicated to correlate with the ability of the transferred T cells to persist and proliferate in vivo, as well as retain their effector function. The CD4+ and CD8+ T cell pools consist of effector memory, central memory, and naive cell subsets that have distinct function and differentiation potential, indicating that these T cell populations may perform differently in ACT (80). Preclinical studies have demonstrated superior antitumor activity in vivo after adoptive transfer of CD8+ CAR-transduced central memory T cells (TCM) compared to CD8+ effector memory or CD8+ naive T cell subsets (80). In a study by Gattinoni et al. (81), it was found that the less differentiated memory stem cells had even more potent antitumor activity
in vivo. Therefore, various studies have developed strategies to manufacture T cell products with memory stem and/or central memory phenotype. For example, the use of IL-7, IL-15, and IL-21 cytokines during T cell ex vivo expansion has been shown to promote memory stem cell phenotype (82, 83). Although many CAR T cell clinical trials to date have transduced cells without preselecting defined T cell subsets, an increasing number of trials are investigating the generation of CAR T cell products using predefined T cell compositions such as CD4, CD8, and/or TCM (84).

Given the emerging preclinical data suggesting that the superior antitumor efficacy of CAR T cells derived from the TCM subset, attempts have been made to enhance the representation of this subset through epigenetic regulation. For example a drug screen revealed that JQ1, an inhibitor of BRD4, could enhance the preservation of TCM ex vivo and consequently enhance their in vivo antitumor efficacy when adoptively transferred (85). The advent of CRISPR screens is likely to reveal additional therapeutic targets to promote this phenotype, which has the potential to enhance CAR T cell engraftment and efficacy in patients. This potential is highlighted by the observation that disruption of the methylcytosine dioxygenase TET2 gene led to improved T cell function (86). The combinatorial strategies of ACT with immune checkpoint inhibitors and/or immune agonists, as well as genetic modification of adoptively transferred T cells, have demonstrated tremendous potential in various preclinical models and are being translated to the clinic. Notably, there has been substantial effort invested in the creation of “off-the-shelf” CAR T cells, including the use of third-party donors, disruption of the TCR using CRISPR-Cas9 or site-specific endonucleases, or the incorporation of a CAR transgene into innate immune cells such as NK cells (90–92). However, it is important to note that innate subsets such as NK cells may be subject to distinct immunosuppressive mechanisms to CAR T cells, and the importance of these pathways will need to be investigated.

In summary, ACT, and more specifically CART T cell therapy, is a highly potent form of cancer treatment with great promise in the solid tumor setting. It is anticipated that further advances in T cell engineering and combination strategies will eventually be able to create lethal tumor-targeted T cells with the ability to overcome the immunosuppressive nature of solid cancers.

REFERENCES AND NOTES


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