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Macrophage-Based Approaches for Cancer Immunotherapy

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ABSTRACT

Adoptive cell therapy with genetically modified T cells has generated exciting outcomes in hematologic malignancies, but its application to solid tumors has proven challenging. This gap has spurred the investigation of alternative immune cells as therapeutics. Macrophages are potent immune effector cells whose functional plasticity leads to antitumor as well as protumor function in different settings, and this plasticity has led to notable efforts to deplete or repolarize tumor-associated macrophages. Alternatively, macrophages could be adoptively transferred after *ex vivo* genetic modification. In this review, we highlight the role of macrophages in solid tumors, the progress made with macrophage-focused immunotherapeutic modalities, and the emergence of chimeric antigen receptor macrophage cell therapy.

Adoptive Cell Therapy for Solid Tumors: T Cells and Beyond

The adoptive transfer of immune cells has been established as a promising approach for the treatment of cancer. While initial studies focused on the transfer of autologous tumor-infiltrating lymphocytes with endogenous antitumor activity (1, 2), advances in viral vector design, molecular biology, and lymphocyte cell culture have contributed to the rapidly growing field of genetically engineered T-cell therapy. Genetic integration of synthetic genes into lymphocytes allows for the generation of large quantities of T cells, which uniformly target a specific tumor antigen, overcoming reliance on endogenous T-cell receptor–mediated antitumor function and expanding the scope of targetable tumor antigens.

One method used for treatment of malignant disease is the introduction of a chimeric antigen receptor (CAR) into bulk peripheral autologous T cells (3). Clinical efficacy with CAR T-cell therapy has thus far been largely restricted to hematologic malignancies. As of August 2020, there are three FDA-approved products for B-cell malignancies, and over 200 active/enrolling clinical trials targeting a variety of hematologic malignancies worldwide.

In contrast, progress in CAR T-cell treatment of solid tumors has been slow to date (4–7). There are several potential causes for poor responses to CAR T-cell therapy in the solid tumor setting. First, effector cells must traffic to and penetrate into the tumor, a process that requires extravasation, chemotaxis, and stromal tissue penetration. Engineered lymphocytes have to traverse abnormal tumor vasculature with reduced adhesion molecules, experience chemokine/chemokine receptor mismatch (8), and must migrate through dense cellular and stromal barriers. Upon ingress into the tumor microenvironment (TME), effector cells encounter unfavorable conditions such as a hypoxic and acidic environment (9), expression of immune checkpoint ligands (10, 11), and an abundance of immunosuppressive cells such as tumor-associated macrophages (TAM), myeloid-derived suppressor cells, and regulatory T cells (Treg; ref. 12). In addition, chronic antigen engagement can lead to T-cell exhaustion, decreasing the effector function of CAR T cells (13). Even if the engineered cells survive in the TME, solid tumors often have heterogeneous surface antigen expression, which can lead to evasion of CAR T-cell detection, incomplete tumor clearance, and eventual outgrowth of antigen-negative tumor cells. This was clearly shown in the treatment of glioblastoma using EGFRvIII-targeting CAR T cells, where expression of EGFRvIII declined in 5 of 7 patients posttreatment (14). Finally, the identification of target antigens with minimal shared normal tissue expression presents an additional hurdle for solid tumor cell therapy (15).

Novel engineering approaches are under investigation to circumvent some of these challenges, such as genetic removal of checkpoint molecules (16), expression of chemokine receptors for tumor homing (17, 18), expression of heparanase to degrade the extracellular matrix (19), or the creation of universal immune receptors capable of targeting multiple tumor antigens to overcome antigen escape (20). While these examples provide some hope that highly engineered T cells could one day prove reliably effective in the treatment of solid tumors, they also highlight the importance of looking beyond T cells for potentially more suitable effector cells.

Although bulk peripheral T cells have been the primary focus of CAR research, the use of chimeric receptors for cancer therapy has been expanded into other lymphoid immune cell types, such as γδ T cells, natural killer T (NKT) cells, and natural killer (NK) cells (21). These lymphocyte subsets possess innate immune functions that can potentially broaden their tumor-killing capabilities beyond those of standard CAR T cells, while additionally providing an avenue toward the production of "off-the-shelf" allogeneic cell products (22–25). In the case of γδ T cells and NKT cells, preclinical studies have demonstrated the feasibility of redirecting effector function with CARs, while also maintaining desirable properties innate to the cells (26, 27). NK cells have been extensively evaluated for CAR-directed cancer therapy. The potential advantages of NK cells over conventional αβ T cells include their endogenous recognition of tumor-associated stress ligands and a reduced risk of cytokine release syndrome (24, 25). Numerous clinical trials have been initiated using CAR NK cells against both solid and hematologic tumor antigens, with complete responses being demonstrated against $CD19^{+}$ heme malignancies (28, 29).

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Though most research in the field to date has focused on the development of cellular therapies from lymphocyte-derived cells, their efficacy in the treatment of solid tumors remains elusive. Using cells of the myeloid lineage—such as monocytes and macrophages—offers a possible solution to the solid tumor-homing challenge, as these cells actively accumulate in tumors and penetrate the dense stromal tissue surrounding tumors. In addition, while lymphocytes provide direct antitumor cytotoxicity, cells of the myeloid lineage combine direct tumoricidal means with the ability to boost endogenous immunity via antigen presentation, making them a unique avenue for antitumor cell therapy development.

Macrophages in Cancer

Macrophages are highly plastic cells that serve a multitude of functions, including tissue development and homeostasis, clearance of cellular debris, elimination of pathogens, and regulation of inflammatory responses (30). Postnatal development of macrophages occurs through the MCSF– or GMCSF–dependent differentiation of circulating monocytes. These cells originate in the bone marrow from myeloid-derived progenitor cells (31). The resulting macrophage can encompass a broad spectrum of phenotypic states, dictated by the makeup of the cytokine milieu and the surrounding tissular niche (32, 33). While the scope of macrophage activation states is complex, it is generally simplified into two categories: M1 classically activated macrophages or M2 alternatively activated macrophages (34).

M1 macrophage polarization is driven by exposure to factors such as GMCSF, IFNγ, TNFα, lipopolysaccharide (LPS), or other pathogen-associated molecular patterns (35, 36). M1 macrophages promote a proinflammatory Th1 response through the secretion of cytokines such as TNFα, IL1β, and IL12, and enhance recruitment of Th1 cells to the site of inflammation through secretion of the chemokines CXCL9 and CXCL10 (37). In addition, M1 macrophages upregulate genes involved in antigen processing and presentation as well as costimulatory molecules to enhance T-cell responses (38). These functions are critical in the response to bacterial and viral pathogens and have the potential to participate in antitumor immunity (39).

M2 macrophage polarization occurs in the presence of MCSF, IL4, IL10, IL13, TGFβ, glucocorticoids, or immune complexes (39). While M2 macrophages have a critical role in normal immune function and homeostasis, such as stimulating Th2 responses, eliminating parasites, immunoregulation, wound healing, and tissue regeneration, certain subsets of M2 macrophages also play a critical role in promoting tumor progression (40). Tumors recruit both circulating monocytes and tissue resident macrophages to the TME and polarize them toward an M2 phenotype, creating TAMs, via a variety of soluble and mechanical factors. TAMs function to enhance tumor progression by promoting genetic instability, angiogenesis, fibrosis, immunosuppression, lymphocyte exclusion, invasion, and metastasis. TAMs are capable of promoting an inflammatory environment by secreting cytokines such as IL17 and IL23, which is believed to increase genetic instability (41, 42). TAMs also play a key role in suppressing endogenous antitumor immunity through upregulation of immunosuppressive surface proteins, secretion of reactive oxygen species, production of cytokines to suppress T-cell function, and secretion of chemokines that recruit Treg cells (42–45). In addition, TAMs promote tumor angiogenesis and metastasis through the secretion of factors such as VEGF and matrix metalloproteinase enzymes that remodel the TME, increase blood vessel formation, and promote tumor cell migration (42).

Thus, a central goal of macrophage-based cancer therapeutics is, stated simply, to reduce antiinflammatory macrophages and increase proinflammatory (antitumor) macrophages.

Targeting TAMs in Cancer: Reduction and Reprogramming

Given the tumor-promoting role of TAMs, a number of strategies have been developed to combat the effects of these cells. Broadly, the strategies can be divided into two groups: reducing the number of TAMs or altering their functionality within the TME. These approaches have been reviewed recently, and hence are briefly summarized here (46, 47).

Limiting the number of TAMs within a tumor can be accomplished via elimination of existent TAMs or inhibition of further TAM recruitment. The most established method of reducing TAM survival is through the blockade of the CSF1 (also known as MCSF)/CSF1R axis, an important ligand-receptor pair for the differentiation and survival of macrophages (48). This approach reduces the number of TAMs by blocking monocyte differentiation while also reducing the survival of existing TAMs. In addition, blockade using a small-molecule inhibitor of CSF1R induces repolarization of TAMs from an M2 toward an M1 phenotype (49). CSF1/CSF1R blockade has been shown to increase tumor sensitivity to other immunotherapies, such as PD-L1 blocking antibodies (50). However, these treatments are not uniformly effective, as CSF1/CSF1R blockade can be compensated for by increasing signaling through other prosurvival pathways (51) or increasing the activity of Tregs in the TME (52). Finally, CSF1/CSF1R blockade can result in the depletion of tissue resident macrophages, which are important for maintaining tissue homeostasis, due to their requirement for CSF1R signaling for survival (53). Recent clinical trials involving CSF1 signaling blockade in combination with anti-PD1 have not shown significant efficacy. Of 88 patients with melanoma or other solid tumors enrolled in a phase I combination study of small-molecule CSF1R inhibitor PLX3397 and pembrolizumab showed a partial response in only 5 of the 88 patients dosed, with another 15 achieving stable disease (NCT02452424). A separate study using the CSF1R targeting antibody cabiralizumab in combination with nivolumab failed to show benefit over standard-of-care chemotherapy in a 160 patient phase II study in pancreatic cancer (NCT03336216), highlighting the difficulty of translating the promising preclinical results of this approach.

Given the role of the chemokine CCL2 in the recruitment of circulating monocytes to tumors, there has been substantial effort in drugging the CCL2/CCR2 axis (54). Multiple studies have shown that blocking CCL2 signaling via either small-molecule inhibitors or neutralizing antibodies can decrease established tumor burden along with the number of metastatic sites in a variety of tumor models (55–57). Despite success in preclinical models, CCL2/CCR2 blockade has failed to demonstrate a similar level of efficacy in clinical trials (58). Computational modeling suggests that CCL2 blockade becomes less effective *in vivo* due to a combination of alterations in the K_D (the ratio of off-rate to the on-rate of ligand-receptor binding) of CCR2 between *in vitro* and *in vivo* contexts (59) and increased production of alternative chemokines in the TME (55, 59). CCL2/CCR2 blockade has no impact on established TAMs, which can still promote tumor progression despite inhibition of further monocyte recruitment (60). Finally, removal of CCL2 blockade therapy causes resumed tumor progression as TAMs are again recruited to tumor sites (61).

Although CSF1/CSF1R and CCL2/CCR2 blockade are the most widely studied axes for TAM depletion, other cytokines have also been shown to have a role in this process. Monocytes have been shown to be recruited to tumors through the interaction of CCL5 with CCR5 (62). Inhibiting the CCL5 axis has been shown to reduce tumor growth and metastasis (62, 63). IL8 (also known as CXCL8) is known to recruit myeloid cells to tumors (64) and inhibition of its signaling through CXCR2 can reduce TAM trafficking (65). Finally, the angiogenic and chemotactic factor CXCL12, which signals through CXCR4, can be targeted to reduce TAM infiltration (66).

In addition to altering TAM recruitment, there is significant interest in "reprogramming" TAMs from tumor supporting to tumor-rejecting cells. These approaches are based on increasing tumor cell phagocytosis, blocking "do not eat me" signals, or triggering proinflammatory signaling pathways in TAMs.

Macrophages are professional phagocytic cells that express both activating and inhibitory receptors for the phagocytosis of opsonized or apoptotic cells (67). Mouse model studies have shown that macrophages are an integral part of the response to antibody-based treatment of hematologic and solid cancers (68). Depletion of macrophages in mice decreased survival during antibody therapy (69), but the loss of NK cells or neutrophils showed no impact (69–71). Antibody-mediated therapy was also found to be more effective when inhibitory Fc receptors, such as CD32b, were knocked out or inhibited (72, 73). In addition to direct killing of tumor cells, macrophages also act as professional antigen-presenting cells. Macrophages can present tumor cell–derived antigens on both MHC class I (74) and MHC class II molecules (75), allowing for activation of an endogenous antitumor T-cell response, amplifying therapeutic efficacy and reducing the risk of tumor cell escape through antigen loss (76). Macrophage antigen presentation is not limited to the tumor site, as macrophages in tumor-draining lymph nodes are also able to prime an adaptive immune response (77).

In contrast to increasing prophagocytic "eat me" signals through the use of opsonizing antibodies, phagocytosis can also be enhanced by reducing antiphagocytic "do not eat me" signals. The most important antiphagocytic axis is based on the binding of CD47 on tumor cells to SIRPα on macrophages. CD47 is highly overexpressed on both heme and solid tumors, reducing the ability of macrophages to phagocytose these cells. Administration of anti-CD47 antibodies can be used to block the interaction between CD47 and SIRPα to increase phagocytosis. This approach has demonstrated efficacy in a variety of heme and solid tumor preclinical models (78–83). A recent clinical trial combining the anti-CD47 antibody 5F9 with the anti-CD20 antibody rituximab demonstrated a 36% complete response rate in patients with B-cell lymphomas (84). In addition to increased direct tumor cell killing, anti-CD47 treatments have been shown to alter TAM phenotypes toward an M1 phenotype (85). Other studies have also shown efficacy by blocking SIRPα on the macrophage (86) or by engineering SIRPα variants with higher binding affinities for CD47 than the WT SIRPα (87). It is imperative to note, however, that CD47/SIRPα blockade does not induce phagocytosis on its own and thus must be combined with an opsonizing agent (87).

Additional therapeutics targeting other important receptors have also been developed in an attempt to reprogram TAMs within the TME. Toll-like receptors (TLR) are a family of receptors involved in innate immune sensing that can alter macrophage phenotype. These pattern recognition receptors can respond to bacterial particles (such as LPS) or bacterial and viral genomes (such as DNA or RNA) to trigger the release of proinflammatory cytokines (88). Intratumoral injection of TLR agonists has been shown to increase monocyte recruitment and infiltration, and to induce repolarization of macrophages away from an M2 TAM phenotype (89). TLR agonists have shown promise in preclinical solid tumor models (90–92). Another target of interest for the reprogramming of TAMs is CD40, which binds to CD40L expressed on activated T cells. CD40 signaling results in the upregulation of costimulatory molecules and proinflammatory cytokines (93). CD40 agonist antibodies can slow tumor progression (94) and sensitize previously resistant tumors to chemotherapy (95). Another molecule of interest for targeting TAMs is TGFβ, which has an antiinflammatory effect and is typically expressed by macrophages during injury resolution. Because macrophages are both a source and a sink for TGFβ, this causes a positive feedback loop for TAMs, which helps to maintain the immunosuppressive environment in the TME by promoting the secretion of additional TGFβ (96). TGFβ reduces the sensitivity of TAMs to type I IFNs and STING agonists, increasing the difficulty of converting TAMs toward an inflammatory phenotype. Blockade of TGFβ, along with treatment with STING agonists, has been shown to mediate tumor regression in mouse models by upregulating expression of type I IFNs (97). Combination therapy of anti-PDL1 and TGFβ blockade mediated durable rejection of tumors in animal models (98). In addition, *in situ* TAM reprogramming has been demonstrated in preclinical models using nanoparticles carrying innate immune stimuli, such as STING agonists or mRNA encoding IRF5 and IKKβ (99, 100).

Despite the successes achieved by the strategies targeting TAMs *in situ*, there are significant drawbacks to pursuing this paradigm. First and most importantly, the TME is made of a multitude of immunosuppressive cells with functional redundancy. These cells all play a role in the progression of disease and it is unlikely that there exists a single cell type that, when targeted, will alter the TME sufficiently as to allow tumor eradication. In addition, any benefits that accrue during the course of treatment may only be transient in nature and not reflect a fundamental alteration of the TME. For example, withdrawing antibodies targeting monocyte trafficking to tumors causes the resumption of this trafficking and increased disease progression (61). Finally, all of the techniques mentioned in this review have only showed limited effectiveness in clinical trials against solid tumors (46), suggesting that a new paradigm is needed for this patient population.

Macrophages as Therapeutics

As an alternative to altering TAMs *in situ*, other groups have attempted to modify macrophages *ex vivo*, with the idea that these "educated" macrophages would naturally traffic to the tumor and alter the TME to allow for an endogenous immune response. The first group to use *ex vivo* cultured macrophages as an anticancer therapeutic was the Andreesen group in Germany. In the late 1980s, they treated 15 patients with advanced cancers, who had failed standard of care, with monocyte-derived macrophage cell therapy. Monocytes were collected via leukapheresis and were cultured with autologous serum for 7 days to allow differentiation into macrophages. Before administration to the patients, the macrophages were "educated" with IFNγ to induce the M1 phenotype. These macrophages were then introduced into patients either via intravenous or intraperitoneal injection—with doses up to 1.7×10^9 cells per injection. Although there was no measurable regression of the primary tumor site, some patients showed stable disease for up to 6 months post therapy. Out of the 7 patients with peritoneal carcinomatosis that received intraperitoneal macrophages, disappearance of ascites was seen in 2. Increased serum IL6 was seen in 7 of 15 patients, suggesting induction of an inflammatory response. Critically, there were no reported side effects other than low-grade

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fever and, in the case of intraperitoneal injections, abdominal discomfort (101). Later studies utilizing a similar procedure for the manufacturing of IFNγ activated macrophages, termed macrophage-activated killer (MAK) cells, demonstrated antitumor activity against cell lines *in vitro* and in preclinical models (102). Notably, Ritchie and colleagues have shown via ^{111}In-oxine radiolabeling of MAK cells that these educated macrophages will actively migrate to sites of metastasis in patients with metastatic ovarian carcinoma. Trafficking occurred for both intravenous and intraperitoneal injections, although it occurred in a higher proportion of patients following intraperitoneal injections. Administration of the macrophages appears to be safe, with no reported high-grade toxicities associated with treatment (103). However, in a head-to-head comparison trial with Bacillus Calmette-Guerin vaccine for bladder cancer, MAK therapy failed to demonstrate improved tumor control (104, 105).

Despite a lack of notable clinical efficacy, these studies have been highly informative for the development of macrophage cell therapies. First, dose-escalation studies have not shown any significant toxicities associated with injection of M1 macrophages. The most frequently reported side effects were low-grade fevers and discomfort at the injection site. However, due to the lack of clinical response, it is possible that the therapeutic level of MAKs is higher than the limit that was administered in these studies. While the cause of limited efficacy in these trials is not well studied, it is plausible that the endogenous antitumor activity of IFNγ-activated macrophages was insufficient to drive meaningful responses. Notably, these nonengineered macrophages did not have a means to recognize tumor-associated antigens and phagocytose cancer cells. In addition, because macrophage polarization is a continuum that changes in response to external cues, it is possible that the TME converted the adoptively transferred macrophages from the IFNγ-primed M1 phenotype toward an M2 TAM phenotype. Together, these results suggest that the addition of targeted activating receptors, together with more permanent methods of macrophage M1 polarization, are required.

CAR Macrophage Cell Therapy for Cancer

To address some of these shortcomings, several groups have published work using genetically engineered monocytes and macrophages for use as antitumor therapeutics (106–110). De Palma and colleagues developed an approach in which the gene for IFNα, which has known antitumor function, was lentivirally transduced into $CD34^+$ hematopoietic stem cells under a Tie2-driven promoter system. The $Tie2^+$ monocyte progeny localized to the tumor site, where they produced IFNα and induced antitumor activity. In addition, these monocytes did not seem to alter normal myelopoiesis or wound healing, suggesting limited off-target effects (111).

More recent work has focused on the engineering of monocyte-derived macrophages. Macrophages are highly resistant to genetic engineering with standard vectors such as lentivirus, retrovirus, and adeno-associated virus. The Landau group developed a modified lentiviral vector, Vpx-LV, which carries viral protein X, which depletes SAMHD1 and permits lentiviral transduction of primary macrophages and dendritic cells (112, 113). The Crane group has published work on macrophages transduced with Vpx-LV, termed genetically engineered macrophages (GEM), and demonstrated robust expression of transgenes such as IL21 and a TGFβ decoy receptor. GEMs persisted *in vivo* and expressed transgenes for extended periods of time (stably for >1 month). GEMs maintained responsiveness to external stimuli such as LPS (109).

To address some of the challenges associated with CAR-T and nonengineered macrophage adoptive cell therapy for solid tumors, we reported the initial development of human CAR macrophages (CAR-M) in 2016 (114). We found that a CD3ζ-based CAR was highly active in human macrophages, capable of driving phagocytosis and killing of target bearing tumor cells in a Syk-dependent manner without the addition of any soluble opsonizing factors (114, 115). CAR-mediated phagocytosis was confirmed against both heme and solid tumor targets. Elegant work published by Morrissey and colleagues in 2018 demonstrated CAR-mediated phagocytosis of antigen-bearing beads and tumor cells utilizing anti-CD19 and anti-CD22 CARs in murine macrophage cell lines and murine bone marrow–derived macrophages—confirming the ability of CARs to induce phagocytic pathways (116).

To establish a translational method for human CAR-M cell therapy, we found that the chimeric adenoviral vector Ad5f35 was able to efficiently and reproducibly transduce primary human monocytes and macrophages, delivering the CAR gene with >75% efficiency and high viability (115). Notably, CAR-M generated with Ad5f35 were shown to eliminate tumor cells more effectively than control or M1 macrophages *in vitro* and *in vivo* (115). CAR-M were able to traffic to established tumors and colocalized with metastatic foci in the lung after intravenous administration without a preconditioning regimen (115). CAR-M treatment induced a significant reduction in tumor burden and improved overall survival compared with mice treated with control macrophages in xenograft models (115).

Transduction of macrophages with Ad5f35 led to the induction of a durable M1 phenotype. Surprisingly, despite the purported plasticity of macrophage phenotype, Ad5f35 transduced macrophages did not convert to M2 upon stimulation with IL4, IL10, IL13, or tumor conditioned media. CAR-M maintained an immunostimulatory M1 phenotype in humanized mice engrafted with tumors, while control donor matched macrophages were converted to M2. In addition, CAR-M induced a proinflammatory signature in the surrounding TME, characterized by upregulation of TNF and MHC genes. Given that solid tumors are rich in TAMs, we evaluated the bidirectional interaction of CAR-M and M2 macrophages. While M2 macrophages failed to convert CAR-M from M1 to M2, CAR-M converted M2 macrophages to M1. In addition, the presence of M2 macrophages did not impact the tumor-killing capacity of CAR-M, highlighting their resistance to the immunosuppressive components of the TME (115).

Finally, CAR-M were shown to interact with cells of the adaptive immune system. CAR-M upregulated antigen presentation pathways and demonstrated heightened T-cell stimulation capacity as compared with control macrophages. Notably, CAR-M were able to present antigens to T cells following phagocytosis. In addition, CAR-M recruited both resting and activated T cells in chemotaxis experiments. Combined, these results demonstrate that CAR-M have the potential to overcome some of the key challenges cell therapies encounter in the solid tumor setting and represent a novel immunotherapeutic platform that can be broadly applied to diverse tumor antigen targets. Notably, while the direct antitumor activity of CAR-M is target dependent, the M1 phenotype is target independent and thus CAR-M have the potential to reprogram the TME and exert antitumor activity in tumors with heterogenous target antigen expression. Given that CAR-M have the ability to induce epitope spreading by priming T-cell responses against tumor neoantigens, CAR-M may reduce the likelihood of antigen escape and antigen-negative relapse. In addition, given the direct

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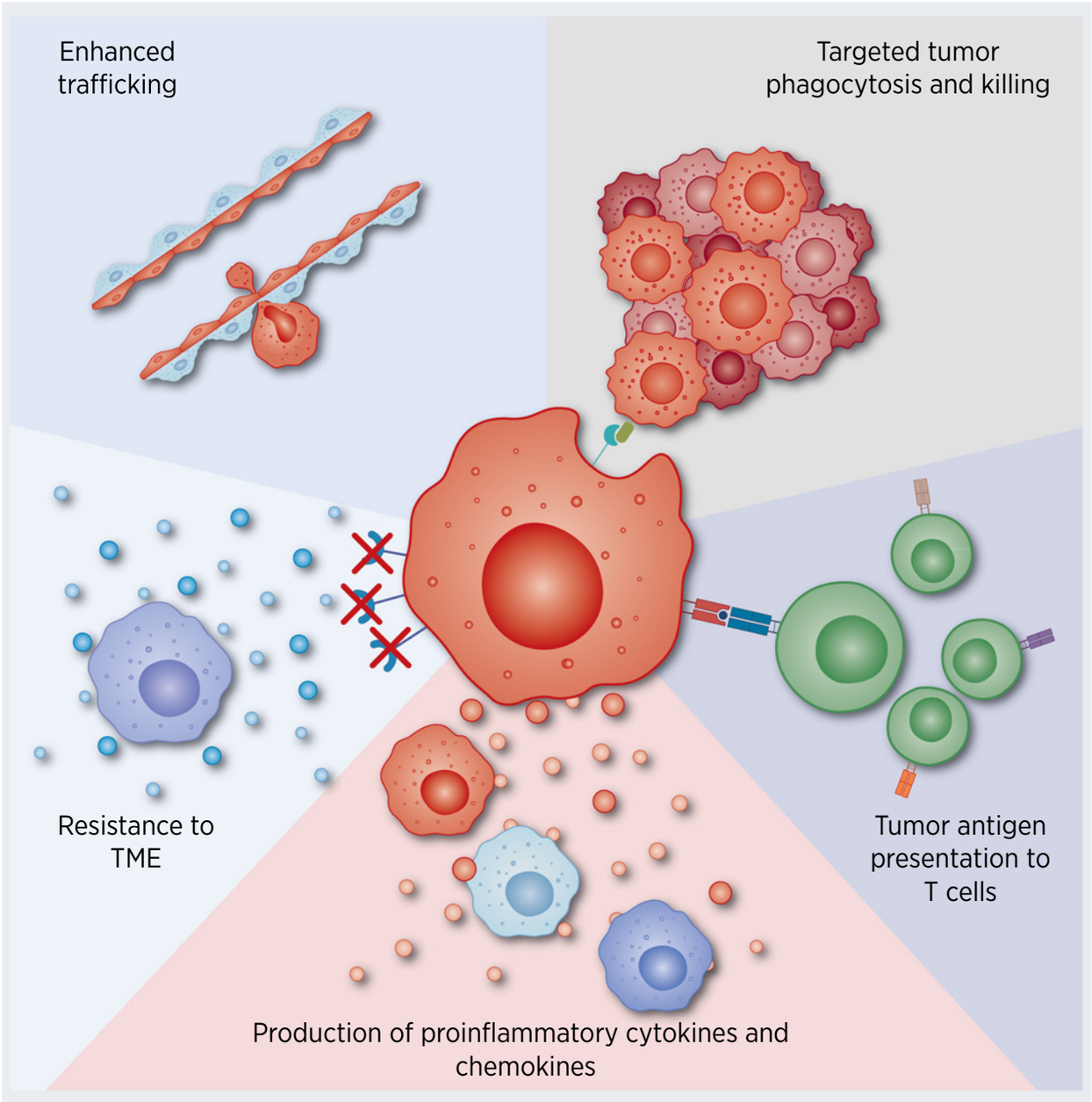

Figure 1.

The pleiotropic antitumor mechanism of CAR-M therapy. CAR-M mount antitumor immunity in numerous ways, which are summarized graphically here. CAR-M leverage the natural tumor-homing ability of myeloid cells to enter solid tumors. Once within the tumor, CAR-M directly kill antigen-expressing tumor cells through phagocytosis and secretion of cytotoxic factors. Given their M1 phenotype, CAR-M secrete cytokines and chemokines that promote a proinflammatory environment and lead to the recruitment of T cells and other leukocytes. When transduced with Ad5f35, CAR-M resist the immunosuppressive TME. Finally, CAR-M serve as an antigen-presenting cell to T cells, allowing for the induction of an adaptive immune response.

interaction between CAR-M and the adaptive immune system, rational combinations with T-cell checkpoint inhibitors are under investigation (115). These engineered monocyte-derived macrophages combine the tumor-trafficking abilities of myeloid cells, a permanent proinflammatory M1 phenotype, CAR-mediated targeted antitumor activity, and professional antigen presentation to mount a multimodal antitumor response (**Fig. 1**; ref. 115).

A critical component for the successful translation of cell therapies to the clinic is the development of a scalable and reproducible manufacturing process. CAR-M therapy is based on a 1-week manufacturing process that starts with a patient's own blood. In brief, monocytes are mobilized with subcutaneous G-CSF administration prior to leukapheresis and $CD14^+$ monocyte selection. Monocytes are differentiated to macrophages *ex vivo* and transduced with Adf535

encoding the CAR transgene. Finally, CAR-M are cryopreserved in infusible media and undergo release testing prior to initiation of therapy.

Macrophages are highly pliable cells, capable of adjusting their identity and function in response to external stimuli. Given their abundance in the TME and established role in tumor progression, there has been significant effort to reduce, reprogram, or disinhibit TAMs. Although macrophages are well known to promote tumor growth and progression, their ability to traffic to both primary tumors and metastases offers a unique opportunity for utility as a "Trojan horse" for cellular therapy. As professional antigen-presenting cells, macrophages bridge innate effector function with adaptive immunity. Advances in gene engineering, such as the discovery of Vpx-LV and Ad5f35 as effective vectors for primary human macrophage engineering, have opened the possibility to using synthetic biology to redirect macrophage effector function against tumors.

Authors' Disclosures

N.R. Anderson, N.G. Minutolo, and M. Klichinsky report being employees of Carisma Therapeutics. M. Klichinsky and S. Gill are co-founders of Carisma Therapeutics. M. Klichinsky and S. Gill hold patents related to CAR-M, which have been licensed to Carisma Therapeutics. S. Gill has received research funding from Carisma Therapeutics. No other disclosures were reported.

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