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Chimeric antigen receptor T cells in solid tumors: a war against the tumor microenvironment

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Chimeric antigen receptor (CAR) T cell is a novel approach, which utilizes anti-tumor immunity for cancer treatment. As compared to the traditional cell-mediated immunity, CAR-T possesses the improved specificity of tumor antigens and independent cytotoxicity from major histocompatibility complex molecules through a monoclonal antibody in addition to the T-cell receptor. CAR-T cell has proven its effectiveness, primarily in hematological malignancies, specifically where the CD19 CAR-T cells were used to treat B-cell acute lymphoblastic leukemia and B-cell lymphomas. Nevertheless, there is little progress in the treatment of solid tumors despite the fact that many CAR agents have been created to target tumor antigens such as CEA, EGFR/EGFRvIII, GD2, HER2, MSLN, MUC1, and other antigens. The main obstruction against the progress of research in solid tumors is the tumor microenvironment, in which several elements, such as poor locating ability, immunosuppressive cells, cytokines, chemokines, immunosuppressive checkpoints, inhibitory metabolic factors, tumor antigen loss, and antigen heterogeneity, could affect the potency of CAR-T cells. To overcome these hurdles, researchers have reconstructed the CAR-T cells in various ways. The purpose of this review is to summarize the current research in this field, analyze the mechanisms of the major barriers mentioned above, outline the main solutions, and discuss the outlook of this novel immunotherapeutic modality.

chimeric antigen T cell, solid tumor, tumor microenvironment

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Introduction

Immunotherapy has made quantum leaps in recent decades, regardless of humoral or cellular immunotherapy. As regards to the former one, monoclonal antibodies are gradually receiving widespread clinical application, with some examples being bevacizumab, rituximab, trastuzumab, and checkpoint inhibitors, including programmed death-1 (PD-1)/programmed death-ligand1 (PD-L1) blockade (pembrolizumab), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) blockade (Ipilimumab) (Gross et al., 1989; Al-Hilli et al., 2019; Amrane et al., 2019; Henon et al., 2019; Sharma et al., 2019; Soumerai et al., 2019; Tian et al., 2019). For the latter one, cellular immunotherapy has witnessed the development and testing of T-cell receptor (TCR)-redirected cell therapy and TCR autologous tumor-infiltrating lymphocytes (TILs) (Takayama et al., 2000; Goff et al., 2016). Nevertheless, both humoral and cellular immunotherapies have their own shortcomings. The effect of monoclonal an-

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tibodies highly depends on the genetic characteristics (tumor cells whose genome contain deficient mismatch repair and microsatellite instability are sensitive), specific antigens of cancer cells, and the surrounding immune microenvironment (Yaghoubi et al., 2019). Moreover, the proportion of patients, who receive long-term benefits, is still limited (Schadendorf et al., 2015). The T-cell-mediated cytotoxicity greatly relies on the human leukocyte antigen (HLA), which is produced by major histocompatibility complex (MHC) (Ti et al., 2018). To make matters complicated, the difficult isolation of TILs from the cancerous tissue poses another obstacle for clinical progression (Lee et al., 2015). Therefore, a new immunotherapy pattern is urgently needed.

Around 25 years ago, scientists have combined variable regions of an antibody with the constant regions of the TCR to create the chimeric antigen receptor (CAR) T cells (CAR-T), which is an all-in-one approach where a T cell can be engineered to possess the antibody's specificity, homing, tissue penetration, and independent cytotoxicity from MHC molecules (Eshhar et al., 1993; Mirzaei et al., 2017). The primary difference between CAR-T and traditional T cells is that the former one is equipped with a CAR part in addition to the TCR complex (Figure 1A–D). The main structure of the CAR includes extracellular, transmembrane, and intracellular domains. The first one is a tumor-antigen-binding domain, which is frequently derived from the variable light (V_L) and variable heavy (V_H) sequences of a monoclonal antibody (scFv) connected by a peptide linker (Muniappan et al., 2000; Sadelain et al., 2003) (Figure 1B). The transmembrane portion originates from CD3-Zeta chain (CD3-ζ), CD4, CD8, CD28, ICOS, etc. (Sadelain et al., 2013; Li and Zhao, 2017; Van Schandevyl and Kerre, 2018). The intracellular domain is initially a CD3ζ intracellular signaling domain capable of activating T cells, and it can be fused with one or more co-stimulatory molecules (Eshhar et al., 1993). Currently, four generations of CAR-Ts exist, and the distinction between each generation is generally the constitution of the intracellular domain. The first-generation CAR (Figure 1B) exclusively consists of CD3-ζ or Fc receptor γ (FcRγ) in an intracellular motif, which results in temporary T-cell activation (Brocker and Karjalainen, 1995). The second (Figure 1C) and third generations (Figure 1D) of CARs were built upon the foundation of the first generation by adding one (second generation) or more co-stimulatory molecules (third generation), such as CD28, 4-1BB, or OX40. These signaling domains induce expansion of T cells, increase cytokine secretion, and enhance their anti-tumor immunity (Finney et al., 2004). The fourth-generation CAR-T cells will be elaborated in the following sections (Van Schandevyl and Kerre, 2018).

Currently, the majority of studies regarding CAR-T are focused on hematological malignancies and have demonstrated excellent progress. Almost half of all CAR-T studies investigated CD19, a surface antigen of various B-cell malignancies (Beatty and O'Hara, 2016). The second generation of CD19-specific CAR-T cells with a 4-1BB co-stimulatory domain has achieved complete remission (CR) rates of 90% in patients with chemotherapy-resistant or refractory (r/t) leukemia or lymphoma. About 70% of patients who achieved CR were stable for about half a year; similarly,

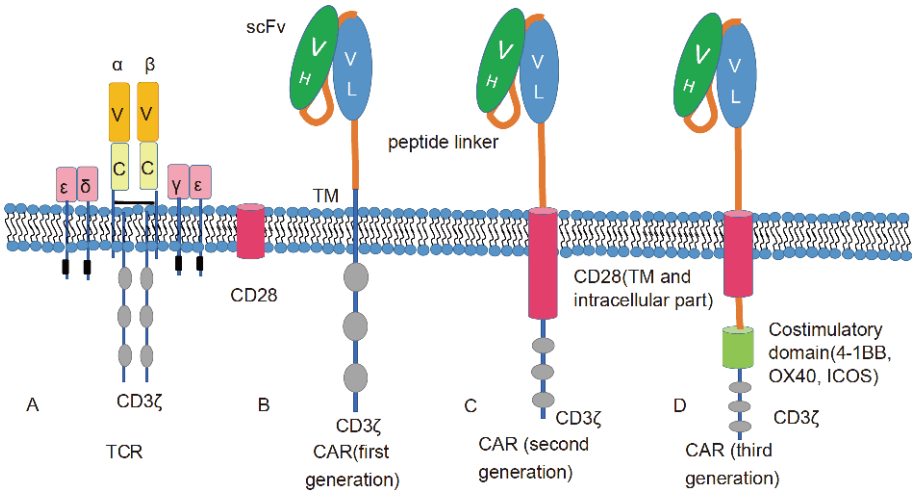

Figure 1 Basic structures of natural T-cell receptor (TCR) complex and CAR. T-cell receptor (A) consists of variable chains called α, β or δ, γ, depending on the cell types. CD3ζ is its constant portion located trans- and intracellularly. On the other hand, CAR replaces the variable chains of T-cell receptor with variable fragments from heavy and light chains of monoclonal antibodies (scFv). All three generations of CARs have a similar extracellular part: variable fragments from heavy and light chains of monoclonal antibodies. Regarding the TM part, the first-generation TM portion generally originates from the natural TCR of CD3ζ, CD4, or CD8 (B); the second- (C) and the third- (D) generation CARs utilize either CD3ζ or CD28. As for the intracellular part, the first generation only possesses CD3ζ to enable the T-cell activation (B), whereas the second (C) and the third (D) generations possess one and two co-stimulatory domains for strengthening the function of T cells, respectively. CAR, chimeric antigen receptor; scFv, mouse monoclonal antibody; TM, transmembrane part; V_L, variable light chain; V_H, variable heavy chain.

other trials have shown that CD19-CD28 CAR-T cells attained an 88% complete response rate in the adult group (Porter et al., 2011; Grupp et al., 2013; Davila et al., 2014; Maude et al., 2014; Lee et al., 2015; Brudno et al., 2016). Unlike hematological research, the CAR-T cell therapy in solid tumors takes place on a new battleground. For several years, various preclinical trials (Table 1) and clinical trials (Tables 2 and 3) are devoted to the research of typical tumor-associated antigens such as carcinoembryonic antigen (CEA), disialoganglioside (GD2), mesothelin (MSLN), human epidermal growth factor receptor-2 (HER2), epidermal growth factor receptor (EGFR), and many other tumor antigens (FAP, IL13Rα2, MUC1, PSCA, PSMA) (Townsend et al., 2018). Generally, most of them are non-specifically expressed on the surface of different cancer cells such as breast cancer, gastrointestinal cancer, pancreatic cancer, mesothelioma, and ovarian cancer, whereas some are organ-specific (i.e., PSCA and PSMA are only expressed in prostate cancer) (Martinez and Moon, 2019). So far, a few side effects of CAR-T cell infusion were recorded: fever, abnormal liver enzyme elevation, pain, and other Grade 1–2 toxicities (abnormal blood routine results) (Neelapu et al., 2018; Wang et al., 2018), whereas a minority of participants suffered from Grade 3–4 adverse events (i.e., leukopenia, hyperbilirubinemia, congestive rashes, systemic subcutaneous hemorrhages, serum cytokine release) (Neelapu et al., 2018; Wang et al., 2018). Hence, there is much room for improvement for the response to treatment. The cases, where the clinical responses were recorded, generally reported no considerable improvements. Unfortunately, most of the patients experienced deteriorating conditions or death due to the solid malignancies during treatment or in the process of follow-up. A number of factors, such as the self-origin of tumor antigens, antigen loss, the downregulation of HLA expression, resistance to CTLs, and environmental suppression, are known to hamper the advancements in the application of CAR-T cells (Sadelain et al., 2003). The purpose of this review is to outline the main issues and solutions of CAR-T cells for the treatment of solid tumors.

Main obstacles of CAR-T cell treatment for solid tumors: solid tumor microenvironment (TME)

Poor tumor locating ability

One of the main reasons for the achievement of promising results by CAR-T cells in hematological malignancies is that the infused cells can directly target the tumor cells located in the bloodstream, thereby negating the need for the CAR-T cells to seek for targets. In contrast, to attack the solid tumor cells, T cells have to first locate the tumor cells and reach the specific tumor sites via homing to the nearby lymph nodes or passing through the blood vessels. Even in the event of reaching the surface of the solid tumor, the dense extracellular matrix would restrict the penetration and infiltration of these CAR-Ts, hence preventing CAR-T cells from gaining access to the target (Mikucki et al., 2015) (Figure 2A). The regional or intratumoral application of adoptive T cells can not only exert an effective and persistent anti-tumor function in the tumor site, circumventing dense extracellular matrix, but also reduce the risk of systemic off-target toxicity (Parente-Pereira et al., 2011; Tchou et al., 2017). Studies have indicated that chemokines play a pivotal role in the restriction of homing abilities (Mullins et al., 2004; Harlin et al., 2009; Mlecnik et al., 2010; Martinet et al., 2012; Evans et al., 2015). Solid tumors can produce and secrete chemokines such as C-C chemokine ligand 2 (CCL2), but their corresponding receptors, chemokine (C-C motif) receptor (CCR) 2b, and CCR4, are sparsely distributed on the membrane of adoptive T cells, thereby causing the homing ability onto the tumor site to be disabled (Moon et al., 2011) (Figure 2B). Conversely, it is known that the major chemokine receptor is C-X-C motif chemokine ligand 3 (CXCL3), which mediates the migration of effector T cells to the tumor sites. It binds to the tumor-derived CXCL9 and CXCL10 (Mikucki et al., 2015). CCL5, which is secreted by tumor cells, can bind with its CCR5 receptor on T cells, thus indicating good T-cell intralesional infiltration and offer promising prognosis in the patients with melanoma and colorectal cancer (Sapoznik et al., 2012; Bedognetti et al., 2013). However, only a small amount of these molecules is expressed by tumor cells (Gobert et al., 2009) (Figure 2C). Some tumors, including prostate cancer, secrete chemokines such as CXCL2, CXCL5, CXCL12, CCL22, and CCL28, which attract myeloid-derived suppressor cells (MDSC) and regulatory T cells (Tregs), the two major immunosuppressive cells neutralizing the cytotoxicity of effector T cells in solid tumors (Gobert et al., 2009; Facciabene et al., 2011; Hillerdal and Essand, 2015) (Figure 2D). These chemokines significantly thwart the homing ability of effector T cells. In some solid tumors, the expression of lymphocyte integrin decreased, including lymphocyte function-associated antigen (LFA-1) and VLA-4. Weak binding of effector T cells to intracellular adhesion molecules 1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) adversely affects the T-cell migration (Kitayama et al., 1999; Kitayama et al., 1999).

Regulatory T cells and cytokines

Treg is a subset of the $CD4^+$ T cell family, accounting for 5% of the entire T-cell population in humans (Frydrychowicz et al., 2017) (Figure 3). This group of T cells feature a high expression of CD25, Forkhead Box Protein 3, and several other activating markers such as glucocorticoid-induced tumor necrosis factor receptor and CTLA-4 (Jonuleit and Schmitt, 2003; Knoechel et al., 2005; Sakaguchi et al., 2008;

Table 1 Summary of bench study of chimeric antigen receptor T cells in solid malignancies[a)]

Tumor-associated antigen	Model	Type of cancer	Generation of CAR-T	Co-stimulating domain	Reference
AFP	Human HCC cell line/female SCID-beige or NSG mice	Hepatocellular cancer	2nd generation	CD28	(Liu et al., 2017)
αvβ3	Tumor cell lines/female NSG mice	Solid tumor	2nd generation	CD28	(Wallstabe et al., 2018)
B7-H3	NALM-6-GL cell lines	Pediatrics solid tumors and CNS malignancies	2nd generation 3rd generation	4-1BB CD28 and 4-1BB	(Majzner et al., 2019)
CD166	Human cell line	Osteosarcoma	2nd generation	4-1BB	(Wang et al., 2019)
CEA	Murine pancreatic carcinoma lines; murine fibrosarcoma lines/ CEAtg mice	Pancreatic carcinoma	2nd generation	CD28	(Chmielewski et al., 2012)
EC17/folate-FITC	Human cell line/NOD/SCID gamma (NSG™) mice	Osteosarcoma	3rd generation (bispecific)	CD28 and 4-1BB	(Lu et al., 2019)
EGFR/MSLN	HEK293FT, ovarian, pancreatic BxPC3 and MCF-7 breast cancer cell lines/ male NSG mice	Solid tumor	2nd generation	CD28/GITR/4-1BB	(Golubovskaya, 2018)
EGFRvIII	Mouse glioblastoma cell line/ mouse model	Glioblastoma	2nd generation	CD28	(Chen et al., 2019)
EpCAM	Human cell lines/NOD/SCID mice	Cervical cancer Non-small cell lung cancer Breast cancer Colon cancer	3rd generation	CD28 and 4-1BB	(Zhang et al., 2019)
EpCAM	PC3, PC3M, Hela, and 293 T cells/male NOD/SCID mice	Prostate cancer	2nd generation	CD28	(Deng et al., 2015)
FAP	HT1080FAP are HT1080 cells/ NSG mice	Malignant pleural mesothelioma	2nd generation	CD28	(Schuberth et al., 2013)
FR-α	Human ovarian cancer cell lines/NSG mice	Solid tumor	2nd generation	4-1BB	(Song et al., 2011)
GD2	Neuroblastoma (NB)-derived cell lines and the leukemia cell line/male NSG mice	Solid tumor	3rd generation	CD28 and 4-1BB/OX40	(Quintarelli et al., 2018)
GPC-3	Human HCC cell lines and 293T/NOD/SCID mice	Hepatocellular carcinoma	1st generation 3rd generation	CD28 CD28 and 4-1BB	(Gao et al., 2014)
GPC-3	HepG2, Hep3B, A549, G401, and HEK 293T cell lines/NSG mice	Hepatocellular carcinoma	1st generation 2nd generation 3rd generation	CD28 CD28/4-1BB CD28 and 4-1BB	(Li et al., 2017)
GPC-3	HCC and lung adenocarcinoma cell lines/NSI mice	Hepatocellular carcinoma	3rd generation	CD28 and 4-1BB	(Jiang et al., 2017)
GPC-3 and ASGR-1	Human HCC cell lines and 293T cell line/NOD/ SCID mice	Hepatocellular carcinoma	3rd generation (bispecific)	CD28 and 4-1BB	(Chen et al., 2017)
Her-2	Patient-derived cell lines/NOG mice	Melanoma (uveal and cutaneous)	2nd generation	CD28	(Forsberg et al., 2019)
Her-2	Human cell lines/NPG mice	Colon cancer	3rd generation	CD28 and 4-1BB	(Teng et al., 2019)
Her-2 and CD24	Human pancreatic adenocarcinoma cell line and human Her2/neu^{+} adenocarcinoma cell line/ male SCID mice	Pancreatic adenocarcinoma	2nd generation (CD24) 3rd generation (Her-2)	CD28 CD28 and 4-1BB	(Maliar et al., 2012)
IL13Rα2	Murine parental GL261 (NIH), and SMA560 glioma cell lines/ mix-gender mice	Glioblastoma	2nd generation	CD28	(Pituch et al., 2018)
MSLN	Human cell lines/NPG mice	Cervical cancer/non-small cell lung cancer	2nd generation	CD28/4-1BB	(Ye et al., 2019)
MSLN	Human erythroleukemia lines and human pancreatic ductal adenocarcinoma cell lines	Solid tumor	2nd generation	4-1BB	(Beatty et al., 2014)

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Tumor-associated antigen	Model	Type of cancer	Generation of CAR-T	Co-stimulating domain	Reference
MSLN	K562-Meso cell line, human mesothelin-engineered murine ovarian cancer cell line/nude mice	Ovarian cancer	2nd generation	4-1BB	(Hung et al., 2018)
MUC1	Tumor cell lines/SCID-beige mice	Solid tumor	2nd generation 3rd generation	CD28 CD28/OX40	(Wilkie et al., 2008)
MUC16	The OV-CAR3, SK-OV3 tumor cell lines, primary ovarian cancer cells/SCID-beige mice	Ovarian cancer	2nd generation	CD28	(Chekmasova et al., 2010)
Tn-MUC/CD19	Human leukemia, pancreatic, and breast cancer cell lines/NSG mice	Solid tumor	2nd generation	4-1BB	(Posey Jr. et al., 2016)
NKG2D	Human gastric cancer cell line/ NSG mice	Gastric cancer	2nd generation	4-1BB	(Tao et al., 2018)
PSMA	Human cell line/nude mice	Prostate cancer	1st generation 2nd generation	CD3ζ CD3ζ and CD28	(Ma et al., 2014)
TAG-72	The epithelial ovarian cancer line OVCAR-3/NSG mice	Ovarian cancer	2nd generation	4-1BB	(Murad et al., 2018)
VEGFR-2	Murine tumor lines/female mice	Solid tumor	3rd generation	CD28 and 4-1BB	(Chinnasamy et al., 2010)

a) AFP, alpha fetoprotein; ASGR-1, Asialoglycoprotein receptor 1; CEA, carcinoembryonic antigen gene family; EpCAM, epithelial cell adhesion molecule; FAP, fibroblast activation protein; FR-α, folate receptor-alpha; FITC, fluorescein isothiocyanate; GD2, disialoganglioside; GPC, glycoprotein precursor; GITR, glucocorticoid-induced TNFR-related protein; Her-2, human epidermal growth factor receptor 2; IL13Rα2, interleukin 13 receptor α2; MSLN, mesothelin; MUC, mucin; NKG2D, natural killer group 2 member D; NSG, NOD/SCID/γ-chain-/-mice; PSMA, prostate-specific membrane antigen; TAG-2, tumor-associated glycoprotein 72; VEGFR-2, vascular endothelial growth factor receptor 2.

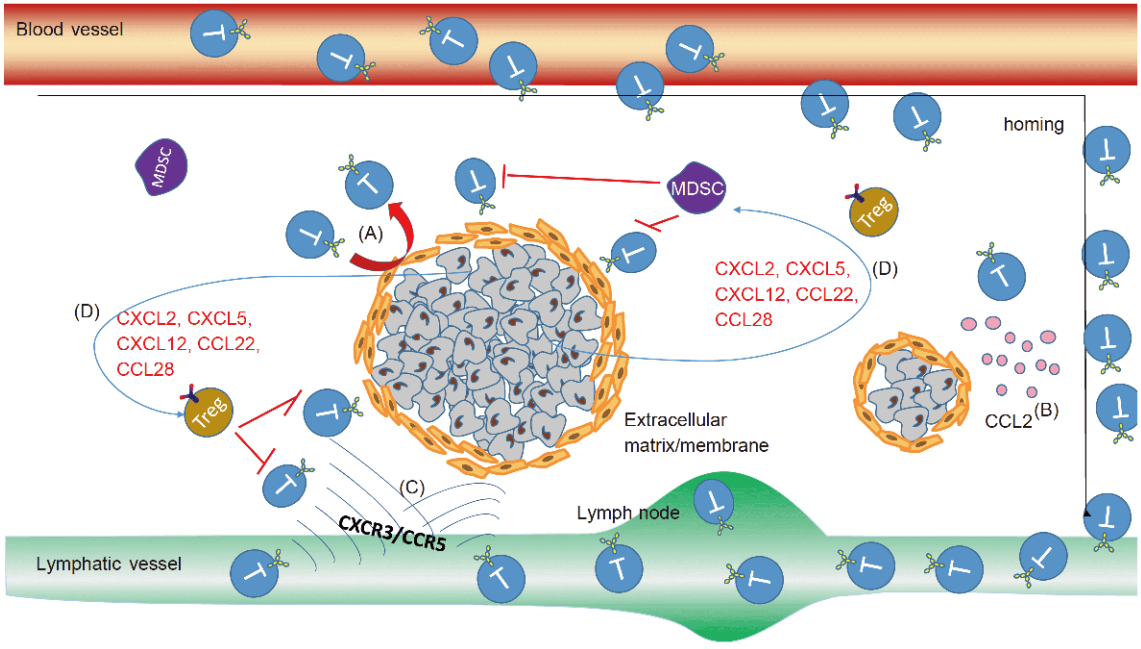

Figure 2 Factors adversely affecting the locating and homing abilities of CAR-T cells in the tumor microenvironment. To battle the solid tumors, CAR-T cells must overcome a few obstacles. The main mechanism is homing to proximal lymph nodes or extravasation through blood vessels. However, ligand-receptor mismatch makes this process much more difficult. Despite arriving at their destination, the dense extracellular matrix poses another hurdle that prevents the CAR-T cells from executing their cytotoxicity (A). As regards to ligand-receptor mismatch, CCL2 is secreted by the tumor cells but its corresponding receptor is absent in T cells, further resulting in homing disability (B). On the contrary, CXCR3 and CCR5, which are expressed on T cells, can bind with CXCL9, CXCL10, and CCL5, which are produced by the tumor cells. However, these tumor-derived molecules are only present in small amounts in the tumor microenvironment (C). The homing of T cells will be more difficult when chemokines, such as CXCL2, CXCL5, CXCL12, CCL22, and CCL28, from tumors introduce two immunosuppressive cells, MDSCs and Tregs, into the solid tumor milieu (D). CCL2, C-C chemokine ligand 2; CCR5, chemokine (C-C motif) receptor; CXCL, C-X-C motif chemokine ligand; CXCR, C-X-C motif chemokine receptor; MDSC, myeloid-derived suppressor cell; T, CAR-T cell; Treg, regulatory T cell.

Table 2 Ongoing trials of CAR-T cells in solid tumor[a)]

Antigen	Type of cancer	Patients (*n*)	Phase	ID	Result
CEA	Liver metastases	5	I	NCT02850536	Active, not recruiting
CEA	Liver metastases	20	I and II	NCT02862704	Ongoing trial
CEA	Lung cancer Colorectal cancer Gastric cancer Breast cancer Pancreatic cancer	75	I	NCT02349724	Ongoing trial
CEA	Solid tumors	6–9 (Actual number is unavailable)	I	NCT00004178	Ongoing trial
cMet	Malignant melanoma Breast cancer	10	I (Early)	NCT03060356	Ongoing trial
EGFR	Advanced glioma	10	I	NCT02331693	Ongoing trial
EGFR	Advanced malignancies	20	I and II	NCT02873390	Ongoing trial
EGFR	Advanced solid tumor	20	I and II	NCT02862028	Ongoing trial
EGFR	Advanced solid tumor	40	I and II	NCT03182816	Ongoing trial
EGFRvIII	Glioma, glioblastoma, Brain cancer	107	I and II	NCT01454596	Ongoing trial
EGFRvIII	Glioblastoma multiforme	20	I and II	NCT03170141	Ongoing trial
EpCAM	Colon cancer Esophageal carcinoma Pancreatic cancer Prostate cancer Gastric cancer Hepatic carcinoma	60	I and II	NCT03013712	Ongoing trial
EPCAM	Liver neoplasms	25	II	NCT02729493	Ongoing trial
EpCAM	Malignant neoplasm of nasopharynx TNM staging distant Metastasis (M) Breast cancer recurrent	30	I	NCT02915445	Ongoing trial
EPCAM	Stomach neoplasms	19	I and II	NCT02725125	Ongoing trial
EphA2	EphA2þ malignant glioma	60	I and II	NCT02575261	Ongoing trial
Erb/4αβ (T4)*	Head and neck squamous cell carcinoma	30	I	NCT01818323	Recruiting
FAP	Malignant pleural mesothelioma	6	I	NCT01722149	Ongoing trial
GD2	Neuroblastoma	5	I	NCT01460901	Ongoing trial
GD2	Neuroblastoma	11	I	NCT01822652	Active, not recruiting
GD2	Neuroblastoma	30	II	NCT02765243	Ongoing trial
GD2	Osteosarcoma Neuroblastoma	26	I	NCT01953900	Ongoing trial
GD2	Sarcoma Osteosarcoma Neuroblastoma Melanoma	15	I	NCT02107963	Ongoing trial
GD2	Solid tumor	100	I and II	NCT02992210	Ongoing trial
GPC3	HCC	13	I	NCT02395250	Ongoing trial
GPC3	HCC	30	I and II	NCT02715362	Ongoing trial
GPC3	HCC	60	I and II	NCT02723942	Ongoing trial
GPC3	Lung squamous cell carcinoma	20	I	NCT02876978	Ongoing trial
GPC3	HCC	10	I and II	NCT03130712	Ongoing trial
GPC3	HCC	30	I	NCT03198546	Ongoing trial
HER2	Breast cancer	60	I and II	NCT02547961	Ongoing trial

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Antigen	Type of cancer	Patients (*n*)	Phase	ID	Result
HER2	Brain tumors	14	I	NCT02442297	Ongoing trial
IL13Rα2	Anaplastic, astrocytoma Anaplastic ependymoma Anaplastic meningioma Anaplastic oligodendroglioma Brain stem glioma Ependymoblastoma Giant cell glioblastoma Glioblastoma Gliosarcoma Grade III meningioma Meningeal hemangiopericytoma Mixed glioma Pineal gland astrocytoma Brain tumor	6	I	NCT01082926	Ongoing trial
MSLN	Malignant pleural disease Mesothelioma metastases Lung cancer Breast Cancer	36	I	NCT02414269	Ongoing trial
MSLN	Malignant mesothelioma	20	I	NCT02580747	Ongoing trial
MSLN	Metastatic pancreatic (ductal) adenocarcinoma Epithelial ovarian cancer Malignant epithelial pleural mesothelioma	19	I	NCT02159716	Ongoing trial
MSLN	Pancreatic cancer	30	I	NCT02706782	Ongoing trial
MSLN	Solid tumor	40	I and II	NCT03030001	Ongoing trial
MSLN	Solid tumor	40	I and II	NCT03182803	Ongoing trial
MSLNCD19	PDAC	4	I	NCT02465983	Ongoing trial
MUC1	Advanced solid tumor	40	I and II	NCT03179007	Ongoing trial
MUC1	Malignant glioma of brain Colorectal carcinoma Gastric carcinoma	20	I and II	NCT02617134	Ongoing trial
MUC16ecto	Solid tumors	30	I	NCT02498912	Ongoing trial
NY-ESO-1	Multiple myeloma Melanoma Synovial sarcoma Myxoid/round cell liposarcoma	18	I	NCT03399448	Ongoing trial
ROR1	Estrogen receptor negative HER2/Neu negative Progesterone receptor negative Recurrent adult acute lymphoblastic leukemia Recurrent mantle cell lymphoma Refractory chronic lymphocytic leukemia Stage IV breast cancer (AJCC v6 and v7) Stage IV non-small cell lung cancer (AJCC v7) triple-negative Breast carcinoma	60	I	NCT02706392	Ongoing trial
PSCA	Lung and other cancers	30	I	NCT03198052	Ongoing trial
PSCA	Non-resectable pancreatic cancer	30	I	NCT02744287	Ongoing trial
PSMA	Prostate cancer	13	I	NCT01140373	Active, not recruiting

a) *, T4 is a kind of autologous T cells genetically equipped with a retroviral vector and it co-expresses two chimeric receptors: T1E28z, consisting of multiple ErbB dimers; 4αβ, a chimeric cytokine receptor that can stimulate expansion and enrichment of T4 (+) T-cells *ex vivo*. CEA, carcinoembryonic antigen; EGFR, epidermal growth factor receptor; EGFRvIII, epidermal growth factor receptor variant III; EPCAM, epithelial cell adhesion molecule; EphA2, EPH receptor A2; FAP, fibroblast activation protein alpha; GD2, disialoganglioside; GPC 3, glypican 3; HCC, hepatoellular carcinoma; HER2, human epidermal growth factor receptor-2; IL13Rα2, interleukin 13 receptor-α2; L1CAM, L1 cell adhesion molecule; MLSN, mesothelin; MUC1, mucin 1, cell surface associated; MUC16ecto, mucin 16 ecto; NY-ESO-1, cancer/testis antigen 1; PDAC, pancreatic ductal adenocarcinoma; PSCA, prostate stem cell antigen; PSMA, prostate-specific membrane antigen; ROR1, receptor tyrosine kinase like orphan receptor 1.

Table 3 Completed clinical trials of CAR-T cells in solid tumor[a)]

Antigen	Type of cancer	Patients (*n*)	Phase	ID	Result	Reference
CAIX	Renal cell carcinoma	12	I	DDHK97-29/ P00.0040C	No clinical response (median overall survival: cohort 1: 9.5 months (range: 3–33 months); cohort 2: 2 months; cohort 3: 12.5 months (6–24 months). Side effects: 2 of 3 patients in cohort 1 developed short-term grade 3–4 liver enzyme elevation. The same number of patients in cohort 2 suffered the same condition whereas no dose-related toxicities or higher than grade 1 liver toxicity were observed.	(Lamers Ch Fau-Sleijfer et al., 2006; Lamers et al., 2013)
CEA	Colorectal cancer	1	I	NCT00673322	Terminated	
CEA	Liver metastases	6	I	NCT01373047	One patient with stable disease (SD) by MRI and PET with other 5 died of progression of disease (PD) Side effects: Fever has been found in 4 patients. One patient suffered from grade 3 elevation of AST and ALT. No grade 4 or 5 adverse events.	(Katz et al., 2015)
CEA	Liver metastases	8	I	NCT02416466	Completed	
CEA	Breast cancer Colorectal cancer Gastric cancer Lung cancer Ovarian cancer Pancreatic cancer Unspecified adult solid tumor (protocol specific)	14	I	NCT01212887	Terminated	
cMet	Triple negative breast cancer; metastatic breast cancer	15	I	NCT01837602	Completed	
EGFR	Advanced biliary tract cancers	19	I	NCT01869166	1 CR, 10 SD (of 17 evaluable patients) The median progression-free survival was 4 months. Toxicities: grade ≥3 lymphopenia (10 of 19); grade ≥3 thrombocytopenia (2 of 19); grade ≥3 acute fever/ chill (3 of 19).	(Guo et al., 2018)
EGFR/CD133	Advanced cholangiocarcinoma	1	I & II	NCT02541370/ NCT01869166	8.5 month PR (CART-EGFR therapy) 4.5 month-lasting PR (CART-CD133 therapy) Side effect: CART-EGFR therapy: mild chills, fever, fatigue, vomiting and muscle soreness, and a 9-day duration of delayed lower fever, accompanied by escalation of IL-6 and C reactive protein (CRP), acute increase of glutamic-pyruvic transaminase and glutamic-oxalacetic transaminase, and grade 2 lichen striatus-like skin pathological changes. CART-CD133 cells: grade 3 systemic subcutaneous hemorrhages and congestive rashes together with serum cytokine release, which needed emergent medical intervention including intravenous methylprednisolone.	(Feng et al., 2017)
EGFR	Non-small-cell lung cancer	11 (median age: 58 years; range: 40–66 years)	I & II	NCT01869166	2 PR, 4 PD, 5 SD Side effect: One patient suffered from grade 3–4 toxicity (serum lipase elevation) and some other patients suffered from grade 1–2 toxicities.	(Feng et al., 2016)
FR-α	Ovarian cancer	14	I	NCT00019136	No reduction in tumor burden was seen in all patients. Side effects: One patient reported no severe CAR-T cell related toxicities. 12 patients experienced grade 1 or 2 toxicity events and 5 patients experienced grade 3–4 toxicity events.	(Kershaw et al., 2006)
GD2	Neuroblastoma	19 (10 females) (median age: 7 years; range: 3–20 years)	I	NCT00085930	Four patients indicated no evidence of disease; 2 patients with CR; 3 people alive with disease whereas 10 patients died of disease. No severe or dose-dependent toxicities have been recorded.	(Pule et al., 2008; Louis et al., 2011)
HER2	Advanced HER-2 positive solid tumors (biliary tract and pancreatic cancers) Chemotherapy refractory HER-2 antibody inhibitor therapy refractory	11	I & II	NCT01935843	1 PR; 5 SD; 5 PD Side effect: One case for grade 3 fever and 1 patient for abnormal transaminase elevation. Other moderate adverse effects include slight skin pruritus and upper gastrointestinal hemorrhage.	(Feng et al., 2017)

(*To be continued on the next page*)

(*Continued*)

Antigen	Type of cancer	Patients (*n*)	Phase	ID	Result	Reference
HER2	Glioblastoma multiforme	17 (median age: 49 years; range: 11–71 years)	I	NCT01109095	1 PR; 7 SD; 8 PD (1 patient was unable to be evaluated) Side effect: No severe adverse effects or cytokine release syndrome within doses of 1×10^{-6}/m^2–1×10^{-8}/m^2	(Ahmed et al., 2015)
HER2	HER2-positive sarcoma (unspecific subtype of cancers)	19 (median age: 17 years; range: 7.7–29.6 years)	I & II	NCT00902044	4 SD (3 patients got their residual tumor removed); 13 PD; 2 cases not evaluable. Side effect: One patient on the highest dose level had fever for 12 h and was resolved with ibuprofen. No other side effects have been recorded.	(Ahmed et al., 2015)
HER2	HER2-positive metastatic cancers (unspecific subtype of cancers)	1 (39 years old)	I & II	NCT00924287	This patient failed to survive the whole procedure of evaluation. This patient suffered from serious adverse events (decreased platelet count, cardiopulmonary arrest, lower gastrointestinal hemorrhage, renal failure, dyspnea)	
IL13Rα2	Glioblastoma	3 (2 females, 57 and 35 years old; 1 male, 57 years old)	I	NCT00730613	According to flow cytometry, qPCR analysis and IHC staining of paraffin embedded histological sections, dramatic down-regulation of IL13Rα2 expression of excised tumor occurred in one patient but treatment failure was indicated by IL13Rα2-negative tumor relapsed. According to MRI, 2 of 3 subjects showed no recurrence while the third subject suffered from IL13Rα2-negative tumor. Side effects: Grade 3 headache occurred in one subject and one grade 3 neurologic event happened in another patient. The third one had no severe adverse events related to CAR-T cell infusion.	(Brown et al., 2015)
L1-CAM	Neuroblastoma	6	I	NCT00006480	1 PR and 5 SD 56 days after CAR-T cell infusion. But all patients died of disease ultimately with only one achieved a relatively long survival. Side effects: One patient suffered from grade 3 pneumonitis and in another subject tumor pain occurred. No grade 4 or 5 adverse event was found.	(Park et al., 2007)
MSLN	Cervical cancer Pancreatic cancer Ovarian cancer Mesothelioma Lung cancer	136	I & II	NCT01583686	Terminated	
MSLN	Pleural mesothelioma	2	I	NCT01355965	One patient experienced a transient partial response (PR) according to imaging result. No detailed clinical outcome presented for another recipient. Side effects: One patient reported minimal arthralgia and fatigue while another one suffered from anaphylaxis and cardiac arrest event after third infusion.	(Maus et al., 2013)
VEGFRII	Metastatic cancer Metastatic melanoma Renal cancer	24 (17 males, 7 females)	I & II	NCT01218867	1 PR, 1 SD, 22 PD. One patient died of disease during treatment. Side effects: All but one patient suffered from adverse events. 23 recipients experienced blood and lymphatic system disorders. More than 4 participants suffered from gastrointestinal events. Two suffered from fever and 8 afflicted by fatigue. Seven suffered from dyspnea. Eight suffered from hypoalbuminemia.	
PSMA	Prostate cancer	6 (5)	I	NCT01929239	Two patients had PR but relapsed after 1–2 months. Other patients failed to meet clinical response. Side effects: No adverse effect was recorded.	(Junghans et al., 2016)

a) CAIX, carboxy-anhydrase IX; CEA, carcinoembryonic antigen; CR, complete response; EGFR, epidermal growth factor receptor; FR-α, folate receptor alpha; GD2, disialoganglioside; GPC 3, glypican 3; HCC, hepatocellular carcinoma; Her-2, Human Epidermal growth factor Receptor-2; IL13Rα2, interleukin 13 receptor-α2; L1CAM, L1 cell adhesion molecule; MLSN, mesothelin; PR, partial response; PSCA, prostate stem cell antigen; SD, stable disease; VEGFR-II, Vascular endothelial growth factor receptor II; PSMA, prostate-specific membrane antigen.

Whiteside, 2014). Treg is a heterogeneous subpopulation, and at least two Treg subsets, naturally occurring Treg cells (nTregs) and inducible or adaptive Tregs (iTregs), have been discovered in humans. They differ by possessing dissimilar types of surface antigens (Miyara and Sakaguchi, 2011). In the solid TME, nTreg triggers immunosuppression by cell-cell contact in the pathways, which are predominated by granzyme B/perforin or Fas/FasL (Jonuleit et al., 2001; Grossman et al., 2004; Strauss et al., 2009). In addition, this pathway is highly dependent on IL-2, but they inversely

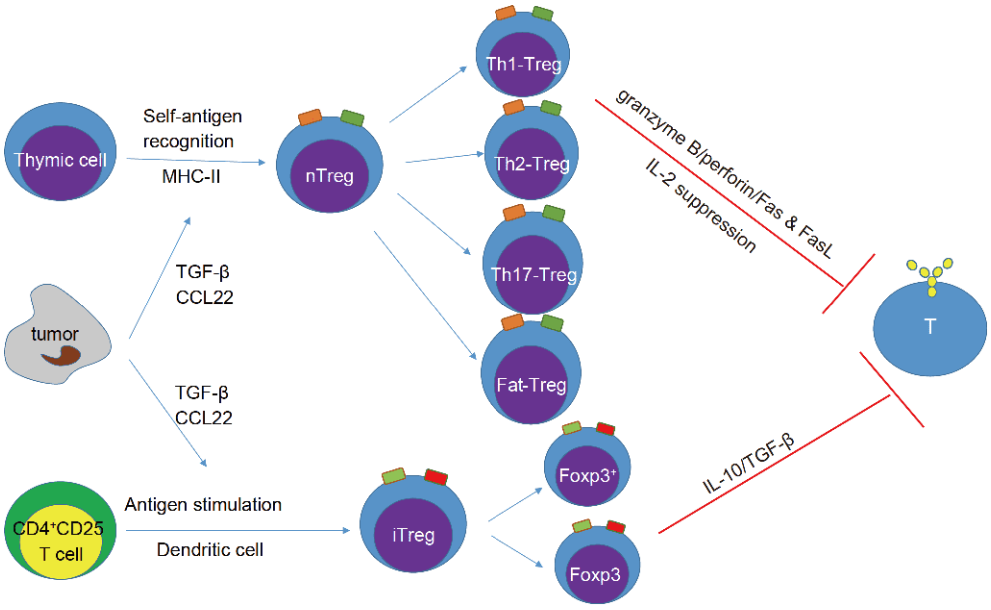

Figure 3 Diagram of the differentiation and major classifications of Tregs and their major functions toward the effector T cells. The Treg family mainly includes two subtypes: nTreg and iTreg. Through positive selection, nTreg can differentiate from $CD4^+CD25^+$ thymic cells and can further develop into several types (Th1, Th2, Th17, Fat, and so on). nTregs can inhibit T-cell function via granzyme B/perforin or Fas/FasL pathways, and the depression of IL-2 secretion of T cells is another option. On the contrary, iTreg is derived from the $CD4^+CD25^-$ mature T cells in the periphery. Instead of killing cells via contact, iTreg generally eliminates the T cells via IL-10 and TGF-β suppression. In contrast to nTreg, iTreg can be further differentiated into $Foxp3^-$. Tumor cells can also stimulate Treg differentiation, thereby adversely affecting the anti-tumor function of T cells. iTreg, inducible or adaptive Treg cells; nTreg, naturally occurring Treg cells; MHC, major histocompatibility complex.

repress the effector T-cell function by inhibiting their expression and production of IL-2 (Shevach, 2002). nTreg can persist and exhibit stable functioning in the periphery (Azimi et al., 2016). It can be further differentiated into Th1-Treg, Th2-Treg, Th17-Treg, Fat-Treg, and Tfr-Treg. In contrast to nTreg, iTreg is derived from $CD4^+CD25^-$ mature T cells in the peripheral tissue following the stimulation of antigens from the antigen-presenting cells (typically immature dendritic cells (DC)) (Steinbrink et al., 1997; Jonuleit et al., 2000; Jonuleit and Schmitt, 2003; Shevach, 2006; Nedoszytko et al., 2017). The functional characteristics of iTreg include independence from cell-cell contact and cytokine-related immunosuppression by IL-10 and transforming growth factor-β (TGF-β) (Jonuleit and Schmitt, 2003). TGF-β is capable of dampening the function of effector T cells and inducing the switching of effector T cells into the regulatory phenotype (Chen et al., 2005; Liu et al., 2007). iTreg can be further classified into two types: $Foxp3^+$ and $Foxp3^-$ (Chen et al., 2003; Sakaguchi et al., 2008; Nedoszytko et al., 2017). According to the measurements from one bench study, in the tumor environment, Treg accounts for approximately 15% of the total $CD4^+$ cells and approximately 20% of TIL and lymphocytes in nodes (Liyanage et al., 2002). Tumor cells also play an important role in Treg's immunosuppression. Researchers have found that cancer cells can secret TGF-β and chemokine CCL22 to trigger Treg differentiation and recruit peripheral Tregs to counteract the anti-tumor function of T cells (Curiel et al., 2004; Chen et al., 2005). Owing to the difficulty of eliminating Tregs in the TME (Byrne et al., 2011) and the high production of TGF-β from Tregs, it is crucial to prevent TGF-β-mediated immunosuppression. Thus, one study designed a dominant-negative TGF-β receptor II on CAR-T cells, thus assuring better proliferation, infiltration, anti-tumor immunity, and persistence in the mouse prostate cancer models (Kloss et al., 2018).

Myeloid-derived suppressor cell (MDSC)

MDSCs include various populations of cells derived from the bone marrow, which includes immature myeloid progenitors for neutrophils, monocytes, and DC (Yang et al., 2004; Ornstein et al., 2018). According to their surface molecules, MDSC can be divided into two subtypes: $CD11b^+Gr1^{hi}$, which represents the immature neutrophil phenotype and is designated polymorphonuclear MDSC (PMN-MDSC), and $CD11b^+Gr1^{low}$, which comprises the cells with a monocyte-like phenotype and is termed monocytic MDSC (m-MDSC) (Movahedi et al., 2008; Sawanobori et al., 2008; Marigo et al., 2010; Ben-Meir et al., 2018) (Figure 4). The immunosuppressive nature of MDSC induces the downregulation of the function of T, B, natural killing (NK), and DC in cancerous or cancer-free peripheral microenvironments (Gabrilovich et al., 2007; Baniyash, 2016; Gabrilovich, 2017; Meirow and Baniyash, 2017). MDSCs mainly attack T cells (Gabrilovich, 2017), and different subpopulations of MDSCs are known to suppress the

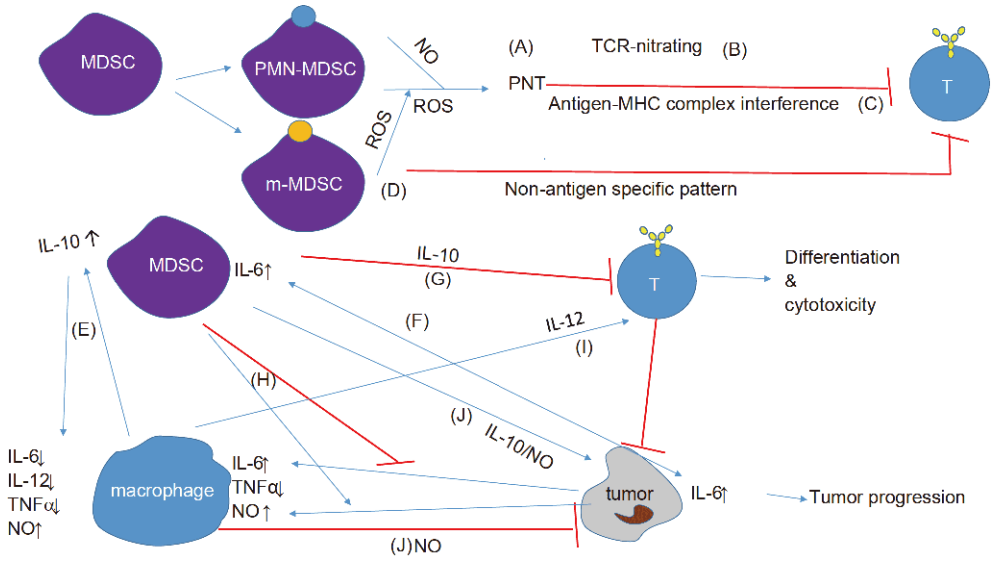

Figure 4 MDSC classification, its immunosuppressive pathway on T cells, and the network among major immune cells in the tumor microenvironment. According to the cell surface biomarkers, MDSC can be categorized into PMN-MDSC and m-MDSC. The former group can negatively affect the potency of T cells by ROS, which can react with NO to produce peroxynitrite (PNT) (A). PNT can nitrate TCR, thereby leading to a weakened combination of TCR and MHC molecules (B). In addition, PNT can disrupt the linkage between MHC and tumor antigens to blunt the alertness of T cells (C). On the contrary, m-MDSC can utilize both antigen- and non-antigen-specific patterns to interfere with the immune capability of T cells (D). The non-antigen-specific pattern mainly includes cytokines such as IL-6, IL-10, IL-12, and TNF-α. Macrophages can stimulate exclusive IL-10 expression in MDSC, whereas IL-10 from MDSC reversely inhibits the expression of IL-6, IL-12, and TNF-α by macrophages, with the exception of increasing NO secretion (E). IL-6 can be generated by MDSC, tumor cells, and macrophages, whereas the tumor cells and MDSC can stimulate each other's yield of IL-6 (F). IL-10 is produced by MDSC and, akin to IL-6, it is also an immunosuppressive molecule that can weaken the T-cell activation (G). MDSC can adversely affect IL-6 production and positively affect the NO production of macrophages in the tumor microenvironment (H). IL-12 is generally secreted by macrophages and can trigger T-cell activation (I). NO is a two-edged sword, possessing both immune activation and depression traits (J). MHC, major histocompatibility complex; M-MDSC, monocytic MDSC; PMN-MDSC, polymorphonuclear MDSC; NO, nitric oxide; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α.

effect of T cells via different mechanisms (Gabrilovich, 2017). It was found that PMN-MDSC neutralizes the immune response via an antigen-specific pattern, whereas the m-MDSC convicted this crime via both antigen or non-antigen-specific patterns (Gabrilovich et al., 2012) (Figure 4A–D). In the antigen-specific immunosuppressive pathway of MDSC, reactive oxygen species (ROS) is a key element including hydrogen peroxide, superoxide anion, and peroxynitrite (PNT) (Gabrilovich, 2017). Superoxide reacts with nitric oxide (NO), a chemical produced by the NO synthase 2 (*nos*2) gene in MDSC, and generates PNT (Figure 4A), a molecule that can directly weaken the function of T cells by nitrating TCR (Figure 4B) and impairs their alertness to the homogenous antigen-MHC complexes (Nagaraj et al., 2007) (Figure 4C). In addition, PNT is also capable of preventing the T-cell migration via the nitration of T-cell specific chemokines (Lu et al., 2011; Molon et al., 2011). To survive this oxidative environment, CAR-T cells have been genetically modified to produce an anti-oxidant intracellular enzyme catalase (CAR-CAT), which have resulted in promising anti-tumor capabilities (Ligtenberg et al., 2016). Additionally, PMN-MDSC can secrete arginase and indoleamine, both of which can decompose amino acids, which are necessary for T-cell growth and activation (Bronte et al., 2003; Yu et al., 2013). This is proven by published results in which the exhaustion of PMN-MDSC protects the efficacy of CAR-T cells (Burga et al., 2015). Cytokines that induce IL-6 and IL-10 also play a key role in mediating MDSC's immunosuppressive function. To promote a pro-TME, MDSC produces the increasing amounts of IL-10, thereby leading to the downregulation of IL-6, IL-12, and tumor necrosis factor-α (TNF-α) and upregulation of NO from macrophages (Beury et al., 2014) (Figure 4E). Macrophages can stimulate the IL-10 production of MDSC (Gabrilovich et al., 2012) (Figure 4E), but extrinsic IL-6 (macrophage-secreting or other source) has no direct effect on IL-10 (Beury et al., 2014). Additionally, tumor cells and MDSC can stimulate IL-6 production from one another, thus contributing to the whole immunosuppressive environment (Sinha et al., 2007; Bunt et al., 2009; Beury et al., 2014) (Figure 4F). Among these cytokines, IL-6 stimulates the tumor cell growth, development, and vascularization while reducing tumor cell death (Becker et al., 2005; Santer et al., 2010; Su et al., 2011). IL-10 supports tumor growth, suppresses T-cell-related cytotoxicity, and prevents tumor-specific helper T type 1 (Th1) responses (Garcia-Hernandez et al., 2002; Kawamura et al., 2002; Yang and Lattime, 2003) (Figure 4G). Interestingly, MDSC can interfere with the communication between tumors and macrophages as it can suppress the yield of macrophages of IL-6 in the tumor's presence while sti-

mulating NO production of macrophages in the tumor tissue (Beury et al., 2014) (Figure 4H). IL-12 from macrophages promotes the differentiation of naïve helper T cells to Th1 phenotype and activates the function of cytotoxic T cells, natural killer T cells, and conventional NK cells (Vom Berg et al., 2013) (Figure 4I). TNF-α can trigger DNA damage of tissue cells, inhibit their apoptosis, and enhance tumor cell invasion and metastasis by increasing the production of cytokines, chemokines, and matrix metalloproteases (Balkwill, 2006). In contrast to the cytokines above, NO possesses both pro-tumor and anti-tumor activities (Figure 4J). The former function is executed when NO is produced by MDSC, and the latter function is activated when it is secreted by M1-like macrophages (Hussain et al., 2004; Bronte and Zanovello, 2005).

Tumor-associated macrophages (TAM)

TAM are present abundantly in the solid TME (Fujimura et al., 2018), especially in inflammation-mediated and hypoxic tumor sites (Qu et al., 2018; Shrivastava et al., 2019). Gentles et al. found that a high burden of TAM is associated with poorer prognosis in most cases of solid tumors (Gentles et al., 2015). Technically, TAM consists of a heterogeneous mixture of immature myeloid cells and are mainly divided into M1- (Figure 5A) and M2-like (Figure 5B) phenotypes, where the latter one is predominant in tumors (Fujimura et al., 2016). In humans, M2 can be further classified into M2a, M2b, and M2c, which are differentiated from M0 by IL-4/IL-13, immune complexes, IL-1β or LPS, and IL-10/transforming growth factor (TGF)-β/glucocorticoids, respectively (Mantovani et al., 2009; Chistiakov et al., 2015). Generally, in the TME, a high level of M1-TAM usually indicates a better outcome for its anti-tumor effect, though, sometimes, it can also trigger tumorigenesis via chronic inflammation (Qian and Pollard, 2010). Normally, it is capable of antigen presentation and high expression of pro-inflammatory cytokines (e.g., IL-1, IL-6, IL-12, IL-18, IL-23, and high levels of IL-1 receptor type I (IL-1RI)) (Santoni et al., 2013; Chavez-Galan et al., 2015). Regarding chemokines, CXCL9 and CXCL10 can be produced for the recruitment of Th1 T cells, which allows the achievement of subsequent killing of tumor (Verreck et al., 2004; Santoni et al., 2013; van Dalen et al., 2019) (Figure 5C). These kinds of TAMs also exhibit potent phagocytosis and cytotoxicity toward tumor cells by secreting NO, reactive oxygen intermediates (ROI), and TNF-α (Mantovani et al., 2005) (Figure 5D). However, M1 cells can be polarized into the M2 type via Th2 cell-derived IL-4, IL-10, and IL-13 (van Dalen et al., 2019) (Figure 5E). M2-TAM tends to dismiss the capability of antigen presentation and tends to secrete immunosuppressive molecules, such as IL-4, IL-10, TGF-β (Figure 5F), adrenomedullin, vascular endothelial growth factors (VEGFs) (Figure 5G) and so forth to help complete angiogenesis, debris scavenging, tissue re-

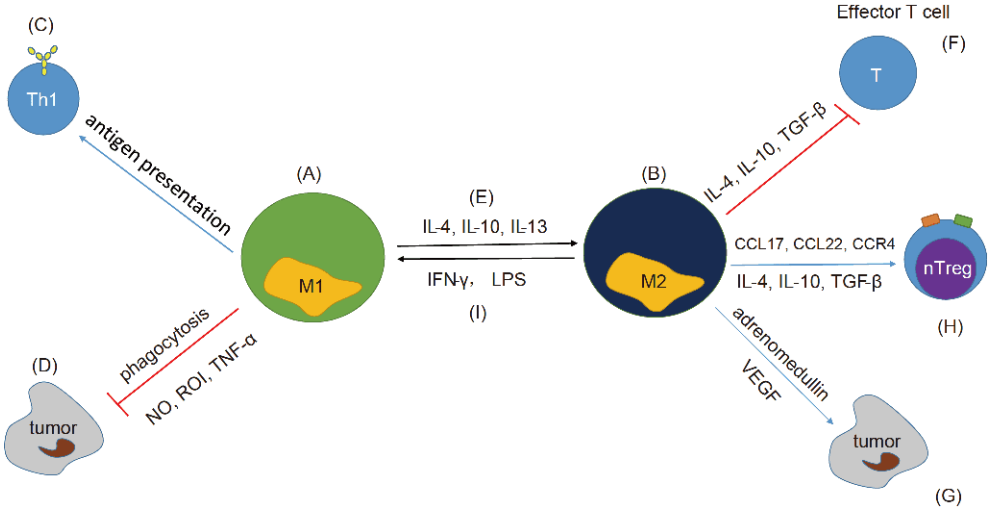

Figure 5 Major types of tumor-associated macrophages (TAM) and the network of interactions between the macrophage, T cell, tumor cell, and Treg. TAMs are divided into two main types: M1 (A) and M2 (B). The M1 TAM is pro-inflammatory while the M2 TAM is anti-inflammatory. The M1 TAM is capable of exerting indirect cytotoxicity via the secretion of cytokines such as IL-1, IL-6, IL-12, and IL-18 or chemokines such as CXCL9 and CXCL10 to stimulate the immune response of Th1 cells (C). Moreover, it can present tumor antigens via phagocytosis and exert direct tumor-cell cytotoxicity via NO and ROI (D). Conversely, M2 cells exert an immunosuppressive trait via the inhibition of the immune response of effector T cells using immunosuppressive molecules: e.g., IL-4, IL-10, and TGF-β (F). Through the excretion of adrenomedullin and VEGF, M2 TAMs support tumor angiogenesis and debris scavenging (G). Furthermore, M2 cells can also induce nTreg functions via the production of cytokines including IL-4, IL-10, and TGF-β or chemokines such as CCL17, CCL22, and CCR4 (H). Importantly, M1 and M2 can transform into one another (polarization) in certain circumstances by the presence of different cytokine patterns in the tumor microenvironment (E, I). IFN-γ, interferon-γ; LPS, lipopolysaccharide; NO, nitric oxide; nTreg, naturally occurring Treg cell; ROI, reactive oxygen intermediate; TGF-β (transforming growth factor beta); TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

modeling, and immune modulation (Wu et al., 2010; Gabrilovich et al., 2012). Studies have proved that M2 TAM can dampen the proliferation and persistence of CAR-T cells (Ruella et al., 2017). Moreover, the M2 phenotype can depress tumor immunity by attracting nTreg infiltration via the production of cytokines (IL-10, TGF-β) or chemokines such as CCL17/CCL22 or CCR4 (Columba-Cabezas et al., 2002) (Figure 5H). As interferon-γ (IFN-γ, derived from Th1 cells, cytotoxic T cells, and NK cells) and lipopolysaccharide (LPS, from bacteria and other pathogens) exists in the TME, the M2 type will be repolarized into the M1 variant (Chavez-Galan et al., 2015) (Figure 5I). To interfere with the pro-tumor activity of TAM, scientists are devoted to discovering targets against them. One of the candidates is colony-stimulating factor 1 receptor (CSF1R), which is especially relevant to M2 TAM (Zhang et al., 2018). A third-generation anti-CSF1R CAR-T cell was tested for their cytotoxicity on macrophages. The results demonstrated that these CAR-T cells are capable of accurately targeting the macrophage antigens. In addition, they also exhibit potent cytotoxicity, which is promising for CAR-T cells to prevent immunosuppression in the TAM-rich microenvironments.

Immunosuppressive checkpoint ligand/receptor

Immunosuppressive checkpoints are a group of auxiliary molecules which predominantly regulate the adaptive immune response, especially in T cells. These newly found molecules can suppress the adaptive immune response initiated by T, B, and other immune cells (Marcucci et al., 2017; Wieder et al., 2018). The immune checkpoint consists of two components: the immune checkpoint receptor and immune checkpoint ligand. Both of these can appear on the surface of APCs, T cells, B cells, or tumor cells (Pardoll, 2012) (Figure 6). Recently, numerous studies have found that solid tumors, including small/non-small cell lung cancer (Garassino et al., 2018), breast cancer (Kumar et al., 2017), melanoma (Cervera-Carrascon et al., 2018), and head and neck squamous cell carcinoma (Chen et al., 2017) express immunosuppressive checkpoints such as PD-1, PD-L1 (B7-H1), PD-L2 (B7-DC) (Shibahara et al., 2018), and CTLA-4 (Lan et al., 2018) (Figure 6). Among these immunosuppressive checkpoints, the interaction of PD-1 and PD-L1, CTLA-4 and CD80/86 are two pairs of well defined molecules in tumor immunity (Wieder et al., 2018). PD-1 is homologous to CD28, a co-stimulatory signal on the surface of T cells, and it is a key regulator of the adaptive immune response. PD-1 is expressed on a variety of host immune cells including T cells (primarily), B cells, dendritic cells, and Langerhans cells (Ishida et al., 1992; Pena-Cruz et al., 2010; Thibult et al., 2013; Lim et al., 2016). PD-1 can be combined with two ligands, PD-L1 and PD-L2, to induce inactivity and apoptosis of $CD4^+$ and $CD8^+$ T cells at the later effector phase while not exerting effects on immunosuppressive T cells such as Tregs (Marmarelis and Aggarwal, 2018; Nishida and Kudo, 2018). In addition, PD-

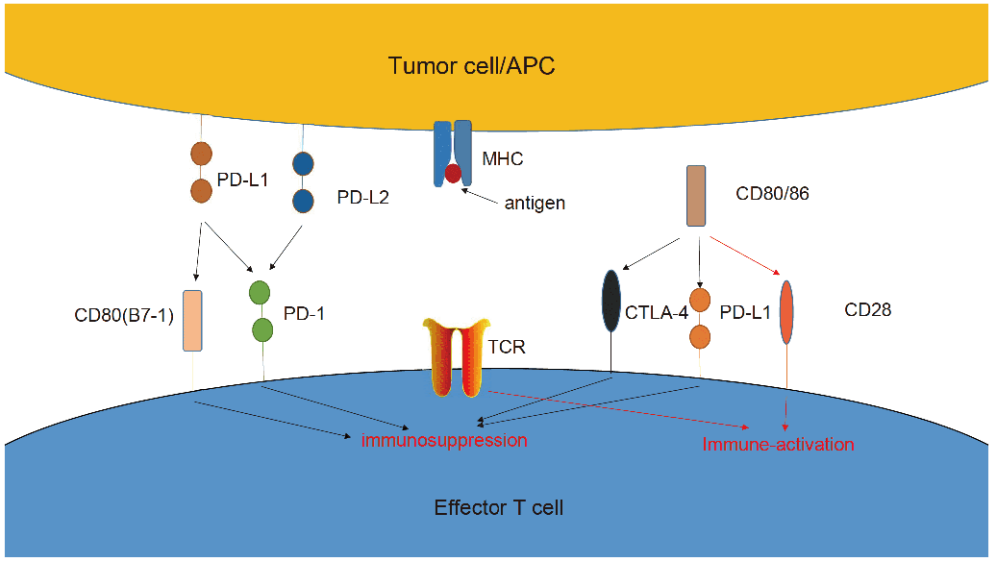

Figure 6 Immunosuppressive checkpoint ligand/receptor expressed on T cells, APCs, and tumor cells. PD-1 is mainly expressed by mature effector T cells, and its combination with PD-L1 can downregulate T-cell activation. PD-L1 can be produced by a variety of immune-related cells and tumor cells. Both PD-L1 and PD-L2 can combine with PD-1, resulting in the inactivation and programmed cell death of effector T cells. Additionally, PD-L1 can also target CD80, another essential immunoregulative molecule which is expressed on T cells, APCs, and tumor cells. Similar to PD-1, CD80 can also counteract T-cell function. CTLA-4 is an important immunosuppressive checkpoint which is predominantly expressed on the surface of Tregs and active $CD4^+$ T cells. It can competitively bind with CD80/86 on APCs to prevent the CD80/86 from binding with CD28, an immune-activating molecule, on T cells. APC, antigen-presenting cell; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; MHC, major histocompatibility complex; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; TCR, T cell receptor.

L1 can also target CD80 (B7-1), another key immune checkpoint molecule which regulates the immune cell response. Both PD-L1 and CD80 can be expressed on the surface of T cells, tumor cells, or antigen-presenting cells (a bidirectional interaction) (Keir et al., 2008). In the tumor microenvironment, IFN-γ, TNF-α, IL-4, and IL-10 can upregulate PD-L1 and PD-L2 expression on various types of host immune cells (Kondo et al., 2010; Sanmamed and Chen, 2014; Shibahara et al., 2018; Tang and Zheng, 2018). Conversely, immunosuppressive IL-10 can be induced by the interaction between PD-1 and PD-L1 (Dong et al., 1999; Trabattoni et al., 2003). CTLA-4 is a biomarker commonly expressed on the surface of Treg cells as well as activated $CD4^+$ T cells. It exhibits competitive binding to CD80/86 (B7 complex) on APCs, preventing CD80/86 from interacting with co-stimulatory signal CD28 on activated T cells for early T-cell activation (Chan et al., 2014; Marmarelis and Aggarwal, 2018). CTLA-4 can also deplete CD80/86 on the APCs by trans-endocytosis, thus avoiding these potential targets from being exposed to other T cells with co-stimulatory signals for T-cell activation (Qureshi et al., 2011). Moreover, CTLA-4 is capable of binding PP2A (protein phosphatase 2) and SHP2 (SH2 domain-containing protein-tyrosine phosphatase-2) to control TCR signaling as well as activating phosphatidylinositide3-kinase (PI3K) to decrease IL-2 production, which leads to the decrease of effector function, proliferation, and survival of effector T cells (Seidel et al., 2018; Shi et al., 2018). In addition to the famous pairs of PD-1/PD-L1 and CTLA-4/B7, T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), lymphocyte-activation gene 3 (LAG-3), as well as T-cell immunoreceptor with Ig and ITIM domains (TIGIT) are three other major immune checkpoints related to immune inhibition and dysfunction in the adaptive immune system (Gardner et al., 2014; Seidel et al., 2018).

Besides immune checkpoints of adaptive immunity, immune checkpoints in the innate immune system should be considered as well. CD47 is highly expressed by tumor cells and it suppresses the phagocytosis of macrophages via binding with signal regulatory protein α (SIRPα) on phagocytes (Tong and Wang, 2018). The interaction between CD47 and SIRPα can also support tumor angiogenesis and damage the anti-tumor function of T cells (Weiskopf, 2017; Tong and Wang, 2018). The aforementioned TIM-3 can also serve as an immunosuppressive molecule in the innate immunity by its high expression on macrophages after TGF-β induction (Yan et al., 2015).

Inhibitory metabolic factors

Although solid tumor cells possess heterogeneity in various mutant genes, mutation sites, and gene expression patterns, all types of solid tumors share some common traits including rapid proliferation, infinite growth, local invasion, and remote metastasis (You and Jones, 2012; Punt et al., 2017; Duan, 2018). Tissue hypoxia is staple during the growth and proliferation of tumor cells, and it is the root of all metabolic changes in the tumor microenvironment. Hypoxia in growing tumors can be categorized into two subtypes: chronic hypoxia, which refers to the constant lack of oxygen supply due to aberrant tumor vasculature, and intermittent hypoxia where the supply from blood vessels are repeatedly turned on and off, leading to fluctuations of oxygen levels in the area surrounding the tumor cells (Thomlinson and Gray, 1955; Brown, 1979). In chronic hypoxia, the response of tumor cells to hypoxia is basically dependent on heterodimeric hypoxia-inducible transcription factor 1 and 2 (HIF1 and HIF2). Both of these are biomarkers which indicate a worse prognosis for patients suffering from various cancers (Aebersold et al., 2001; Burri et al., 2003; Holmquist-Mengelbier et al., 2006). Once the tumor cells receive a signal of hypoxia, the genes of HIF are stabilized. This is followed by the binding of HIFαβ to hypoxia response element and activation of the subsequent transcription cascade (Semenza, 2003). HIF1-α can also induce the upregulation of glucose transporter-1 (GLUT-1) and execute increased glycolysis in tumor cells (Hanahan and Weinberg, 2011). Lactate is the main product of glycolysis during hypoxia. It weakens the survival and anti-tumor function of T cells and NK cells while downregulating the secretion of TNF by macrophages (Dietl et al., 2010; Brand et al., 2016). This has been demonstrated by a study in which the inhibition of lactate dehydrogenase remarkably enhances the vulnerability of tumors to adoptive T-cell therapy *in vitro* and *in vivo* (Cascone et al., 2018). To make matters worse, one study suggested that a low-oxygen environment can hinder the expansion, cytokine production, and differentiation as well as induce a higher CD4:CD8 ratio of CAR-T cells, and this is the reason that CAR-T cells are usually disabled in the treatment of patients suffering from solid tumors (Berahovich et al., 2019). Besides this, hypoxia has an influence on the expression level of immune checkpoint molecules on both T cells and tumor cells. For T cells, low-oxygen levels inhibit the expression of effector T-bet, a T-cell transcription factor, inversely causing an increased expression of CTLA-4 and LAG-3, which results in the disability of $CD8^+$ T cells (Doedens et al., 2013). Intermittent hypoxia, also known as acute or cyclic hypoxia, has the possibility of strengthening the livelihood of cancer cells, inducing autophagy, preventing ionizing radiation, and selecting for tumor cells which possess stem cell characteristics in different cancers including gastric cancer, lung cancer, pancreatic cancer, melanoma, and neuroblastoma (Liu et al., 2010; Rofstad et al., 2010; Bhaskara et al., 2012; Miao et al., 2014; Zhu et al., 2014). Some links between hypoxia and immunosuppressive cells have been proposed. A number of published works have

shown that HIF-1α, which is induced in hypoxic milieu, can remarkably elevate the production of inducible nitric oxide synthase (iNOS) and arginase (ARG)-1 in MDSC. iNOS can generate NO, which, in turn, can induce T-cell apoptosis as well as block the binding sites of TCR with tumor antigens (Chouaib et al., 2018). ARG-1 can assist MDSCs to competitively consume and deplete extrinsic L-arginine, which is essential for the development of T cells and TCR expression (Ezernitchi et al., 2006; Rodriguez et al., 2007). Another supportive point is that miR-210 induced by hypoxia can potentiate MDSC-mediated T-cell suppression, whereas MDSC functions are hindered by miR-210 (Noman et al., 2015). In addition, HIF1α also enables MDSCs to differentiate into immunosuppressive TAMs (Corzo et al., 2010).

Tumor antigen loss and antigen heterogeneity

Natural selection is a universal occurrence, and medicine is also not exempted from its effects. When antibiotics are abused, more antibiotic-resistant pathogens will emerge and neutralize the effect of these drugs in treating infections. Likewise, the persisting pressure exerted by CAR-T cells will lead to the survival of a specific group of tumor cells, which employ camouflage by downregulating or even ceasing the expression of their surface antigens (Chen et al., 2018; Majzner and Mackall, 2018). This was demonstrated by several studies in both hematological malignancies and solid tumors (Maude et al., 2014; Brown et al., 2015; Gardner et al., 2016; O'Rourke et al., 2017; Ebert et al., 2018; Maude et al., 2018). Basically, there are two major pathways of antigen loss: antigen escape and lineage switch (Majzner et al., 2017). Antigen escape, also known as isoform switch, happens when the tumor cells present another pattern of targeted antigens without being recognized by CAR-T cells. This process is likely to be related to gene mutation in the locus of the antigen (Sotillo et al., 2015; Majzner and Mackall, 2018). Lineage switch, on the other hand, is an alternate route of differentiation of tumor cells. The main cause is uncertain, but there are some possible causes including survival clones in distinct phenotypes, progenitors capable of two-way differentiation, cell reprogramming, or de-differentiation (Gardner et al., 2016; Majzner et al., 2017). A latest theory is put forward: trogocytosis. Using animal models, scientists found that CARs can cause active, reversible antigen loss through the "snatching" of tumor antigens by T cells. As a result, specific tumor antigen density is reduced, whereas the CAR-T cells become more susceptible to other fraternal CAR-T cells because the tumor antigen is now expressed on the cell surface membrane of T cells, resulting in immune disability and T-cell exhaustion (Hamieh et al., 2019). In the case of heterogeneity, there are two major understandings defining it. One is the change in the expression level and the distribution of neoantigens of the tumor cell surface, and the other is the diversity of tumor biomarkers (Ye et al., 2018). The former one has been previously mentioned, where there is an overlap with antigen loss (Chen et al., 2018; Majzner and Mackall, 2018). The latter one is a typical feature of solid tumors (Sun et al., 2016; Tirosh et al., 2016; Ye et al., 2018). In the process of carcinogenesis, initial tumor clones come into contact with stromal and immune cells and convert them into premalignant cells (Sabaawy, 2013). With subsequent changes in the genotype and phenotype, the premalignant cells will differentiate into various tumor clones, presenting diverse surface markers or intracellular neoantigens (Sabaawy, 2013; Ye et al., 2018). The symbiosis between the malignant and non-malignant cells therefore leads to tumorigenesis (Ye et al., 2018). Thus, the network of tumor growth and development in different sites confuses the recognition of CAR-T cell to target and eliminate the "enemy" (Jimenez-Sanchez et al., 2017; Zheng et al., 2017).

In summary, there is a network in which all related cells or cytokines exert their effect on traditional effector T cells. Likewise, CAR-T cells can also be adversely affected by these negative factors because their genome is highly homogeneous to $CD4^+$ or $CD8^+$ T cells. Tregs can directly crash CAR-T cells by granzymeB, perforins, and FasL, or indirectly inhibit the function of CAR-T cells via the depletion of IL-2 or the secretion of IL-10 or TGF-β. The immunosuppressive capacity of Tregs can be strengthened by IL-10 and TGF-β from M2-type TAMs. It is possible for TGF-β to induce $CD4^+CD25^-$ effector T cells, converting them into $CD4^+CD25^+$ Tregs. MDSCs suppress CAR-T cells by IL-10, enzymes, ROS, or being stimulated by macrophages. MDSCs can also inhibit CAR-T function by communicating with tumor cells via IL-10, NO, IL-6, etc. TAM is a two-edged sword to CAR-T. The T-cell-beneficial M1 type can boost tumor antigenicity by presenting antigens to Th1 cells, which can activate CAR-T cell function. Alternatively, it can directly swallow and eliminate tumor cells or kill them via cytotoxic substances such as NO, ROI, and TNF-α. M2-type TAMs, on the other hand, inhibit CAR-T cell function via chemokines, assisting tumor cell growth and proliferation, or activating Th2 and nTreg to inhibit CAR-T cell functions. Besides the surroundings of tumor cells, the tumor cells themselves can suppress CAR-T cell function by ligation of their immunosuppressive checkpoint ligands with their corresponding receptors expressed on CAR-T cells such as PD-1, CD80, CTLA-4, and so forth. Additionally, cancer cells can expedite Treg differentiation to weaken CAR-T anti-tumor function. Besides this, they can hide themselves via discarding their representative antigens or ceaselessly changing their antigens, confusing the specific attacks of CAR-T cells. Hence, solid tumor tissue and their surrounding immunosuppressive network are obstacles for CAR-T cell therapy.

Up-to-date technology of CAR-T cells to overcome the barriers in treating solid tumors

To surmount all the barriers mentioned above, brand-new adoptive immune cells have been designed and updated over the years. Nevertheless, mounting evidence suggests that the immunosuppressive tumor microenvironment will result in poor expansion and persistence of CAR-T cells in the human body (John et al., 2013; Cherkassky et al., 2016; Avanzi et al., 2018). On the basis of the previous three generations of CARs, the fourth generation of CAR-T cells, also designated T cells redirected for universal cytokine-mediated killing (TRUCKs) or "armored T cells," is created by genetically adding an inducible cytokine-producing cassette (e.g., IL-12). Cytokines can be produced and secreted right after TRUCK cells specifically combine with antigens on tumor cells (Van Schandevyl and Kerre, 2018) (Figure 7). The cytokines can activate CAR-T cells, TILs, and other innate immune cells such as anti-tumor macrophages and NK cells (Van Schandevyl and Kerre, 2018). The cassettes include proteins such as cytokines (IL-12, IL-15, IL-18, IL-21) (Chmielewski and Abken, 2012; Yeku and Brentjens, 2016; Krenciute et al., 2017; Yeku et al., 2017; Avanzi et al., 2018) and ligands of receptors on other immune cells or tumor cells (CD40L) (Yeku and Brentjens, 2016). Some bench studies demonstrated that CAR-T cells equipped with cytokines can reverse the immunosuppressive tumor microenvironment not only by enhancing their own lifespan and expansion, but also by activating the immune effector cells and promoting their growth and proliferation (Kerkar et al., 2011; Koneru et al., 2015; Yeku and Brentjens, 2016; Avanzi et al., 2018). In addition, cytokines secreted by T cells can reduce, eliminate, or even reverse the function of immunosuppressive cells such as Tregs or TAMs (Pegram et al., 2012). The precise attacks launched by armored T cells can circumvent the severe treatment-related toxicity and mortality caused by previous systemic injections of large doses of IL-12 (Leonard et al., 1997; Lacy et al., 2009). Besides this, T cells armored with other molecules such as CD40L or 4-1BBL can augment the vulnerability of tumor cells to immune attacks (Curran et al., 2015) and stimulate more pro-inflammatory cytokines which are beneficial for T-cell activation, persistence, and expansion (Zhao et al., 2015). Facing immunosuppressive environments filled with various kinds of anti-inflammatory cytokines or other common inhibitory molecules (e.g., IL-4, IL-10, TGF-β, and PD-1), scientists have engineered T cells with mutated genes capable of expressing invalid receptors of these cytokines or even enabling T cells to swap immunosuppressive signals to pro-inflammatory ones so that the adoptive immune cells can break through this immunosuppressive barrier secreted by tumor cells (Foster et al., 2008; Leen et al., 2014; Cherkassky et al., 2016; Kloss et al., 2018). One study even forces the T cells to express and secrete PD-1/PD-L1-blocking monoclonal antibodies both in an autocrine and paracrine manner to enhance T cell effects (Suarez et al., 2016; Rafiq et al., 2018). To overcome the difficulty of CAR-T migration to tumor sites, furnishing CAR-T cells with genes of CCR, CXCR, or other receptors to chemokines secreted by solid tumors is another option to improve the homing of T cells and the capability of migration and achieving access to tumor cells (Craddock et al., 2010; Moon et al., 2011; Knochelmann et al., 2018).

To avoid ineffective treatment due to antigen loss, bispe-

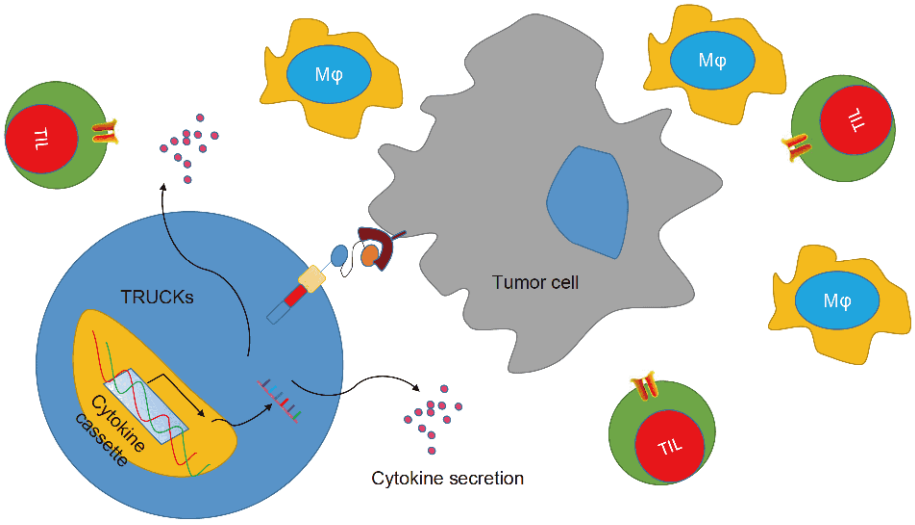

Figure 7 The fourth generation of CAR-T cells and its function in tumor immunity. Based on the previous three generations, the fourth generation of CAR-T cells, also known as "T cells redirected for universal cytokine-mediated killing (TRUCKs)" or "armored T cells," introduces a gene of inducible cytokine cassette (e.g., IL-12) to the T-cell genome. Cytokines can be produced and secreted as soon as TRUCK cells specifically combine with antigens on tumor cells. The cytokines can stimulate CAR-T cells, TILs, and other innate immune cells such as macrophages. Mφ, macrophage; TIL, tumor-infiltrating lymphocyte; TRUCKs, T cells redirected for universal cytokine-mediated killing.

cific CAR-T cells can launch a double attack on the evasive tumor cells (Zhao et al., 2018). Bispecific CAR-T comprises two patterns. One is a CAR monomer where two different scFvs exist "hand-in-hand" on a single cell (dual antigen target tandem CAR therapy, TanCAR) or when two separate CAR monomers with different scFvs exist on one cell (dual signaling CAR therapy) (Figure 8A). The other one is the formation of T-cell pools with different types of CARs infused sequentially or simultaneously (dual antigen target CAR therapy) (Chen et al., 2018; Zhao et al., 2018). However, instability of the scFv sections persists because of the shortage of structural support by the IgG constant regions or even the presence of CAR monomers on a single cell, causing oligomerization with each other, clustering, and ligand-independent tonic signaling (a kind of signaling which originates internally rather than being dependent on external antigens) (Ajina and Maher, 2018). The selection of a proper coexisting epitope is another important topic to be discussed (Sadelain, 2016).

Due to the limitations and difficulties in using CAR-T cells, combination therapy is another alternative in clinical oncology (Park et al., 2007; Lamers et al., 2016). Globally, clinical trials have been assessing the results of the combination of CAR-T cells with other treatments, including chemotherapy, other types of immunotherapy, and other treatments (Xu et al., 2018). In chemotherapy, the drugs can mitigate tumor volume (Alizadeh et al., 2014; Sistigu et al., 2014), making tumor cells more vulnerable to the attack of the CTLs (Ramakrishnan et al., 2012; Parente-Pereira et al., 2013), thereby enhancing the exposure of tumor antigens and aiding the antigen presentation by dendritic cells and TILs (Senovilla et al., 2012; Ma et al., 2013; Garg et al., 2015). Moreover, certain chemotherapeutic agents (i.e., cyclophosphamide, docetaxel, doxorubicin, fluorouracil, and gemcitabine) can disable the function of immunosuppressive cells like Tregs and MDSCs without disrupting the effects of adoptive T cells when the drugs are given in a low dose (Traverso et al., 2012; Alizadeh et al., 2014). Finally, the assistance of CAR-T cell therapy with checkpoint-inhibitor blockades (anti-CTLA-4, anti-PD-1, and anti-PD-L1) is highlighted in recent years, and some agents such as anti-CTLA-4 antibodies (ipilimumab and tremelimumab) and anti-PD-1 antibodies (nivolumab, pembrolizumab, and pidilizumab) have been approved by the US Food and Drug Administration (John et al., 2013; Xu et al., 2018). These agents, especially anti-PD-1, can specifically prevent immunosuppression and exhaustion of T cells by tumor cells and extend the persistence of effector T cells *in vivo* (John et al., 2013; Burga et al., 2015). In addition to antibodies, some studies have found that some microRNAs or even lncRNAs are influential on or related to immune processes (Fan et al., 2019) and carcinogenesis, and there is no exception to CAR-T cell treatment (Xu et al., 2019; Zhang et al., 2019). For

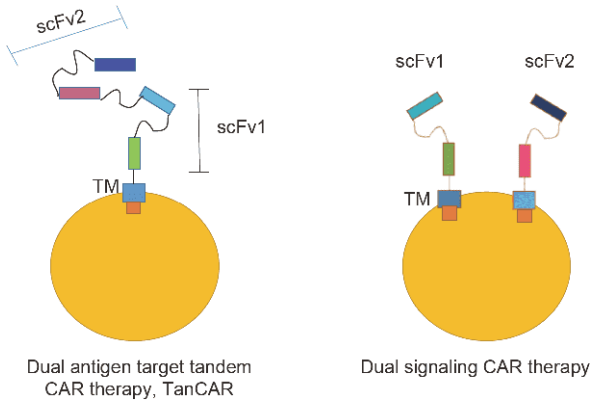

Figure 8 Two kinds of bispecific T cells. Dual antigen targets tandem CAR (TanCAR) is a brand-new T cell on which a CAR monomer has two scFvs in a hand-in-hand pattern (tandem). Dual signaling CAR is another kind, where two separate CARs have one different scFv, respectively. scFv, mouse monoclonal antibody; TM, transmembrane part.

instance, using an RNA interference technique including microRNA (miRNA) is another option for combination therapy, in that miRNA can inhibit some inhibitory molecules in tumor cells and indirectly enhance CAR-T cell cytotoxicity (Huang et al., 2018; Ji et al., 2019).

In contrast to the "addition protocol," namely to add more elements to CAR-T cells or to apply other agents to optimize its function, the "subtraction protocol" has been used to remove some unnecessary or harmful elements from the T cells, offering another direction to further enhance the antitumor effect. The key element of this subtraction protocol lies in the gene-editing technique. Among these techniques, the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system is of great importance. The gene of PD-1 can be edited from the exhausted CAR-T cells using the CRISPR/Cas9 system, where some clinical trials utilizing this technology are currently underway (Menger et al., 2016; Su et al., 2016; Rupp et al., 2017; Hu et al., 2019) (https://clinicaltrials.gov/ct2/show/NCT03399448?term=NCT03399448&draw=2&rank=1). However, some scientists have suggested that the intervention of PD-1 genes in T cells will adversely affect their survival and will conversely promote further exhaustion of naïve T cells (Odorizzi et al., 2015). Besides the examples mentioned above, knockout of diacylglycerol kinase (DGK), a TCR-inhibitory checkpoint, is another option to add CAR-T cell resistance to immunosuppressive molecules such as TGF-β and prostaglandin E2 (PGE2) by strengthening the TCR signaling (Riese et al., 2013; Jung et al., 2018). Similarly, deletion of other "enemy molecules" of the T cells via the CRISPR/Cas9 system including LAG-3 and FASL (indicators of differentiation or apoptosis of T cells) (Eyquem et al., 2017) is also a promising approach to enhance the persistence and antitumor effects of CAR-T cells (Peter et al., 2015; Klebanoff et al., 2016). Besides this, RNA interference is another well-known technology in compliance with the "subtraction

protocol," and it has made a breakthrough especially in the target therapy of tumors (Saw and Song, 2019). Some researchers have been devoted to delivering CAR-T cells with small-interfering RNAs (siRNAs) to downregulate PD-1 and CTLA-4. Through electroporation, this type of CAR-T acquired more cytotoxicity and successfully avoided activation-induced upregulation of these two immunosuppressive checkpoint receptors (Simon et al., 2018).

Conclusion

CAR-T cell therapy has made quantum leaps in recent years, especially in the treatment of hematological malignancies. Through this regimen, patients with acute lymphoblastic leukemia report a CR rate of 90% and sustained remissions of up to two years (Maude et al., 2014). Nevertheless, multiple challenges remain in the treatment of solid tumors. It seems that discovering a novel neoantigen is still a main goal for researchers, and this represents the principle of precision medicine. However, the contribution of innate immunity toward immunotherapy should not be overlooked. In the TME, we can readily identify that almost half of all the factors impacting the function of T cells are related to the innate immune system. Also, the high heterogeneity caused by gene mutation and rapid antigen escape is overtaking our speed of searching for a novel antigen. In addition, when we reconstruct CAR-T cells, we should not neglect the importance of the TCR. One study has reported the creation of a receptor that is TCR-dependent but MHC-independent, designated T cell antigen coupler (TAC) (Helsen et al., 2018). It has three domains: an antigen-binding domain, a TCR-recruitment domain, and a co-receptor domain (hinge, transmembrane, and cytosolic regions). This TAC not only demonstrated successful avoidance of CAR toxicity (Abate-Daga and Davila, 2016) and the exertion of a milder T-cell response, but it also exhibited stronger infiltration into tumors and a more powerful anti-tumor activity (Helsen et al., 2018). This study reminds us that we can potentially achieve more with the reconstruction of CAR-T cells, surpassing the invention of "tri-/quadra-specific" CAR-T cells by the addition of more monoclonal antibodies. A possible future for CAR-T cell development is to combine its highly specific traits (monoclonal antibody) with more elements of non-specific ones (such as TRUCKs, i.e., to recruit other kinds of immune cells into the battlefield). Of course, this progress is currently based on the level of gene-editing for the time being. If possible, we strive to work on the genome level, fusing innate and adaptive immunity into T cells. For example, we can empower CAR-T cells with the ability of antigen presentation or phagocytosis, or even to produce and secrete antibodies instead of requiring direct contact with cancer cells, which can avoid the problems of homing and infiltration. Above all, CAR-T cells are not just soldiers, they can be leaders to stimulate, activate, and preside over the entire immune system. This represents our ultimate goal in tumor immunity: to win the war against the tumor microenvironment.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

References

Abate-Daga, D., and Davila, M.L. (2016). CAR models: next-generation CAR modifications for enhanced T-cell function. Mol Ther Oncolytics 3, 16014.

Aebersold, D.M., Burri, P., Beer, K.T., Laissue, J., Djonov, V., Greiner, R. H., and Semenza, G.L. (2001). Expression of hypoxia-inducible factor-1alpha: a novel predictive and prognostic parameter in the radiotherapy of oropharyngeal cancer. Cancer Res 61, 2911–2916.

Ahmed, N., Brawley, V., Hegde, M., Bielamowicz, K., Wakefield, A., Ghazi, A., Ashoori, A., Diouf, O., Gerken, C., Landi, D., et al. (2015). Autologous HER2 CMV bispecific CAR T cells are safe and demonstrate clinical benefit for glioblastoma in a Phase I trial. J Immunother Cancer 3, O11.

Ahmed, N., Brawley, V.S., Hegde, M., Robertson, C., Ghazi, A., Gerken, C., Liu, E., Dakhova, O., Ashoori, A., Corder, A., et al. (2015). Human epidermal growth factor receptor 2 (HER2)-specific chimeric antigen receptor-modified T cells for the immunotherapy of HER2-positive sarcoma. J Clin Oncol 33, 1688–1696.

Ajina, A., and Maher, J. (2018). Strategies to address chimeric antigen receptor tonic signaling. Mol Cancer Ther 17, 1795–1815.

Al-Hilli, Z., Choong, G., Keeney, M.G., Visscher, D.W., Ingle, J.N., Goetz, M.P., and Jakub, J.W. (2019). Metaplastic breast cancer has a poor response to neoadjuvant systemic therapy. Breast Cancer Res Treat 176, 709–716.

Alizadeh, D., Trad, M., Hanke, N.T., Larmonier, C.B., Janikashvili, N., Bonnotte, B., Katsanis, E., and Larmonier, N. (2014). Doxorubicin eliminates myeloid-derived suppressor cells and enhances the efficacy of adoptive T-cell transfer in breast cancer. Cancer Res 74, 104–118.

Amrane, K., Ollivier, L., Salaun, P.Y., Metges, J.P., and Abgral, R. (2019). Complete metabolic response assessed by FDG PET/CT to FOLFIRI-Aflibercept in second-line treatment of metastatic colorectal cancer. Clin Nucl Med 1.

Avanzi, M.P., Yeku, O., Li, X., Wijewarnasuriya, D.P., van Leeuwen, D.G., Cheung, K., Park, H., Purdon, T.J., Daniyan, A.F., Spitzer, M.H., et al. (2018). Engineered tumor-targeted T cells mediate enhanced anti-tumor efficacy both directly and through activation of the endogenous immune system. Cell Rep 23, 2130–2141.

Azimi, M., Aslani, S., Mortezagholi, S., Salek, A., Javan, M.R., Rezaiemanesh, A., Ghaedi, M., Gholamzad, M., and Salehi, E. (2016). Identification, isolation, and functional assay of regulatory T cells. Immunol Invest 45, 584–602.

Balkwill, F. (2006). TNF-α in promotion and progression of cancer. Cancer Metast Rev 25, 409–416.

Baniyash, M. (2016). Myeloid-derived suppressor cells as intruders and targets: clinical implications in cancer therapy. Cancer Immunol Immunother 65, 857–867.

Beatty, G.L., Haas, A.R., Maus, M.V., Torigian, D.A., Soulen, M.C., Plesa, G., Chew, A., Zhao, Y., Levine, B.L., Albelda, S.M., et al. (2014). Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce antitumor activity in solid malignancies. Cancer Immunol Res 2, 112–120.

Beatty, G.L., and O'Hara, M. (2016). Chimeric antigen receptor-modified T cells for the treatment of solid tumors: defining the challenges and next steps. Pharmacol Therapeutics 166, 30–39.

Becker, C., Fantini, M.C., Wirtz, S., Nikolaev, A., Lehr, H.A., Galle, P.R., Rose-John, S., and Neurath, M.F. (2005). IL-6 signaling promotes tu-

mor growth in colorectal cancer. Cell Cycle 4, 217–220.
Bedognetti, D., Spivey, T.L., Zhao, Y., Uccellini, L., Tomei, S., Dudley, M. E., Ascierto, M.L., De Giorgi, V., Liu, Q., Delogu, L.G., et al. (2013). CXCR3/CCR5 pathways in metastatic melanoma patients treated with adoptive therapy and interleukin-2. Br J Cancer 109, 2412–2423.
Ben-Meir, K., Twaik, N., and Baniyash, M. (2018). Plasticity and biological diversity of myeloid derived suppressor cells. Curr Opin Immunol 51, 154–161.
Berahovich, R., Liu, X., Zhou, H., Tsadik, E., Xu, S., Golubovskaya, V., and Wu, L. (2019). Hypoxia selectively impairs CAR-T cells *in vitro*. Cancers 11, 602.
Beury, D.W., Parker, K.H., Nyandjo, M., Sinha, P., Carter, K.A., and Ostrand-Rosenberg, S. (2014). Cross-talk among myeloid-derived suppressor cells, macrophages, and tumor cells impacts the inflammatory milieu of solid tumors. J Leukocyte Biol 96, 1109–1118.
Bhaskara, V.K., Mohanam, I., Rao, J.S., and Mohanam, S. (2012). Intermittent hypoxia regulates stem-like characteristics and differentiation of neuroblastoma cells. PLoS ONE 7, e30905.
Brand, A., Singer, K., Koehl, G.E., Kolitzus, M., Schoenhammer, G., Thiel, A., Matos, C., Bruss, C., Klobuch, S., Peter, K., et al. (2016). LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK cells. Cell Metab 24, 657–671.
Brocker, T., and Karjalainen, K. (1995). Signals through T cell receptor-zeta chain alone are insufficient to prime resting T lymphocytes.. J Exp Med 181, 1653–1659.
Bronte, V., Serafini, P., Mazzoni, A., Segal, D.M., and Zanovello, P. (2003). L-arginine metabolism in myeloid cells controls T-lymphocyte functions. Trends Immunol 24, 301–305.
Bronte, V., and Zanovello, P. (2005). Regulation of immune responses by L-arginine metabolism. Nat Rev Immunol 5, 641–654.
Brown, C.E., Badie, B., Barish, M.E., Weng, L., Ostberg, J.R., Chang, W. C., Naranjo, A., Starr, R., Wagner, J., Wright, C., et al. (2015). Bioactivity and safety of IL13R2-redirected chimeric antigen receptor $CD8^+$ T cells in patients with recurrent glioblastoma. Clin Cancer Res 21, 4062–4072.
Brown, J.M. (1979). Evidence for acutely hypoxic cells in mouse tumours, and a possible mechanism of reoxygenation. Br J Radiol 52, 650–656.
Brudno, J.N., Somerville, R.P.T., Shi, V., Rose, J.J., Halverson, D.C., Fowler, D.H., Gea-Banacloche, J.C., Pavletic, S.Z., Hickstein, D.D., Lu, T.L., et al. (2016). Allogeneic T cells that express an anti-CD19 chimeric antigen receptor induce remissions of B-cell malignancies that progress after allogeneic hematopoietic stem-cell transplantation without causing graft-versus-host disease. J Clin Oncol 34, 1112–1121.
Bunt, S.K., Clements, V.K., Hanson, E.M., Sinha, P., and Ostrand-Rosenberg, S. (2009). Inflammation enhances myeloid-derived suppressor cell cross-talk by signaling through Toll-like receptor 4. J Leukocyte Biol 85, 996–1004.
Burga, R.A., Thorn, M., Point, G.R., Guha, P., Nguyen, C.T., Licata, L.A., DeMatteo, R.P., Ayala, A., Joseph Espat, N., Junghans, R.P., et al. (2015). Liver myeloid-derived suppressor cells expand in response to liver metastases in mice and inhibit the anti-tumor efficacy of anti-CEA CAR-T. Cancer Immunol Immunother 64, 817–829.
Burri, P., Djonov, V., Aebersold, D.M., Lindel, K., Studer, U., Altermatt, H. J., Mazzucchelli, L., Greiner, R.H., and Gruber, G. (2003). Significant correlation of hypoxia-inducible factor-1α with treatment outcome in cervical cancer treated with radical radiotherapy. Int J Radiat Oncol Biol Phys 56, 494–501.
Byrne, W.L., Mills, K.H.G., Lederer, J.A., and O'Sullivan, G.C. (2011). Targeting regulatory T cells in cancer. Cancer Res 71, 6915–6920.
Cascone, T., McKenzie, J.A., Mbofung, R.M., Punt, S., Wang, Z., Xu, C., Williams, L.J., Wang, Z., Bristow, C.A., Carugo, A., et al. (2018). Increased tumor glycolysis characterizes immune resistance to adoptive T cell therapy. Cell Metab 27, 977–987.e4.
Cervera-Carrascon, V., Siurala, M., Santos, J.M., Havunen, R., Tähtinen, S., Karell, P., Sorsa, S., Kanerva, A., and Hemminki, A. (2018). TNFa and IL-2 armed adenoviruses enable complete responses by anti-PD-1 checkpoint blockade. Oncoimmunology 7, e1412902.
Chan, D.V., Gibson, H.M., Aufiero, B.M., Wilson, A.J., Hafner, M.S., Mi, Q.S., and Wong, H.K. (2014). Differential CTLA-4 expression in human $CD4^+$ versus $CD8^+$ T cells is associated with increased NFAT1 and inhibition of $CD4^+$ proliferation. Genes Immun 15, 25–32.
Chavez-Galan, L., Olleros, M.L., Vesin, D., and Garcia, I. (2015). Much more than M1 and M2 macrophages, there are also $CD169^+$ and TCR^+ macrophages. Front Immunol 6.
Chekmasova, A.A., Rao, T.D., Nikhamin, Y., Park, K.J., Levine, D.A., Spriggs, D.R., and Brentjens, R.J. (2010). Successful eradication of established peritoneal ovarian tumors in SCID-Beige mice following adoptive transfer of T cells genetically targeted to the MUC16 antigen. Clin Cancer Res 16, 3594–3606.
Chen, C., Li, K., Jiang, H., Song, F., Gao, H., Pan, X., Shi, B., Bi, Y., Wang, H., Wang, H., et al. (2017). Development of T cells carrying two complementary chimeric antigen receptors against glypican-3 and asialoglycoprotein receptor 1 for the treatment of hepatocellular carcinoma. Cancer Immunol Immunother 66, 475–489.
Chen, M., Sun, R., Shi, B., Wang, Y., Di, S., Luo, H., Sun, Y., Li, Z., Zhou, M., and Jiang, H. (2019). Antitumor efficacy of chimeric antigen receptor T cells against EGFRvIII-expressing glioblastoma in C57BL/6 mice. Biomed Pharmacother 113, 108734.
Chen, M.L., Pittet, M.J., Gorelik, L., Flavell, R.A., Weissleder, R., von Boehmer, H., and Khazaie, K. (2005). Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF-beta signals *in vivo*. Proc Natl Acad Sci USA 102, 419–424.
Chen, N., Li, X., Chintala, N.K., Tano, Z.E., and Adusumilli, P.S. (2018). Driving CARs on the uneven road of antigen heterogeneity in solid tumors. Curr Opin Immunol 51, 103–110.
Chen, W.J., Jin, W., Hardegen, N., Lei, K.J., Li, L., Marinos, N., McGrady, G., and Wahl, S.M. (2003). Conversion of peripheral $CD4^+$ $CD25^-$ naive T cells to $CD4^+$ $CD25^+$ regulatory T cells by TGF-β induction of transcription factor *Foxp3*. J Exp Med 198, 1875–1886.
Chen, W.C., Chu, P.Y., Lee, Y.T., Lu, W.B., Liu, C.Y., Chang, P.M.H., and Yang, M.H. (2017). Pembrolizumab for recurrent/metastatic head and neck squamous cell carcinoma in an Asian population. Medicine 96, e9519.
Cherkassky, L., Morello, A., Villena-Vargas, J., Feng, Y., Dimitrov, D.S., Jones, D.R., Sadelain, M., and Adusumilli, P.S. (2016). Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. J Clin Invest 126, 3130–3144.
Chinnasamy, D., Yu, Z., Theoret, M.R., Zhao, Y., Shrimali, R.K., Morgan, R.A., Feldman, S.A., Restifo, N.P., and Rosenberg, S.A. (2010). Gene therapy using genetically modified lymphocytes targeting VEGFR-2 inhibits the growth of vascularized syngenic tumors in mice. J Clin Invest 120, 3953–3968.
Chistiakov, D.A., Bobryshev, Y.V., and Orekhov, A.N. (2015). Changes in transcriptome of macrophages in atherosclerosis. J Cell Mol Med 19, 1163–1173.
Chmielewski, M., and Abken, H. (2012). CAR T cells transform to trucks: chimeric antigen receptor-redirected T cells engineered to deliver inducible IL-12 modulate the tumour stroma to combat cancer. Cancer Immunol Immunother 61, 1269–1277.
Chmielewski, M., Hahn, O., Rappl, G., Nowak, M., Schmidt-Wolf, I.H., Hombach, A.A., and Abken, H. (2012). T cells that target carcinoembryonic antigen eradicate orthotopic pancreatic carcinomas without inducing autoimmune colitis in mice. Gastroenterology 143, 1095–1107.e2.
Chouaib, S., Umansky, V., and Kieda, C. (2018). The role of hypoxia in shaping the recruitment of proangiogenic and immunosuppressive cells in the tumor microenvironment. Contemp Oncol (Pozn) 2018(1), 7–13.
Columba-Cabezas, S., Serafini, B., Ambrosini, E., Sanchez, M., Penna, G., Adorini, L., and Aloisi, F. (2002). Induction of macrophage-derived chemokine/CCL22 expression in experimental autoimmune encephalomyelitis and cultured microglia: implications for disease regulation. J Neuroimmunol 130, 10–21.
Corzo, C.A., Condamine, T., Lu, L., Cotter, M.J., Youn, J.I., Cheng, P., Cho, H.I., Celis, E., Quiceno, D.G., Padhya, T., et al. (2010). HIF-1α

regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. J Exp Med 207, 2439–2453.
Craddock, J.A., Lu, A., Bear, A., Pule, M., Brenner, M.K., Rooney, C.M., and Foster, A.E. (2010). Enhanced tumor trafficking of GD2 chimeric antigen receptor T cells by expression of the chemokine receptor CCR2b. J Immunother 33, 780–788.
Curiel, T.J., Coukos, G., Zou, L., Alvarez, X., Cheng, P., Mottram, P., Evdemon-Hogan, M., Conejo-Garcia, J.R., Zhang, L., Burow, M., et al. (2004). Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 10, 942–949.
Curran, K.J., Seinstra, B.A., Nikhamin, Y., Yeh, R., Usachenko, Y., van Leeuwen, D.G., Purdon, T., Pegram, H.J., and Brentjens, R.J. (2015). Enhancing antitumor efficacy of chimeric antigen receptor T cells through constitutive CD40L expression. Mol Ther 23, 769–778.
Davila, M.L., Riviere, I., Wang, X., Bartido, S., Park, J., Curran, K., Chung, S.S., Stefanski, J., Borquez-Ojeda, O., Olszewska, M., et al. (2014). Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Transl Med 6, 224ra25.
Deng, Z., Wu, Y., Ma, W., Zhang, S., and Zhang, Y.Q. (2015). Adoptive T-cell therapy of prostate cancer targeting the cancer stem cell antigen EpCAM. BMC Immunol 16, 1.
Dietl, K., Renner, K., Dettmer, K., Timischl, B., Eberhart, K., Dorn, C., Hellerbrand, C., Kastenberger, M., Kunz-Schughart, L.A., Oefner, P.J., et al. (2010). Lactic acid and acidification inhibit TNF secretion and glycolysis of human monocytes. J Immunol 184, 1200–1209.
Doedens, A.L., Phan, A.T., Stradner, M.H., Fujimoto, J.K., Nguyen, J.V., Yang, E., Johnson, R.S., and Goldrath, A.W. (2013). Hypoxia-inducible factors enhance the effector responses of CD8$^+$ T cells to persistent antigen. Nat Immunol 14, 1173–1182.
Dong, H., Zhu, G., Tamada, K., and Chen, L. (1999). B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med 5, 1365–1369.
Duan, H. (2018). Novel therapeutic strategies for solid tumor based on body's intrinsic antitumor immune system. Cell Physiol Biochem 47, 441–457.
Ebert, L.M., Yu, W., Gargett, T., and Brown, M.P. (2018). Logic-gated approaches to extend the utility of chimeric antigen receptor T-cell technology. Biochem Soc Trans 46, 391–401.
Eshhar, Z., Waks, T., Gross, G., and Schindler, D.G. (1993). Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors.. Proc Natl Acad Sci USA 90, 720–724.
Evans, S.S., Repasky, E.A., and Fisher, D.T. (2015). Fever and the thermal regulation of immunity: the immune system feels the heat. Nat Rev Immunol 15, 335–349.
Eyquem, J., Mansilla-Soto, J., Giavridis, T., van der Stegen, S.J.C., Hamieh, M., Cunanan, K.M., Odak, A., Gönen, M., and Sadelain, M. (2017). Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature 543, 113–117.
Ezernitchi, A.V., Vaknin, I., Cohen-Daniel, L., Levy, O., Manaster, E., Halabi, A., Pikarsky, E., Shapira, L., and Baniyash, M. (2006). TCR ζ down-regulation under chronic inflammation is mediated by myeloid suppressor cells differentially distributed between various lymphatic organs. J Immunol 177, 4763–4772.
Facciabene, A., Peng, X., Hagemann, I.S., Balint, K., Barchetti, A., Wang, L.P., Gimotty, P.A., Gilks, C.B., Lal, P., Zhang, L., et al. (2011). Tumour hypoxia promotes tolerance and angiogenesis via CCL28 and Treg cells. Nature 475, 226–230.
Fan, J., Zhang, X., Nie, X., Li, H., Yuan, S., Dai, B., Zhan, J., Wen, Z., Jiang, J., Chen, C., et al. (2020). Nuclear miR-665 aggravates heart failure via suppressing phosphatase and tensin homolog transcription. Sci China Life Sci 63, doi:10.1007/s11427-018-9515-1.
Feng, K., Guo, Y., Dai, H., Wang, Y., Li, X., Jia, H., and Han, W. (2016). Chimeric antigen receptor-modified T cells for the immunotherapy of patients with EGFR-expressing advanced relapsed/refractory non-small cell lung cancer. Sci China Life Sci 59, 468–479.
Feng, K., Liu, Y., Guo, Y., Qiu, J., Wu, Z., Dai, H., Yang, Q., Wang, Y., and Han, W. (2017). Phase I study of chimeric antigen receptor modified T cells in treating HER2-positive advanced biliary tract cancers and pancreatic cancers. Protein Cell 9, 838–847.
Feng, K.C., Guo, Y.L., Liu, Y., Dai, H.R., Wang, Y., Lv, H.Y., Huang, J.H., Yang, Q.M., and Han, W.D. (2017). Cocktail treatment with EGFR-specific and CD133-specific chimeric antigen receptor-modified T cells in a patient with advanced cholangiocarcinoma. J Hematol Oncol 10, 4.
Finney, H.M., Akbar, A.N., and Lawson, A.D.G. (2004). Activation of resting human primary T cells with chimeric receptors: costimulation from CD28, inducible costimulator, CD134, and CD137 in series with signals from the TCRζ chain. J Immunol 172, 104–113.
Forsberg, E.M.V., Lindberg, M.F., Jespersen, H., Alsén, S., Bagge, R.O., Donia, M., Svane, I.M., Nilsson, O., Ny, L., Nilsson, L.M., et al. (2019). HER2 CAR-T cells eradicate uveal melanoma and T-cell therapy-resistant human melanoma in IL2 transgenic NOD/SCID IL2 receptor knockout mice. Cancer Res 79, 899–904.
Foster, A.E., Dotti, G., Lu, A., Khalil, M., Brenner, M.K., Heslop, H.E., Rooney, C.M., and Bollard, C.M. (2008). Antitumor activity of EBV-specific T lymphocytes transduced with a dominant negative TGF-beta receptor. J Immunother 31, 500–505.
Frydrychowicz, M., Boruczkowski, M., Kolecka-Bednarczyk, A., and Dworacki, G. (2017). The dual role of Treg in cancer. Scand J Immunol 86, 436–443.
Fujimura, T., Kakizaki, A., Furudate, S., Kambayashi, Y., and Aiba, S. (2016). Tumor-associated macrophages in skin: how to treat their heterogeneity and plasticity. J Dermatol Sci 83, 167–173.
Fujimura, T., Kambayashi, Y., Fujisawa, Y., Hidaka, T., and Aiba, S. (2018). Tumor-associated macrophages: therapeutic targets for skin cancer. Front Oncol 8.
Gabrilovich, D.I. (2017). Myeloid-derived suppressor cells. Cancer Immunol Res 5, 3–8.
Gabrilovich, D.I., Bronte, V., Chen, S.H., Colombo, M.P., Ochoa, A., Ostrand-Rosenberg, S., and Schreiber, H. (2007). The terminology issue for myeloid-derived suppressor cells. Cancer Res 67, 425.
Gabrilovich, D.I., Ostrand-Rosenberg, S., and Bronte, V. (2012). Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol 12, 253–268.
Gao, H., Li, K., Tu, H., Pan, X., Jiang, H., Shi, B., Kong, J., Wang, H., Yang, S., Gu, J., et al. (2014). Development of T cells redirected to glypican-3 for the treatment of hepatocellular carcinoma. Clin Cancer Res 20, 6418–6428.
Garassino, M.C., Cho, B.C., Kim, J.H., Mazières, J., Vansteenkiste, J., Lena, H., Corral Jaime, J., Gray, J.E., Powderly, J., Chouaid, C., et al. (2018). Durvalumab as third-line or later treatment for advanced non-small-cell lung cancer (ATLANTIC): an open-label, single-arm, phase 2 study. Lancet Oncol 19, 521–536.
Garcia-Hernandez, M.L., Hernandez-Pando, R., Gariglio, P., and Berumen, J. (2002). Interleukin-10 promotes B16-melanoma growth by inhibition of macrophage functions and induction of tumour and vascular cell proliferation. Immunology 105, 231–243.
Gardner, D., Jeffery, L.E., and Sansom, D.M. (2014). Understanding the CD28/CTLA-4 (CD152) pathway and its implications for costimulatory blockade. Am J Transplant 14, 1985–1991.
Gardner, R., Finney, O., Smithers, H., Leger, K.J., Annesley, C.E., Summers, C., Brown, C., Mgebroff, S., Lindgren, C., Spratt, K., et al. (2016). CD19CAR T cell products of defined CD4:CD8 composition and transgene expression show prolonged persistence and durable MRD-negative remission in pediatric and young adult B-cell ALL. Blood 128, 219.
Gardner, R., Wu, D., Cherian, S., Fang, M., Hanafi, L.A., Finney, O., Smithers, H., Jensen, M.C., Riddell, S.R., Maloney, D.G., et al. (2016). Acquisition of a CD19-negative myeloid phenotype allows immune escape of MLL-rearranged B-ALL from CD19 CAR-T-cell therapy. Blood 127, 2406–2410.
Garg, A.D., Galluzzi, L., Apetoh, L., Baert, T., Birge, R.B., Bravo-San

Pedro, J.M., Breckpot, K., Brough, D., Chaurio, R., Cirone, M., et al. (2015). Molecular and translational classifications of DAMPs in immunogenic cell death. Front Immunol 6, 588.
Gentles, A.J., Newman, A.M., Liu, C.L., Bratman, S.V., Feng, W., Kim, D., Nair, V.S., Xu, Y., Khuong, A., Hoang, C.D., et al. (2015). The prognostic landscape of genes and infiltrating immune cells across human cancers. Nat Med 21, 938–945.
Gobert, M., Treilleux, I., Bendriss-Vermare, N., Bachelot, T., Goddard-Leon, S., Arfi, V., Biota, C., Doffin, A.C., Durand, I., Olive, D., et al. (2009). Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome. Cancer Res 69, 2000–2009.
Goff, S.L., Dudley, M.E., Citrin, D.E., Somerville, R.P., Wunderlich, J.R., Danforth, D.N., Zlott, D.A., Yang, J.C., Sherry, R.M., Kammula, U.S., et al. (2016). Randomized, prospective evaluation comparing intensity of lymphodepletion before adoptive transfer of tumor-infiltrating lymphocytes for patients with metastatic melanoma. J Clin Oncol 34, 2389–2397.
Golubovskaya, V.M. (2018). GITR domain inside CAR co-stimulates activity of CAR-T cells against cancer. Front Biosci 23, 2245–2254.
Gross, G., Waks, T., and Eshhar, Z. (1989). Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proc Natl Acad Sci USA 86, 10024–10028.
Grossman, W.J., Verbsky, J.W., Barchet, W., Colonna, M., Atkinson, J.P., and Ley, T.J. (2004). Human T regulatory cells can use the perforin pathway to cause autologous target cell death. Immunity 21, 589–601.
Grupp, S.A., Kalos, M., Barrett, D., Aplenc, R., Porter, D.L., Rheingold, S. R., Teachey, D.T., Chew, A., Hauck, B., Wright, J.F., et al. (2013). Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med 368, 1509–1518.
Guo, Y., Feng, K., Liu, Y., Wu, Z., Dai, H., Yang, Q., Wang, Y., Jia, H., and Han, W. (2018). Phase I study of chimeric antigen receptor-modified T cells in patients with EGFR-positive advanced biliary tract cancers. Clin Cancer Res 24, 1277–1286.
Hamieh, M., Dobrin, A., Cabriolu, A., van der Stegen, S.J.C., Giavridis, T., Mansilla-Soto, J., Eyquem, J., Zhao, Z., Whitlock, B.M., Miele, M.M., et al. (2019). CAR T cell trogocytosis and cooperative killing regulate tumour antigen escape. Nature 568, 112–116.
Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. Cell 144, 646–674.
Harlin, H., Meng, Y., Peterson, A.C., Zha, Y., Tretiakova, M., Slingluff, C., McKee, M., and Gajewski, T.F. (2009). Chemokine expression in melanoma metastases associated with $CD8^+$ T-cell recruitment. Cancer Res 69, 3077–3085.
Helsen, C.W., Hammill, J.A., Lau, V.W.C., Mwawasi, K.A., Afsahi, A., Bezverbnaya, K., Newhook, L., Hayes, D.L., Aarts, C., Bojovic, B., et al. (2018). The chimeric TAC receptor co-opts the T cell receptor yielding robust anti-tumor activity without toxicity. Nat Commun 9, 3049.
Henon, C., Blay, J.Y., Massard, C., Mir, O., Bahleda, R., Dumont, S., Postel-Vinay, S., Adam, J., Soria, J.C., and Le Cesne, A. (2019). Long lasting major response to pembrolizumab in a thoracic malignant rhabdoid-like SMARCA4-deficient tumor. Ann Oncol 30, 1401–1403.
Hillerdal, V., and Essand, M. (2015). Chimeric antigen receptor-engineered T cells for the treatment of metastatic prostate cancer. Biodrugs 29, 75–89.
Holmquist-Mengelbier, L., Fredlund, E., Löfstedt, T., Noguera, R., Navarro, S., Nilsson, H., Pietras, A., Vallon-Christersson, J., Borg, A., Gradin, K., et al. (2006). Recruitment of HIF-1α and HIF-2α to common target genes is differentially regulated in neuroblastoma: HIF-2α promotes an aggressive phenotype. Cancer Cell 10, 413–423.
Hu, B., Zou, Y., Zhang, L., Tang, J., Niedermann, G., Firat, E., Huang, X., and Zhu, X. (2019). Nucleofection with Plasmid DNA for CRISPR/Cas9-Mediated Inactivation of Programmed Cell Death Protein 1 in CD133-Specific CAR T Cells. Human Gene Ther 30, 446–458.
Huang, Q., Xia, J., Wang, L., Wang, X., Ma, X., Deng, Q., Lu, Y., Kumar, M., Zhou, Z., Li, L., et al. (2018). miR-153 suppresses IDO1 expression and enhances CAR T cell immunotherapy. J Hematol Oncol 11, 58.
Hung, C.F., Xu, X., Li, L., Ma, Y., Jin, Q., Viley, A., Allen, C., Natarajan, P., Shivakumar, R., Peshwa, M.V., et al. (2018). Development of anti-human mesothelin-targeted chimeric antigen receptor messenger RNA-transfected peripheral blood lymphocytes for ovarian cancer therapy. Hum Gene Ther 29, 614–625.
Hussain, S.P., Trivers, G.E., Hofseth, L.J., He, P., Shaikh, I., Mechanic, L. E., Doja, S., Jiang, W., Subleski, J., Shorts, L., et al. (2004). Nitric oxide, a mediator of inflammation, suppresses tumorigenesis. Cancer Res 64, 6849–6853.
Ishida, Y., Agata, Y., Shibahara, K., and Honjo, T. (1992). Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J 11, 3887–3895.
Ji, X., Li, H., Zhang, W., Wang, J., Liang, L., Zou, C., Yu, Z., Liu, S., and Zhang, K.Q. (2020). The lifestyle transition of Arthrobotrys oligospora is mediated by microRNA-like RNAs. Sci China Life Sci 63, doi:10.1007/s11427-018-9437-7.
Jiang, Z., Jiang, X., Chen, S., Lai, Y., Wei, X., Li, B., Lin, S., Wang, S., Wu, Q., Liang, Q., et al. (2017). Anti-GPC3-CAR T cells suppress the growth of tumor cells in patient-derived xenografts of hepatocellular carcinoma. Front Immunol 7.
Jimenez-Sanchez, A., Memon, D., Pourpe, S., Veeraraghavan, H., Li, Y., Vargas, H.A., Gill, M.B., Park, K.J., Zivanovic, O., Konner, J., et al. (2017). Heterogeneous tumor-immune microenvironments among differentially growing metastases in an ovarian cancer patient. Cell 170, 927–938.e20.
John, L.B., Devaud, C., Duong, C.P.M., Yong, C.S., Beavis, P.A., Haynes, N.M., Chow, M.T., Smyth, M.J., Kershaw, M.H., and Darcy, P.K. (2013). Anti-PD-1 antibody therapy potently enhances the eradication of established tumors by gene-modified T cells. Clin Cancer Res 19, 5636–5646.
Jonuleit, H., and Schmitt, E. (2003). The regulatory T cell family: distinct subsets and their interrelations. J Immunol 171, 6323–6327.
Jonuleit, H., Schmitt, E., Schuler, G., Knop, J., and Enk, A.H. (2000). Induction of interleukin 10-producing, nonproliferating $CD4^+$ T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. J Exp Med 192, 1213–1222.
Jonuleit, H., Schmitt, E., Stassen, M., Tuettenberg, A., Knop, J., and Enk, A.H. (2001). Identification and functional characterization of human $CD4^+CD25^+$ T cells with regulatory properties isolated from peripheral blood. J Exp Med 193, 1285–1294.
Jung, I.Y., Kim, Y.Y., Yu, H.S., Lee, M., Kim, S., and Lee, J. (2018). CRISPR/Cas9-mediated knockout of DGK improves antitumor activities of human T cells. Cancer Res 78, 4692–4703.
Junghans, R.P., Ma, Q., Rathore, R., Gomes, E.M., Bais, A.J., Lo, A.S.Y., Abedi, M., Davies, R.A., Cabral, H.J., Al-Homsi, A.S., et al. (2016). Phase I trial of anti-PSMA designer CAR-T cells in prostate cancer: possible role for interacting interleukin 2-T cell pharmacodynamics as a determinant of clinical response. Prostate 76, 1257–1270.
Katz, S.C., Burga, R.A., McCormack, E., Wang, L.J., Mooring, W., Point, G.R., Khare, P.D., Thorn, M., Ma, Q., Stainken, B.F., et al. (2015). Phase I hepatic immunotherapy for metastases study of intra-arterial chimeric antigen receptor-modified T-cell therapy for CEA^+ liver metastases. Clin Cancer Res 21, 3149–3159.
Kawamura, K., Bahar, R., Natsume, W., Sakiyama, S., and Tagawa, M. (2002). Secretion of interleukin-10 from murine colon carcinoma cells suppresses systemic antitumor immunity and impairs protective immunity induced against the tumors. Cancer Gene Ther 9, 109–115.
Keir, M.E., Butte, M.J., Freeman, G.J., and Sharpe, A.H. (2008). PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 26, 677–704.
Kerkar, S.P., Goldszmid, R.S., Muranski, P., Chinnasamy, D., Yu, Z., Reger, R.N., Leonardi, A.J., Morgan, R.A., Wang, E., Marincola, F.M., et al. (2011). IL-12 triggers a programmatic change in dysfunctional myeloid-derived cells within mouse tumors. J Clin Invest 121, 4746–4757.
Kershaw, M.H., Westwood, J.A., Parker, L.L., Wang, G., Eshhar, Z., Mavroukakis, S.A., White, D.E., Wunderlich, J.R., Canevari, S.,

Rogers-Freezer, L., et al. (2006). A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin Cancer Res 12, 6106–6115.
Kitayama, J., Nagawa, H., Nakayama, H., Tuno, N., Shibata, Y., and Muto, T. (1999). Functional expression of β1 and β2 integrins on tumor infiltrating lymphocytes (TILs) in colorectal cancer. J Gastroenterol 34, 327–333.
Kitayama, J., Tuno, N., Nakayama, H., Shibata, Y., Muto, T., and Nagawa, H. (1999). Functional down-regulation ofβ1 andβ2 integrins on lamina propria lymphocytes (LPL) and tumor-infiltrating lymphocytes (TIL) in colorectal cancer patients. Ann Surg Oncol 6, 500–506.
Klebanoff, C.A., Scott, C.D., Leonardi, A.J., Yamamoto, T.N., Cruz, A.C., Ouyang, C., Ramaswamy, M., Roychoudhuri, R., Ji, Y., Eil, R.L., et al. (2016). Memory T cell-driven differentiation of naive cells impairs adoptive immunotherapy. J Clin Invest 126, 318–334.
Kloss, C.C., Lee, J., Zhang, A., Chen, F., Melenhorst, J.J., Lacey, S.F., Maus, M.V., Fraietta, J.A., Zhao, Y., and June, C.H. (2018). Dominant-negative TGF-β receptor enhances PSMA-targeted human CAR T cell proliferation and augments prostate cancer eradication. Mol Ther 26, 1855–1866.
Knochelmann, H.M., Smith, A.S., Dwyer, C.J., Wyatt, M.M., Mehrotra, S., and Paulos, C.M. (2018). CAR T cells in solid tumors: blueprints for building effective therapies. Front Immunol 9, 1740.
Knoechel, B., Lohr, J., Kahn, E., Bluestone, J.A., and Abbas, A.K. (2005). Sequential development of interleukin 2-dependent effector and regulatory T cells in response to endogenous systemic antigen. J Exp Med 202, 1375–1386.
Kondo, A., Yamashita, T., Tamura, H., Zhao, W., Tsuji, T., Shimizu, M., Shinya, E., Takahashi, H., Tamada, K., Chen, L., et al. (2010). Interferon-γ and tumor necrosis factor-α induce an immunoinhibitory molecule, B7-H1, via nuclear factor-κB activation in blasts in myelodysplastic syndromes. Blood 116, 1124–1131.
Koneru, M., Purdon, T.J., Spriggs, D., Koneru, S., and Brentjens, R.J. (2015). IL-12 secreting tumor-targeted chimeric antigen receptor T cells eradicate ovarian tumors *in vivo*. Oncoimmunology 4, e994446.
Krenciute, G., Prinzing, B.L., Yi, Z., Wu, M.F., Liu, H., Dotti, G., Balyasnikova, I.V., and Gottschalk, S. (2017). Transgenic expression of IL15 improves antiglioma activity of IL13Rα2-CAR T cells but results in antigen loss variants. Cancer Immunol Res 5, 571–581.
Kumar, S., Davra, V., E. Obr, A., Geng, K., L. Wood, T., De Lorenzo, M.S., and Birge, R.B. (2017). Crk adaptor protein promotes PD-L1 expression, EMT and immune evasion in a murine model of triple-negative breast cancer. Oncoimmunology 7, e1376155.
Lacy, M.Q., Jacobus, S., Blood, E.A., Kay, N.E., Rajkumar, S.V., and Greipp, P.R. (2009). Phase II study of interleukin-12 for treatment of plateau phase multiple myeloma (E1A96): a trial of the Eastern Cooperative Oncology Group. Leukemia Res 33, 1485–1489.
Lamers, C.H.J., Sleijfer, S., Vulto, A.G., Kruit, W.H.J., Kliffen, M., Debets, R., Gratama, J.W., Stoter, G., and Oosterwijk, E. (2006). Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: first clinical experience. J Clin Oncol 24, e20–e22.
Lamers, C.H.J., Klaver, Y., Gratama, J.W., Sleijfer, S., and Debets, R. (2016). Treatment of metastatic renal cell carcinoma (mRCC) with CAIX CAR-engineered T-cells—a completed study overview. Biochem Soc Trans 44, 951–959.
Lamers, C.H., Sleijfer, S., van Steenbergen, S., van Elzakker, P., van Krimpen, B., Groot, C., Vulto, A., den Bakker, M., Oosterwijk, E., Debets, R., et al. (2013). Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: clinical evaluation and management of on-target toxicity. Mol Ther 21, 904–912.
Lan, G., Li, J., Wen, Q., Lin, L., Chen, L., Chen, L., and Chen, X. (2018) Cytotoxic T lymphocyte associated antigen 4 expression predicts poor prognosis in luminal B HER2-negative breast cancer. Oncol Lett .
Lee, D.W., Kochenderfer, J.N., Stetler-Stevenson, M., Cui, Y.K., Delbrook, C., Feldman, S.A., Fry, T.J., Orentas, R., Sabatino, M., Shah, N.N., et al. (2015). T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet 385, 517–528.
Lee, J.H., Lee, J.H., Lim, Y.S., Yeon, J.E., Song, T.J., Yu, S.J., Gwak, G.Y., Kim, K.M., Kim, Y.J., Lee, J.W., et al. (2015). Adjuvant immunotherapy with autologous cytokine-induced killer cells for hepatocellular carcinoma. Gastroenterology 148, 1383–1391.e6.
Leen, A.M., Sukumaran, S., Watanabe, N., Mohammed, S., Keirnan, J., Yanagisawa, R., Anurathapan, U., Rendon, D., Heslop, H.E., Rooney, C.M., et al. (2014). Reversal of tumor immune inhibition using a chimeric cytokine receptor. Mol Ther 22, 1211–1220.
Leonard, J.P., Sherman, M.L., Fisher, G.L., Buchanan, L.J., Larsen, G., Atkins, M.B., Sosman, J.A., Dutcher, J.P., Vogelzang, N.J., and Ryan J. L. (1997). Effects of single-dose interleukin-12 exposure on interleukin-12-associated toxicity and interferon-gamma production. Blood 90, 2541–2548.
Li, H., and Zhao, Y. (2017). Increasing the safety and efficacy of chimeric antigen receptor T cell therapy. Protein Cell 8, 573–589.
Li, W., Guo, L., Rathi, P., Marinova, E., Gao, X., Wu, M.F., Liu, H., Dotti, G., Gottschalk, S., Metelitsa, L.S., et al. (2017). Redirecting T cells to Glypican-3 with 4-1BB zeta chimeric antigen receptors results in Th1 polarization and potent antitumor activity. Hum Gene Ther 28, 437–448.
Ligtenberg, M.A., Mougiakakos, D., Mukhopadhyay, M., Witt, K., Lladser, A., Chmielewski, M., Riet, T., Abken, H., and Kiessling, R. (2016). Coexpressed catalase protects chimeric antigen receptor-redirected T cells as well as bystander cells from oxidative stress-induced loss of antitumor activity. J Immunol 196, 759–766.
Lim, T.S., Chew, V., Sieow, J.L., Goh, S., Yeong, J.P.S., Soon, A.L., and Ricciardi-Castagnoli, P. (2016). PD-1 expression on dendritic cells suppresses $CD8^+$ T cell function and antitumor immunity. Oncoimmunology 5, e1085146.
Liu, H., Xu, Y., Xiang, J., Long, L., Green, S., Yang, Z., Zimdahl, B., Lu, J., Cheng, N., Horan, L.H., et al. (2017). Targeting alpha-fetoprotein (AFP)-MHC complex with CAR T-cell therapy for liver cancer. Clin Cancer Res 23, 478–488.
Liu, V.C., Wong, L.Y., Jang, T., Shah, A.H., Park, I., Yang, X., Zhang, Q., Lonning, S., Teicher, B.A., and Lee, C. (2007). Tumor evasion of the immune system by converting $CD4^+CD25^-$ T cells into $CD4^+CD25^+$ T regulatory cells: role of tumor-derived TGF-β. J Immunol 178, 2883–2892.
Liu, Y., Song, X., Wang, X., Wei, L., Liu, X., Yuan, S., and Lv, L. (2010). Effect of chronic intermittent hypoxia on biological behavior and hypoxia-associated gene expression in lung cancer cells. J Cell Biochem 111, 554–563.
Liyanage, U.K., Moore, T.T., Joo, H.G., Tanaka, Y., Herrmann, V., Doherty, G., Drebin, J.A., Strasberg, S.M., Eberlein, T.J., Goedegebuure, P.S., et al. (2002). Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma. J Immunol 169, 2756–2761.
Louis, C.U., Savoldo, B., Dotti, G., Pule, M., Yvon, E., Myers, G.D., Rossig, C., Russell, H.V., Diouf, O., Liu, E., et al. (2011). Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. Blood 118, 6050–6056.
Lu, T., Ramakrishnan, R., Altiok, S., Youn, J.I., Cheng, P., Celis, E., Pisarev, V., Sherman, S., Sporn, M.B., and Gabrilovich, D. (2011). Tumor-infiltrating myeloid cells induce tumor cell resistance to cytotoxic T cells in mice. J Clin Invest 121, 4015–4029.
Lu, Y.J., Chu, H., Wheeler, L.W., Nelson, M., Westrick, E., Matthaei, J.F., Cardle, I.I., Johnson, A., Gustafson, J., Parker, N., et al. (2019). Preclinical evaluation of bispecific adaptor molecule controlled folate receptor CAR-T cell therapy with special focus on pediatric malignancies. Front Oncol 9, 151.
Ma, Q., Gomes, E.M., Lo, A.S.Y., and Junghans, R.P. (2014). Advanced generation anti-prostate specific membrane antigen designer T cells for prostate cancer immunotherapy. Prostate 74, 286–296.
Ma, Y., Adjemian, S., Mattarollo, S.R., Yamazaki, T., Aymeric, L., Yang, H., Portela Catani, J.P., Hannani, D., Duret, H., Steegh, K., et al. (2013).

Anticancer chemotherapy-induced intratumoral recruitment and differentiation of antigen-presenting cells. Immunity 38, 729–741.
Majzner, R.G., Heitzeneder, S., and Mackall, C.L. (2017). Harnessing the immunotherapy revolution for the treatment of childhood cancers. Cancer Cell 31, 476–485.
Majzner, R.G., and Mackall, C.L. (2018). Tumor antigen escape from CAR T-cell therapy. Cancer Discov 8, 1219–1226.
Majzner, R.G., Theruvath, J.L., Nellan, A., Heitzeneder, S., Cui, Y., Mount, C.W., Rietberg, S.P., Linde, M.H., Xu, P., Rota, C., et al. (2019). CAR T cells targeting B7-H3, a pan-cancer antigen, demonstrate potent preclinical activity against pediatric solid tumors and brain tumors. Clin Cancer Res 25, 2560–2574.
Maliar, A., Servais, C., Waks, T., Chmielewski, M., Lavy, R., Altevogt, P., Abken, H., and Eshhar, Z. (2012). Redirected T cells that target pancreatic adenocarcinoma antigens eliminate tumors and metastases in mice. Gastroenterology 143, 1375–1384.e5.
Mantovani, A., Garlanda, C., and Locati, M. (2009). Macrophage diversity and polarization in atherosclerosis. Arterioscler Thromb Vasc Biol 29, 1419–1423.
Mantovani, A., Sica, A., and Locati, M. (2005). Macrophage polarization comes of age. Immunity 23, 344–346.
Marcucci, F., Rumio, C., and Corti, A. (2017). Tumor cell-associated immune checkpoint molecules—drivers of malignancy and stemness. Biochim Biophys Acta Rev Cancer 1868, 571–583.
Marigo, I., Bosio, E., Solito, S., Mesa, C., Fernandez, A., Dolcetti, L., Ugel, S., Sonda, N., Bicciato, S., Falisi, E., et al. (2010). Tumor-induced tolerance and immune suppression depend on the C/EBPβ transcription factor. Immunity 32, 790–802.
Marmarelis, M.E., and Aggarwal, C. (2018). Combination immunotherapy in non-small cell lung cancer. Curr Oncol Rep 20, 55.
Martinet, L., Le Guellec, S., Filleron, T., Lamant, L., Meyer, N., Rochaix, P., Garrido, I., and Girard, J.P. (2012). High endothelial venules (HEVs) in human melanoma lesions. Oncoimmunology 1, 829–839.
Martinez, M., and Moon, E.K. (2019). CAR T cells for solid tumors: new strategies for finding, infiltrating, and surviving in the tumor microenvironment. Front Immunol 10, 128.
Maude, S.L., Frey, N., Shaw, P.A., Aplenc, R., Barrett, D.M., Bunin, N.J., Chew, A., Gonzalez, V.E., Zheng, Z., Lacey, S.F., et al. (2014). Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 371, 1507–1517.
Maude, S.L., Laetsch, T.W., Buechner, J., Rives, S., Boyer, M., Bittencourt, H., Bader, P., Verneris, M.R., Stefanski, H.E., Myers, G.D., et al. (2018). Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med 378, 439–448.
Maus, M.V., Haas, A.R., Beatty, G.L., Albelda, S.M., Levine, B.L., Liu, X., Zhao, Y., Kalos, M., and June, C.H. (2013). T cells expressing chimeric antigen receptors can cause anaphylaxis in humans. Cancer Immunol Res 1, 26–31.
Meirow, Y., and Baniyash, M. (2017). Immune biomarkers for chronic inflammation related complications in non-cancerous and cancerous diseases. Cancer Immunol Immunother 66, 1089–1101.
Menger, L., Sledzinska, A., Bergerhoff, K., Vargas, F.A., Smith, J., Poirot, L., Pule, M., Hererro, J., Peggs, K.S., and Quezada, S.A. (2016). TALEN-mediated inactivation of PD-1 in tumor-reactive lymphocytes promotes intratumoral T-cell persistence and rejection of established tumors. Cancer Res 76, 2087–2093.
Miao, Z.F., Zhao, T.T., Wang, Z.N., Xu, Y.Y., Mao, X.Y., Wu, J.H., Liu, X. Y., Xu, H., You, Y., and Xu, H.M. (2014). Influence of different hypoxia models on metastatic potential of SGC-7901 gastric cancer cells. Tumor Biol 35, 6801–6808.
Mikucki, M.E., Fisher, D.T., Matsuzaki, J., Skitzki, J.J., Gaulin, N.B., Muhitch, J.B., Ku, A.W., Frelinger, J.G., Odunsi, K., Gajewski, T.F., et al. (2015). Non-redundant requirement for CXCR3 signalling during tumoricidal T-cell trafficking across tumour vascular checkpoints. Nat Commun 6, 7458.
Mirzaei, H.R., Rodriguez, A., Shepphird, J., Brown, C.E., and Badie, B. (2017). Chimeric antigen receptors T cell therapy in solid tumor: challenges and clinical applications. Front Immunol 8, 1850.
Miyara, M., and Sakaguchi, S. (2011). Human FoxP3^{+}CD4^{+} regulatory T cells: their knowns and unknowns. Immunol Cell Biol 89, 346–351.
Mlecnik, B., Tosolini, M., Charoentong, P., Kirilovsky, A., Bindea, G., Berger, A., Camus, M., Gillard, M., Bruneval, P., Fridman, W.H., et al. (2010). Biomolecular network reconstruction identifies T-cell homing factors associated with survival in colorectal cancer. Gastroenterology 138, 1429–1440.
Molon, B., Ugel, S., Del Pozzo, F., Soldani, C., Zilio, S., Avella, D., De Palma, A., Mauri, P.L., Monegal, A., Rescigno, M., et al. (2011). Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. J Exp Med 208, 1949–1962.
Moon, E.K., Carpenito, C., Sun, J., Wang, L.C.S., Kapoor, V., Predina, J., Powell, D.J., Riley, J.L., June, C.H., and Albelda, S.M. (2011). Expression of a functional CCR2 receptor enhances tumor localization and tumor eradication by retargeted human T cells expressing a mesothelin-specific chimeric antibody receptor. Clin Cancer Res 17, 4719–4730.
Movahedi, K., Guilliams, M., Van den Bossche, J., Van den Bergh, R., Gysemans, C., Beschin, A., De Baetselier, P., and Van Ginderachter, J. A. (2008). Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell-suppressive activity. Blood 111, 4233–4244.
Mullins, I.M., Slingluff, C.L., Lee, J.K., Garbee, C.F., Shu, J., Anderson, S. G., Mayer, M.E., Knaus, W.A., and Mullins, D.W. (2004). CXC chemokine receptor 3 expression by activated CD8^{+} T cells is associated with survival in melanoma patients with stage III disease. Cancer Res 64, 7697–7701.
Muniappan, A., Banapour, B., Lebkowski, J., and Talib, S. (2000). Ligand-mediated cytolysis of tumor cells: use of heregulin-ζ chimeras to redirect cytotoxic T lymphocytes. Cancer Gene Ther 7, 128–134.
Murad, J.P., Kozlowska, A.K., Lee, H.J., Ramamurthy, M., Chang, W.C., Yazaki, P., Colcher, D., Shively, J., Cristea, M., Forman, S.J., et al. (2018). Effective targeting of TAG72+ peritoneal ovarian tumors via regional delivery of CAR-engineered T cells. Front Immunol 9, 2268.
Nagaraj, S., Gupta, K., Pisarev, V., Kinarsky, L., Sherman, S., Kang, L., Herber, D.L., Schneck, J., and Gabrilovich, D.I. (2007). Altered recognition of antigen is a mechanism of CD8^{+} T cell tolerance in cancer. Nat Med 13, 828–835.
Nedoszytko, B., Sokołowska-Wojdyło, M., Renke, J., Lange, M., Trzonkowski, P., Sobjanek, M., Szczerkowska-Dobosz, A., Niedoszytko, M., Górska, A., Romantowski, J., et al. (2017). The role of regulatory T cells and genes involved in their differentiation in pathogenesis of selected inflammatory and neoplastic skin diseases. Part III: Polymorphisms of genes involved in Tregs' activation and function. Postepy Dermatol Alergol 34, 517–525.
Neelapu, S.S., Tummala, S., Kebriaei, P., Wierda, W., Gutierrez, C., Locke, F.L., Komanduri, K.V., Lin, Y., Jain, N., Daver, N., et al. (2018). Chimeric antigen receptor T-cell therapy—assessment and management of toxicities. Nat Rev Clin Oncol 15, 47–62.
Nishida, N., and Kudo, M. (2018). Immune checkpoint blockade for the treatment of human hepatocellular carcinoma. Hepatol Res 48, 622–634.
Noman, M.Z., Janji, B., Hu, S., Wu, J.C., Martelli, F., Bronte, V., and Chouaib, S. (2015). Tumor-promoting effects of myeloid-derived suppressor cells are potentiated by hypoxia-induced expression of miR-210. Cancer Res 75, 3771–3787.
O'Rourke, D.M., Nasrallah, M.L.P., Desai, A., Melenhorst, J.J., Mansfield, K., Morrissette, J.J.D., Martinez-Lage, M., Brem, S., Maloney, E., Shen, A., et al. (2017). A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. Sci Transl Med 9, eaaa0984.
Odorizzi, P.M., Pauken, K.E., Paley, M.A., Sharpe, A., and Wherry, E.J. (2015). Genetic absence of PD-1 promotes accumulation of terminally differentiated exhausted CD8^{+} T cells. J Exp Med 212, 1125–1137.
Ornstein, M.C., Diaz-Montero, C.M., Rayman, P., Elson, P., Haywood, S.,

Finke, J.H., Kim, J.S., Pavicic Jr, P.G., Lamenza, M., Devonshire, S., et al. (2018). Myeloid-derived suppressors cells (MDSC) correlate with clinicopathologic factors and pathologic complete response (pCR) in patients with urothelial carcinoma (UC) undergoing cystectomy. Urol Oncol 36, 405–412.

Pardoll, D.M. (2012). The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 12, 252–264.

Parente-Pereira, A.C., Burnet, J., Ellison, D., Foster, J., Davies, D.M., van der Stegen, S., Burbridge, S., Chiapero-Stanke, L., Wilkie, S., Mather, S., et al. (2011). Trafficking of CAR-engineered human T cells following regional or systemic adoptive transfer in SCID beige mice. J Clin Immunol 31, 710–718.

Parente-Pereira, A.C., Whilding, L.M., Brewig, N., van der Stegen, S.J.C., Davies, D.M., Wilkie, S., van Schalkwyk, M.C.I., Ghaem-Maghami, S., and Maher, J. (2013). Synergistic chemoimmunotherapy of epithelial ovarian cancer using ErbB-retargeted T cells combined with carboplatin. J Immunol 191, 2437–2445.

Park, J.R., Digiusto, D.L., Slovak, M., Wright, C., Naranjo, A., Wagner, J., Meechoovet, H.B., Bautista, C., Chang, W.C., Ostberg, J.R., et al. (2007). Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma. Mol Ther 15, 825–833.

Pegram, H.J., Lee, J.C., Hayman, E.G., Imperato, G.H., Tedder, T.F., Sadelain, M., and Brentjens, R.J. (2012). Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning. Blood 119, 4133–4141.

Pena-Cruz, V., McDonough, S.M., Diaz-Griffero, F., Crum, C.P., Carrasco, R.D., and Freeman, G.J. (2010). PD-1 on immature and PD-1 ligands on migratory human Langerhans cells regulate antigen-presenting cell activity. J Invest Dermatol 130, 2222–2230.

Peter, M.E., Hadji, A., Murmann, A.E., Brockway, S., Putzbach, W., Pattanayak, A., and Ceppi, P. (2015). The role of CD95 and CD95 ligand in cancer. Cell Death Differ 22, 549–559.

Pituch, K.C., Miska, J., Krenciute, G., Panek, W.K., Li, G., Rodriguez-Cruz, T., Wu, M., Han, Y., Lesniak, M.S., Gottschalk, S., et al. (2018). Adoptive transfer of IL13Rα2-specific chimeric antigen receptor T cells creates a pro-inflammatory environment in glioblastoma. Mol Ther 26, 986–995.

Porter, D.L., Levine, B.L., Kalos, M., Bagg, A., and June, C.H. (2011). Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med 365, 725–733.

Posey Jr., A.D., Schwab, R.D., Boesteanu, A.C., Steentoft, C., Mandel, U., Engels, B., Stone, J.D., Madsen, T.D., Schreiber, K., Haines, K.M., et al. (2016). Engineered CAR T cells targeting the cancer-associated Tn-glycoform of the membrane mucin MUC1 control adenocarcinoma. Immunity 44, 1444–1454.

Pule, M.A., Savoldo, B., Myers, G.D., Rossig, C., Russell, H.V., Dotti, G., Huls, M.H., Liu, E., Gee, A.P., Mei, Z., et al. (2008). Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. Nat Med 14, 1264–1270.

Punt, C.J.A., Koopman, M., and Vermeulen, L. (2017). From tumour heterogeneity to advances in precision treatment of colorectal cancer. Nat Rev Clin Oncol 14, 235–246.

Qian, B.Z., and Pollard, J.W. (2010). Macrophage diversity enhances tumor progression and metastasis. Cell 141, 39–51.

Qu, X., Tang, Y., and Hua, S. (2018). Immunological approaches towards cancer and inflammation: a cross talk. Front Immunol 9, 563.

Quintarelli, C., Orlando, D., Boffa, I., Guercio, M., Polito, V.A., Petretto, A., Lavarello, C., Sinibaldi, M., Weber, G., Del Bufalo, F., et al. (2018). Choice of costimulatory domains and of cytokines determines CAR T-cell activity in neuroblastoma. Oncoimmunology 7, e1433518.

Qureshi, O.S., Zheng, Y., Nakamura, K., Attridge, K., Manzotti, C., Schmidt, E.M., Baker, J., Jeffery, L.E., Kaur, S., Briggs, Z., et al. (2011). Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. Science 332, 600–603.

Rafiq, S., Yeku, O.O., Jackson, H.J., Purdon, T.J., van Leeuwen, D.G., Drakes, D.J., Song, M., Miele, M.M., Li, Z., Wang, P., et al. (2018). Targeted delivery of a PD-1-blocking scFv by CAR-T cells enhances anti-tumor efficacy in vivo. Nat Biotechnol 36, 847–856.

Ramakrishnan, R., Huang, C., Cho, H.I., Lloyd, M., Johnson, J., Ren, X., Altiok, S., Sullivan, D., Weber, J., Celis, E., et al. (2012). Autophagy induced by conventional chemotherapy mediates tumor cell sensitivity to immunotherapy. Cancer Res 72, 5483–5493.

Riese, M.J., Wang, L.C.S., Moon, E.K., Joshi, R.P., Ranganathan, A., June, C.H., Koretzky, G.A., and Albelda, S.M. (2013). Enhanced effector responses in activated $CD8^+$ T cells deficient in diacylglycerol kinases. Cancer Res 73, 3566–3577.

Rodriguez, P.C., Quiceno, D.G., and Ochoa, A.C. (2007). L-arginine availability regulates T-lymphocyte cell-cycle progression. Blood 109, 1568–1573.

Rofstad, E.K., Gaustad, J.V., Egeland, T.A.M., Mathiesen, B., and Galappathi, K. (2010). Tumors exposed to acute cyclic hypoxic stress show enhanced angiogenesis, perfusion and metastatic dissemination. Int J Cancer 127, 1535–1546.

Ruella, M., Klichinsky, M., Kenderian, S.S., Shestova, O., Ziober, A., Kraft, D.O., Feldman, M., Wasik, M.A., June, C.H., and Gill, S. (2017). Overcoming the immunosuppressive tumor microenvironment of Hodgkin lymphoma using chimeric antigen receptor T cells. Cancer Discov 7, 1154–1167.

Rupp, L.J., Schumann, K., Roybal, K.T., Gate, R.E., Ye, C.J., Lim, W.A., and Marson, A. (2017). CRISPR/Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of human chimeric antigen receptor T cells. Sci Rep 7, 737.

Sabaawy, H.E. (2013). Genetic heterogeneity and clonal evolution of tumor cells and their impact on precision cancer medicine. J Leuk 01.

Sadelain, M. (2016). Tales of antigen evasion from CAR therapy. Cancer Immunol Res 4, 473.

Sadelain, M., Brentjens, R., and Rivière, I. (2013). The basic principles of chimeric antigen receptor design. Cancer Discov 3, 388–398.

Sadelain, M., Rivière, I., and Brentjens, R. (2003). Targeting tumours with genetically enhanced T lymphocytes. Nat Rev Cancer 3, 35–45.

Sakaguchi, S., Yamaguchi, T., Nomura, T., and Ono, M. (2008). Regulatory T cells and immune tolerance. Cell 133, 775–787.

Sanmamed, M.F., and Chen, L. (2014). Inducible expression of B7-H1 (PD-L1) and its selective role in tumor site immune modulation. Cancer J 20, 256–261.

Santer, F.R., Malinowska, K., Culig, Z., and Cavarretta, I.T. (2010). Interleukin-6 trans-signalling differentially regulates proliferation, migration, adhesion and maspin expression in human prostate cancer cells. Endocr Relat Cancer 17, 241–253.

Santoni, M., Massari, F., Amantini, C., Nabissi, M., Maines, F., Burattini, L., Berardi, R., Santoni, G., Montironi, R., Tortora, G., et al. (2013). Emerging role of tumor-associated macrophages as therapeutic targets in patients with metastatic renal cell carcinoma. Cancer Immunol Immunother 62, 1757–1768.

Sapoznik, S., Ortenberg, R., Galore-Haskel, G., Kozlovski, S., Levy, D., Avivi, C., Barshack, I., Cohen, C.J., Besser, M.J., Schachter, J., et al. (2012). CXCR1 as a novel target for directing reactive T cells toward melanoma: implications for adoptive cell transfer immunotherapy. Cancer Immunol Immunother 61, 1833–1847.

Saw, P.E., and Song, E.W. (2020). siRNA therapeutics: a clinical reality. Sci China Life Sci 63, doi:10.1007/s11427-018-9438-y .

Sawanobori, Y., Ueha, S., Kurachi, M., Shimaoka, T., Talmadge, J.E., Abe, J., Shono, Y., Kitabatake, M., Kakimi, K., Mukaida, N., et al. (2008). Chemokine-mediated rapid turnover of myeloid-derived suppressor cells in tumor-bearing mice. Blood 111, 5457–5466.

Schadendorf, D., Hodi, F.S., Robert, C., Weber, J.S., Margolin, K., Hamid, O., Patt, D., Chen, T.T., Berman, D.M., and Wolchok, J.D. (2015). Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. J Clin Oncol 33, 1889–1894.

Schuberth, P.C., Hagedorn, C., Jensen, S.M., Gulati, P., van den Broek, M., Mischo, A., Soltermann, A., Jüngel, A., Marroquin Belaunzaran, O.,

Stahel, R., et al. (2013). Treatment of malignant pleural mesothelioma by fibroblast activation protein-specific re-directed T cells. J Transl Med 11, 187.
Seidel, J.A., Otsuka, A., and Kabashima, K. (2018). Anti-PD-1 and anti-CTLA-4 therapies in cancer: mechanisms of action, efficacy, and limitations. Front Oncol 8, 86.
Semenza, G.L. (2003). Targeting HIF-1 for cancer therapy. Nat Rev Cancer 3, 721–732.
Senovilla, L., Vitale, I., Martins, I., Tailler, M., Pailleret, C., Michaud, M., Galluzzi, L., Adjemian, S., Kepp, O., Niso-Santano, M., et al. (2012). An immunosurveillance mechanism controls cancer cell ploidy. Science 337, 1678–1684.
Sharma, P., Siefker-Radtke, A., de Braud, F., Basso, U., Calvo, E., Bono, P., Morse, M.A., Ascierto, P.A., Lopez-Martin, J., Brossart, P., et al. (2019). Nivolumab alone and with ipilimumab in previously treated metastatic urothelial carcinoma: CheckMate 032 nivolumab 1 mg/kg plus ipilimumab 3 mg/kg expansion cohort results. J Clin Oncol 37, 1608–1616.
Shevach, E.M. (2002). $CD4^+CD25^+$ suppressor T cells: more questions than answers. Nat Rev Immunol 2, 389–400.
Shevach, E.M. (2006). From vanilla to 28 flavors: multiple varieties of T regulatory cells. Immunity 25, 195–201.
Shi, T., Ma, Y., Yu, L., Jiang, J., Shen, S., Hou, Y., and Wang, T. (2018). Cancer immunotherapy: a focus on the regulation of immune checkpoints. IJMS 19, 1389.
Shibahara, D., Tanaka, K., Iwama, E., Kubo, N., Ota, K., Azuma, K., Harada, T., Fujita, J., Nakanishi, Y., and Okamoto, I. (2018). Intrinsic and extrinsic regulation of PD-L2 expression in oncogene-driven non-small cell lung cancer. J Thorac Oncol 13, 926–937.
Shrivastava, R., Asif, M., Singh, V., Dubey, P., Ahmad Malik, S., Lone, M. U.D., Tewari, B.N., Baghel, K.S., Pal, S., Nagar, G.K., et al. (2019). M2 polarization of macrophages by Oncostatin M in hypoxic tumor microenvironment is mediated by mTORC2 and promotes tumor growth and metastasis. Cytokine 118, 130–143.
Simon, B., Harrer, D.C., Schuler-Thurner, B., Schaft, N., Schuler, G., Dörrie, J., and Uslu, U. (2018). The siRNA-mediated downregulation of PD-1 alone or simultaneously with CTLA-4 shows enhanced *in vitro* CAR-T-cell functionality for further clinical development towards the potential use in immunotherapy of melanoma. Exp Dermatol 27, 769–778.
Sinha, P., Clements, V.K., Bunt, S.K., Albelda, S.M., and Ostrand-Rosenberg, S. (2007). Cross-talk between myeloid-derived suppressor cells and macrophages subverts tumor immunity toward a type 2 response. J Immunol 179, 977–983.
Sistigu, A., Yamazaki, T., Vacchelli, E., Chaba, K., Enot, D.P., Adam, J., Vitale, I., Goubar, A., Baracco, E.E., Remédios, C., et al. (2014). Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. Nat Med 20, 1301–1309.
Song, D.G., Ye, Q., Carpenito, C., Poussin, M., Wang, L.P., Ji, C., Figini, M., June, C.H., Coukos, G., and Powell, D.J. (2011). *In vivo* persistence, tumor localization, and antitumor activity of CAR-engineered T cells is enhanced by costimulatory signaling through CD137 (4-1BB). Cancer Res 71, 4617–4627.
Sotillo, E., Barrett, D.M., Black, K.L., Bagashev, A., Oldridge, D., Wu, G., Sussman, R., Lanauze, C., Ruella, M., Gazzara, M.R., et al. (2015). Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. Cancer Discov 5, 1282–1295.
Soumerai, J.D., Davids, M.S., Werner, L., Fisher, D.C., Armand, P., Amrein, P.C., Neuberg, D., Hochberg, E.P., Brown, J.R., and Abramson, J.S. (2019). Phase 1 study of lenalidomide, bendamustine, and rituximab in previously untreated patients with chronic lymphocytic leukemia. Leuk Lymphoma 60, 2931–2938.
Steinbrink, K., Wölfl, M., Jonuleit, H., Knop, J., and Enk, A.H. (1997). Induction of tolerance by IL-10-treated dendritic cells. J Immunol 159, 4772–4780.
Strauss, L., Bergmann, C., and Whiteside, T.L. (2009). Human circulating $CD4^+CD25^{high}$ $Foxp3^+$ regulatory T cells kill autologous $CD8^+$ but not $CD4^+$ responder cells by Fas-mediated apoptosis. J Immunol 182, 1469–1480.
Su, S., Hu, B., Shao, J., Shen, B., Du, J., Du, Y., Zhou, J., Yu, L., Zhang, L., Chen, F., et al. (2016). CRISPR-Cas9 mediated efficient PD-1 disruption on human primary T cells from cancer patients. Sci Rep 6, 20070.
Su, Y.W., Xie, T.X., Sano, D., and Myers, J.N. (2011). IL-6 stabilizes Twist and enhances tumor cell motility in head and neck cancer cells through activation of casein kinase 2. PLoS ONE 6, e19412.
Suarez, E.R., Chang, D.K., Sun, J., Sui, J., Freeman, G.J., Signoretti, S., Zhu, Q., and Marasco, W.A. (2016). Chimeric antigen receptor T cells secreting anti-PD-L1 antibodies more effectively regress renal cell carcinoma in a humanized mouse model. Oncotarget 7, 34341–34355.
Sun, C., Dotti, G., and Savoldo, B. (2016). Utilizing cell-based therapeutics to overcome immune evasion in hematologic malignancies. Blood 127, 3350–3359.
Takayama, T., Sekine, T., Makuuchi, M., Yamasaki, S., Kosuge, T., Yamamoto, J., Shimada, K., Sakamoto, M., Hirohashi, S., Ohashi, Y., et al. (2000). Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomised trial. Lancet 356, 802–807.
Tang, F., and Zheng, P. (2018). Tumor cells versus host immune cells: whose PD-L1 contributes to PD-1/PD-L1 blockade mediated cancer immunotherapy? Cell Biosci 8, 34.
Tao, K., He, M., Tao, F., Xu, G., Ye, M., Zheng, Y., and Li, Y. (2018). Development of NKG2D-based chimeric antigen receptor-T cells for gastric cancer treatment. Cancer Chemother Pharmacol 82, 815–827.
Tchou, J., Zhao, Y., Levine, B.L., Zhang, P.J., Davis, M.M., Melenhorst, J. J., Kulikovskaya, I., Brennan, A.L., Liu, X., Lacey, S.F., et al. (2017). Safety and efficacy of intratumoral injections of chimeric antigen receptor (CAR) T cells in metastatic breast cancer. Cancer Immunol Res 5, 1152–1161.
Teng, R., Zhao, J., Zhao, Y., Gao, J., Li, H., Zhou, S., Wang, Y., Sun, Q., Lin, Z., Yang, W., et al. (2019). Chimeric antigen recepto-modified T cells repressed solid tumors and their relapse in an established patient-derived colon carcinoma xenograft model. J Immunother 42, 33–42.
Thibult, M.L., Mamessier, E., Gertner-Dardenne, J., Pastor, S., Just-Landi, S., Xerri, L., Chetaille, B., and Olive, D. (2013). PD-1 is a novel regulator of human B-cell activation. Int Immunol 25, 129–137.
Thomlinson, R.H., and Gray, L.H. (1955). The histological structure of some human lung cancers and the possible implications for radiotherapy. Br J Cancer 9, 539–549.
Ti, D., Niu, Y., Wu, Z., Fu, X., and Han, W. (2018). Genetic engineering of T cells with chimeric antigen receptors for hematological malignancy immunotherapy. Sci China Life Sci 61, 1320–1332.
Tian, M., Shi, Y., Liu, W., and Fan, J. (2019). Immunotherapy of hepatocellular carcinoma: strategies for combinatorial intervention. Sci China Life Sci 62, 1138–1143.
Tirosh, I., Izar, B., Prakadan, S.M., Wadsworth, M.H., Treacy, D., Trombetta, J.J., Rotem, A., Rodman, C., Lian, C., Murphy, G., et al. (2016). Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. Science 352, 189–196.
Tong, B., and Wang, M. (2018). CD47 is a novel potent immunotherapy target in human malignancies: current studies and future promises. Future Oncol 14, 2179–2188.
Townsend, M.H., Shrestha, G., Robison, R.A., and O'Neill, K.L. (2018). The expansion of targetable biomarkers for CAR T cell therapy. J Exp Clin Cancer Res 37, 163.
Trabattoni, D., Saresella, M., Biasin, M., Boasso, A., Piacentini, L., Ferrante, P., Dong, H., Maserati, R., Shearer, G.M., Chen, L., et al. (2003). B7-H1 is up-regulated in HIV infection and is a novel surrogate marker of disease progression. Blood 101, 2514–2520.
Traverso, I., Fenoglio, D., Negrini, S., Parodi, A., Battaglia, F., Kalli, F., Conteduca, G., Tardito, S., Traverso, P., Indiveri, F., et al. (2012). Cyclophosphamide inhibits the generation and function of $CD8^+$ regulatory T cells. Hum Immunol 73, 207–213.

van Dalen, F.J., van Stevendaal, M.H.M.E., Fennemann, F.L., Verdoes, M., and Ilina, O. (2019). Molecular Repolarisation of Tumour-Associated Macrophages. Molecules 24, 9.

Van Schandevyl, S., and Kerre, T. (2018). Chimeric antigen receptor T-cell therapy: design improvements and therapeutic strategies in cancer treatment. Acta Clin Belg 1–7.

Verreck, F.A.W., de Boer, T., Langenberg, D.M.L., Hoeve, M.A., Kramer, M., Vaisberg, E., Kastelein, R., Kolk, A., de Waal-Malefyt, R., and Ottenhoff, T.H.M. (2004). Human IL-23-producing type 1 macrophages promote but IL-10-producing type 2 macrophages subvert immunity to (myco)bacteria. Proc Natl Acad Sci USA 101, 4560–4565.

Vom Berg, J., Vrohlings, M., Haller, S., Haimovici, A., Kulig, P., Sledzinska, A., Weller, M., and Becher, B. (2013). Intratumoral IL-12 combined with CTLA-4 blockade elicits T cell-mediated glioma rejection. J Exp Med 210, 2803–2811.

Wallstabe, L., Mades, A., Frenz, S., Einsele, H., Rader, C., and Hudecek, M. (2018). CAR T cells targeting α_v β_3 integrin are effective against advanced cancer in preclinical models. Adv Cell Gene Ther 1, e11.

Wang, Y., Chen, M., Wu, Z., Tong, C., Dai, H., Guo, Y., Liu, Y., Huang, J., Lv, H., Luo, C., et al. (2018). CD133-directed CAR T cells for advanced metastasis malignancies: A phase I trial. Oncoimmunology 7, e1440169.

Wang, Y., Yu, W., Zhu, J., Wang, J., Xia, K., Liang, C., and Tao, H. (2019). Anti-CD166/4-1BB chimeric antigen receptor T cell therapy for the treatment of osteosarcoma. J Exp Clin Cancer Res 38, 168.

Weiskopf, K. (2017). Cancer immunotherapy targeting the CD47/SIRPα axis. Eur J Cancer 76, 100–109.

Whiteside, T.L. (2014). Regulatory T cell subsets in human cancer: are they regulating for or against tumor progression? Cancer Immunol Immunother 63, 67–72.

Wieder, T., Eigentler, T., Brenner, E., and Röcken, M. (2018). Immune checkpoint blockade therapy. J Allergy Clin Immunol 142, 1403–1414.

Wilkie, S., Picco, G., Foster, J., Davies, D.M., Julien, S., Cooper, L., Arif, S., Mather, S.J., Taylor-Papadimitriou, J., Burchell, J.M., et al. (2008). Retargeting of human T cells to tumor-associated MUC1: the evolution of a chimeric antigen receptor. J Immunol 180, 4901–4909.

Wu, A., Wei, J., Kong, L.Y., Wang, Y., Priebe, W., Qiao, W., Sawaya, R., and Heimberger, A.B. (2010). Glioma cancer stem cells induce immunosuppressive macrophages/microglia. Neuro-Oncol 12, 1113–1125.

Xu, J., Wang, Y., Shi, J., Liu, J., Li, Q., and Chen, L. (2018). Combination therapy: A feasibility strategy for CAR-T cell therapy in the treatment of solid tumors (Review). Oncol Lett 16, 2063–2070.

Xu, W., Deng, B., Lin, P., Liu, C., Li, B., Huang, Q., Zhou, H., Yang, J., and Qu, L. (2020). Ribosome profiling analysis identified a KRAS-interacting microprotein that represses oncogenic signaling in hepatocellular carcinoma cells. Sci China Life Sci 63, doi:10.1007/s11427-019-9580-5.

Yaghoubi, N., Soltani, A., Ghazvini, K., Hassanian, S.M., and Hashemy, S. I. (2019). PD-1/PD-L1 blockade as a novel treatment for colorectal cancer. Biomed Pharmacother 110, 312–318.

Yan, W., Liu, X., Ma, H., Zhang, H., Song, X., Gao, L., Liang, X., and Ma, C. (2015). Tim-3 fosters HCC development by enhancing TGF-β-mediated alternative activation of macrophages. Gut 64, 1593–1604.

Yang, A.S., and Lattime E.C. (2003). Tumor-induced interleukin 10 suppresses the ability of splenic dendritic cells to stimulate CD4 and CD8 T-cell responses. Cancer Res 63, 2150–2157.

Yang, L., DeBusk, L.M., Fukuda, K., Fingleton, B., Green-Jarvis, B., Shyr, Y., Matrisian, L.M., Carbone, D.P., and Lin, P.C. (2004). Expansion of myeloid immune suppressor Gr^+CD11b^+ cells in tumor-bearing host directly promotes tumor angiogenesis. Cancer Cell 6, 409–421.

Ye, B., Stary, C.M., Li, X., Gao, Q., Kang, C., and Xiong, X. (2018). Engineering chimeric antigen receptor-T cells for cancer treatment. Mol Cancer 17, 32.

Ye, L., Lou, Y., Lu, L., and Fan, X. (2019). Mesothelin-targeted second generation CAR-T cells inhibit growth of mesothelin-expressing tumors *in vivo*. Exp Ther Med 17, 739–747.

Yeku, O.O., and Brentjens, R.J. (2016). Armored CAR T-cells: utilizing cytokines and pro-inflammatory ligands to enhance CAR T-cell anti-tumour efficacy. Biochem Soc Trans 44, 412–418.

Yeku, O.O., Purdon, T.J., Koneru, M., Spriggs, D., and Brentjens, R.J. (2017). Armored CAR T cells enhance antitumor efficacy and overcome the tumor microenvironment. Sci Rep 7, 10541.

You, J.S., and Jones, P.A. (2012). Cancer genetics and epigenetics: two sides of the same coin? Cancer Cell 22, 9–20.

Yu, J., Du, W., Yan, F., Wang, Y., Li, H., Cao, S., Yu, W., Shen, C., Liu, J., and Ren, X. (2013). Myeloid-derived suppressor cells suppress antitumor immune responses through IDO expression and correlate with lymph node metastasis in patients with breast cancer. J Immunol 190, 3783–3797.

Zhang, B.L., Li, D., Gong, Y.L., Huang, Y., Qin, D.Y., Jiang, L., Liang, X., Yang, X., Gou, H.F., Wang, Y.S., et al. (2019). Preclinical evaluation of chimeric antigen receptor-modified T cells specific to epithelial cell adhesion molecule for treating colorectal cancer. Hum Gene Ther 30, 402–412.

Zhang, P., Zhao, S., Wu, C., Li, J., Li, Z., Wen, C., Hu, S., An, G., Meng, H., Zhang, X., et al. (2018). Effects of CSF1R-targeted chimeric antigen receptor-modified NK92MI T cells on tumor-associated macrophages. Immunotherapy 10, 935–949.

Zhang, Q., Hu, H., Chen, S.Y., Liu, C.J., Hu, F.F., Yu, J., Wu, Y., and Guo, A.Y. (2019). Transcriptome and regulatory network analyses of CD19-CAR-T immunotherapy for B-ALL. Genom Proteom Bioinf 17, 190–200.

Zhao, Z., Chen, Y., Francisco, N.M., Zhang, Y., and Wu, M. (2018). The application of CAR-T cell therapy in hematological malignancies: advantages and challenges. Acta Pharm Sin B 8, 539–551.

Zhao, Z., Condomines, M., van der Stegen, S.J.C., Perna, F., Kloss, C.C., Gunset, G., Plotkin, J., and Sadelain, M. (2015). Structural design of engineered costimulation determines tumor rejection kinetics and persistence of CAR T cells. Cancer Cell 28, 415–428.

Zheng, C., Zheng, L., Yoo, J.K., Guo, H., Zhang, Y., Guo, X., Kang, B., Hu, R., Huang, J.Y., Zhang, Q., et al. (2017). Landscape of infiltrating T cells in liver cancer revealed by single-cell sequencing. Cell 169, 1342–1356.e16.

Zhu, H., Wang, D., Zhang, L., Xie, X., Wu, Y., Liu, Y., Shao, G., and Su, Z. (2014). Upregulation of autophagy by hypoxia-inducible factor-1α promotes EMT and metastatic ability of $CD133^+$ pancreatic cancer stem-like cells during intermittent hypoxia. Oncol Rep 32, 935–942.