INNOVATIONS JOURNALS

Review Article

A Comprehensive Exploration of Agents Targeting Tumor Microenvironment: Challenges and Future Perspectives

Carlos Diego Holanda Lopes,[1] Camila Braganca Xavier,[2] Carlos Torrado,[2] Ana Carolina Veneziani,[1] Thais Baccili Cury Megid[3]

[1]Princess Margaret Cancer Centre, University of Toronto, Toronto, ON, Canada
[2]University of Texas MD Anderson Cancer Center, Houston, TX, USA
[3]Dr. Léon-Richard Oncology Centre, Vitalité Health Network, Moncton, NB, Canada

Address correspondence to Camila Braganca Xavier (cbxavier@mdanderson.org).

Source of Support: None. Conflict of Interest: None.

Submitted: Jun 9, 2024; First Revision Received: Aug 13, 2024; Accepted: Aug 20, 2024; First Published: Nov 1, 2024.

Lopes CDH, Xavier CB, Torrado C, Veneziani AC, Megid TBC. A comprehensive exploration of agents targeting tumor microenvironment: challenges and future perspectives. *J Immunother Precis Oncol*. 2024; 7:283–299. DOI: 10.36401/JIPO-24-23.

ABSTRACT

The tumor microenvironment (TME) encompasses the complex and diverse surroundings in which tumors arise. Emerging insights highlight the TME's critical role in tumor development, progression, metastasis, and treatment response. Consequently, the TME has attracted significant research and clinical interest, leading to the identification of numerous novel therapeutic targets. Advances in molecular technologies now enable detailed genomic and transcriptional analysis of cancer cells and the TME and the integration of microenvironmental data to the tumor genomic landscape. This comprehensive review discusses current progress in targeting the TME for drug development, addressing associated challenges, strategies for modulating the pro-tumor microenvironment, and the discovery of new targets.

Keywords: tumor microenvironment, targets, challenges, mechanisms, resistance

INTRODUCTION

The tumor microenvironment (TME) plays a crucial role in regulating the basic survival of tumor cells and supporting their functions.[1] The development and persistence of cancer—such as maintaining cell proliferation, evading apoptosis, stimulating angiogenesis, promoting invasion and metastasis, inciting tumor-promoting inflammation, and evading immune surveillance—are influenced, to varying degrees, by the complex interactions within the TME.[2] A significant portion of patients with metastatic disease will inevitably develop resistance to treatment following cancer therapy, and a comprehensive understanding of the dynamic changes occurring within the TME during tumor progression is essential for the development of targeted therapeutic approaches.[2,3] This resistance arises from a spectrum of biological mechanisms, encompassing DNA repair, genetic and epigenetic alterations, metabolic reprogramming, heightened angiogenesis, and modifications in the TME[2,3] (Figure 1). This comprehensive review was performed using the PubMed database, and articles approaching TME components were non-systematically pooled by the authors. We aimed to delve into the latest advancements in TME research, outline the obstacles and potential pathways for TME modulation, and provide new perspectives and potential breakthroughs in cancer therapy, particularly in overcoming treatment resistance associated with the TME.[4] In addition, we examined the literature covering the emerging technologies and the role of artificial intelligence in unraveling the complexities of the TME.

TARGETING TME COMPONENTS

Dendritic Cells

The engagement of the FMS-like receptor tyrosine kinase-3 (FLT3) with its ligand, FLT3L, plays a crucial role in regulating dendritic cells (DCs). When FLT3L is administered, it leads to the expansion of circulating DCs in vivo, followed by their migration to various tissues.[5] This process not only increases the number of DCs in the TME but also promotes DC maturation, enhancing the priming of antitumor T cells. Recombinant FLT3L, such as CDX-301, has been demonstrated to expand DCs and hematopoietic precursors in healthy human volunteers.[6] Although phase 1 and 2 trials (ClinicalTrials.gov ID: NCT00003431) have confirmed

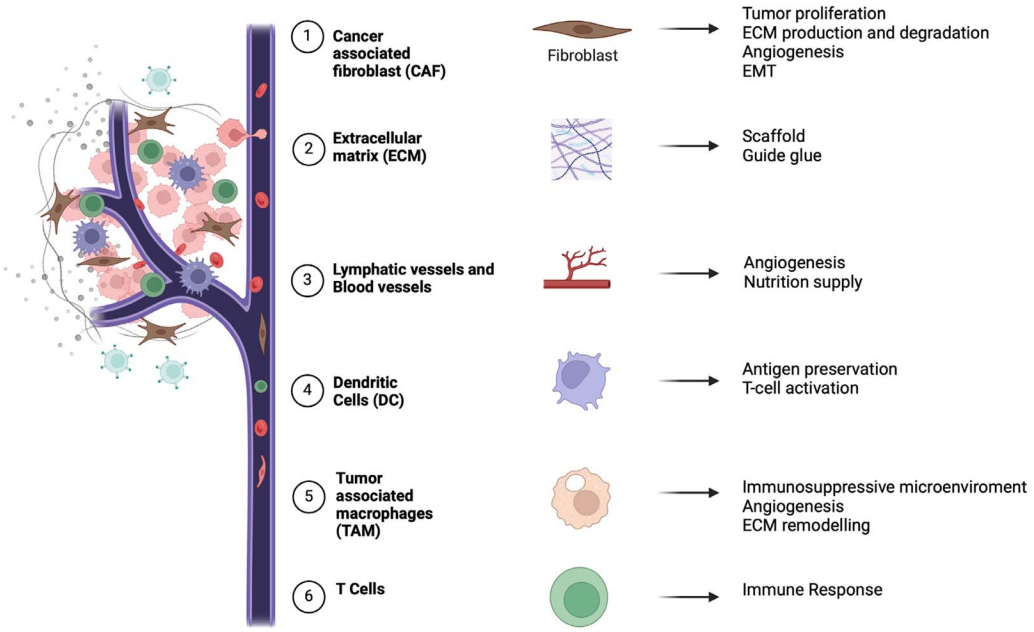

Figure 1. The tumor microenvironment components. The tumor microenvironment is a complex network of diverse cells and secreted factors that serve as targets for anticancer treatments. It includes various cell types like cancer cells, immune cells (such as T and B lymphocytes, TAMs, DCs, NK cells, myeloid-derived suppressor cells, neutrophils, and eosinophils), stromal cells (like CAFs, pericytes, and mesenchymal stromal cells), as well as vascular networks and tissue-specific cells such as neurons and adipocytes. These cells release components like ECM, growth factors, cytokines, and EVs, crucial for communication within the TME and beyond. CAF: cancer-associated fibroblast; DC: dendritic cell; ECM: extracellular matrix; NK: natural killer; TAM: tumor-associated macrophage; TME: tumor microenvironment.

the immunogenicity and safety of CDX-301, its efficacy as a monotherapy in tumor remission remains to be established. A strategy combining radiation and CDX-301 is currently being studied in phase II trials for patients with advanced non–small cell lung cancer (NSCLC) (NCT04491084). In addition, a combination approach involving radiotherapy, FLT3L, and the costimulatory molecule CD40 is undergoing phase II trials in patients with lung cancer (NCT04491084).

Granulocyte-macrophage colony-stimulating factor (GM-CSF) regulates the development of myeloid cell types in response to stress, infections, and cancers. Imbalances in GM-CSF levels can either hinder or promote cancer progression, depending on factors like the amount of GM-CSF, cancer type, and tumor environment.[7] Treatments targeting granulocyte-macrophage colony-stimulating factor (GM-CSF) act as a booster of antitumor immunity by promoting the differentiation of DCs. Current clinical and preclinical approaches have evaluated treatments like GM-CSF monotherapy as well as GM-CSF combined with chemotherapy, monoclonal antibodies, or cancer vaccines.

DC vaccines involve loading DCs with tumor-associated antigens (TAAs) to trigger an immune response in patients, promoting the development of T cells for a targeted antitumor effect.[8] DCs are derived from CD34-positive precursor cells or monocytes and activated by Toll-like receptor (TLR) agonists and cytokines like interleukin 1 beta (IL-1β), interleukin 6 (IL-6), tumor necrosis factor alfa (TNF-α), and prostaglandin E2 (PGE2). This activation leads to their maturation into functional DCs. Mature DCs express major histocompatibility complex (MHC), costimulatory molecules, and cytokines, ultimately inducing a T helper 1 cells (Th1) immune response.[9] The effectiveness of inducing DC maturation is a critical determinant in the success or failure of DC vaccine treatments.[10]

Sipuleucel-T, a DC-based immunotherapy, for instance, comprises autologous peripheral blood mononuclear cells enriched for antigen-presenting cells, which are cultured ex vivo with recombinant PA2024 protein as a source of antigens to enhance their T lymphocyte activation properties. Following reinfusion into patients with asymptomatic prostate cancer with metastatic castration-resistant disease, this approach led to a modest median overall survival improvement of 4.1 months compared with placebo, resulting in United States Food and Drug Administration (FDA) approval for use in this setting.[11]

Recent progress in novel DC vaccination approaches holds significant promise, particularly in treating solid tumors. As an example, in individuals with melanoma, the combination of a high-dose systemic interferon alfa-2b (IFN-α2b) and a DC vaccine demonstrates a significant extension in both overall survival and progression-free survival compared with patients treated with the DC vaccine alone.[10] An overview of DC-based therapeutics in solid tumors is provided in Table 1.

Blood Vessels

Hypoxia, a hallmark of the TME of various cancer types, can attenuate the efficacy of chemotherapy and

Table 1. Dendritic-cell–based therapeutics in solid tumors

Target and Drug Combination or Vaccine Type	Therapeutic Strategy	Type of Cancer	ClinicalTrials.gov ID
FLT3L THERAPIES			
	FLT3L in treating patients with metastatic colorectal cancer	Metastatic colorectal cancer	NCT00003431[117]
	CD40 agonist antibody (CDX-1140) alone vs. combination with recombinant FLT3L (CDX-301) vs. combination with pembrolizumab vs. combination with chemotherapy	Multiple cancer types	NCT03329950[118]
	FLT3L (CDX-301) with CD40 agonist antibody (CDX-1140) and stereotactic radiotherapy vs. stereotactic radiotherapy alone	Non-small cell lung cancer	NCT04491084[119]
	CDX-1401 and Poly-ICLC vaccine therapy with or without CDX-301 in treating patients with stage IIB-IV melanoma	Stage IIB-IV melanoma	NCT02129075[120]
	Dose escalation of adenovirus gene transfer that drives direct tumor killing and FLT3L expression	Malignant glioma and glioblastoma multiforme	NCT01811992[121]
	Recombinant FLT3L alone or with melanoma-associated peptides	Stage IV melanoma, stage IV renal cell cancer, recurrent renal cell cancer and recurrent melanoma	NCT00019396[122]
GM-CSF THERAPIES			
Monotherapy	GM-CSF monotherapy	Prostate cancer; ovarian cancer; kidney cancer; colon and rectal cancer	NCT00908141[123] NCT00157573[124] NCT00006483[125]
GM-CSF with chemotherapy	GM-CSF + different chemotherapy	Colon and rectal cancer	NCT00257322 [126]
	GM-CSF + docetaxel GM-CSF + mitoxantrone	Prostate cancer	NCT00488982[127] NCT00477087 [128]
GM-CSF with monoclonal antibody	Leukine (sargramostim) + herceptin	Breast cancer	NCT00429104[129]
	Leukine (sargramostim) + edrecolomab	Colorectal cancer	NCT00002664[130]
	GM-CSF + nivolumab + ipilimumab	Metastatic cutaneous melanoma	NCT02339571[131]
	PSA/IL-2/GM-CSF (complete vaccine)	Prostate cancer	NCT02058680[132] NCT00140374[133] NCT00799110[134] NCT01479244[135] NCT00448409[136]
	GVAX/ vaccine (GM-CSF secreting prostate cancer vaccine)	Prostate cancer	NCT00003002[137]
	GM-CSF + dendritic cell/tumor fusion vaccine		
	Leukine (sargramostim) + NeuVaxTM vaccine (E75 synthetic peptide combined with GM-CSF)	Ovarian cancer Primary peritoneal cancer Fallopian tube cancer	NCT03645148[138]
	GM-CSF + TroVax/ (vaccinia virus encoding the human oncofetal antigen 5T4)	Breast cancer with low to intermediate HER2 expression	NCT01789099[139]
	Leukine (sargramostim) + HER-2/neu peptide vaccine	Prostate cancer Breast cancer Lung cancer	NCT04382664[140]
	Leukine (sargramostim) + iNeo Vac P01 (peptide vaccine)	Ovarian cancer Pancreatic cancer	NCT04040231[141]
	GM-CSF + UV1 synthetic peptide vaccine	Non–small cell lung cancer	NCT03600350[142]

Table 1 continues on next page

Table 1. Continued

Target and Drug Combination or Vaccine Type	Therapeutic Strategy	Type of Cancer	ClinicalTrials.gov ID
GM-CSF with cancer vaccines and monoclonal antibody	GM-CSF + UV1 vaccine + ipilimumab + nivolumab	Melanoma Lung cancer	NCT04382664[140]
	Leukine (sargramostim) + galinpepimut-S (WT1 peptide vaccine) + nivolumab	Mesothelioma Pleural mesothelioma Wilms tumor	NCT04300244[143] NCT04040231[144]
	Leukine (sargramostim) + pTVG-HP (plasmid DNA vaccine) + nivolumab	Prostate cancer	NCT03600350[142]
DENDRITIC CELL VACCINES			
Conventional MoDC-based	Sipuleucel-T and ipilimumab	Advanced prostate cancer	NCT01832870[145]
Conventional MoDC-based	Combining sipuleucel-T (SipT) and ipilimumab (Ipi)	Metastatic castration-resistant prostate cancer (mCRPC)	NCT01804465[146]
Conventional MoDC-based	Glycosylated recombinant human interleukin-7 (CYT107) after vaccine therapy	mCRPC	NCT01881867[147]
Conventional MoDC-based	Sipuleucel-T or placebo	mCRPC	NCT00065442[11]
Biomaterial-based	WDVAX vaccine	Metastatic melanoma	NCT01753089[148]
mRNA-based	mRNA electroporated autologous dendritic cells	Stage III/IV melanoma	NCT01676779[149]
DCsEV-based	tumor antigen-loaded dendritic cell-derived exosomes	Non–small cell lung cancer	NCT01159288[150]

CD40: cluster of differentiation 40; CYT107: glycosylated recombinant human interleukin-7; DCsEV: dendritic cell-derived exosomes; FLT3L: fms-like tyrosine kinase 3 ligand; GM-CSF: granulocyte-macrophage colony-stimulating factor; MHC: major histocompatibility complex; NCT: national clinical trial; PGE2: prostaglandin E2; SipT: sipuleucel-T; Th1: T helper 1; TLR: toll-like receptor; VEGF: vascular endothelial growth factor; VEGFR: vascular endothelial growth factor receptor; WT1: wilms' tumor 1; MoDC: monocyte derived dendritic cells; mRNA: messenger RNA; WDVAX: poly(lactic-co-glycolic acid-based scaffold vaccine.

radiotherapy, trigger the secretion of immune-suppressive cytokines, and promote the recruitment and proliferation of immune-regulatory cell population. [12–14] More recently, studies have been exploring the idea of vascular normalization. One of the notable advantages of vascular normalization is its potential for synergizing with other anticancer treatments, including chemotherapy, radiotherapy, and immunotherapy.[15–17] One classic example from clinical practice is the metastatic clear cell renal cell carcinoma (mRCC) treatment, in which combinations of anti-vascular endothelial growth factor (VEGF) agents with immune checkpoint inhibitors (ICIs) provide superior disease control than to single-agent VEGF-targeted therapies, signaling a shift in first-line treatment approaches.[18] Achieving vascular normalization poses certain challenges. The optimal dose of anti-angiogenic therapy and the timing of effective normalization, known as the "normalization window," are tightly limited and vary between individuals, which presents obstacles to its clinical application.[19] Thus far, most of the mechanistic understanding regarding vessel normalization has been derived from inhibiting VEGF signaling pathways with moderate-to-low doses of monoclonal antibodies or small-molecule inhibitors targeting tyrosine kinase receptors (Table 2). However, given that VEGF is a critical survival factor for endothelial cells, sustained inhibition, even at low doses, ultimately results in vessel demise or the increased expression of alternative angiogenic factors.[20] Furthermore, the resistance to VEGF targeting regulated by immunosuppression suggests the involvement of additional regulators in tumor promotion through immunomodulation. These findings indicate that analyzing the immune profile within the altered TME could offer the potential for enhancing treatment efficacy through a combination of vessel-targeting and immunotherapy strategies.[21] Another potential mechanism that indirectly reduces the proliferation of endothelial cells is by acting on the mTOR/AP-1/VEGF pathway and consequently inhibiting angiogenesis.[22] In addition, the hypoxia-inducible factor (HIF)-2α inhibitor belzutifan recently demonstrated a significant clinical benefit among patients with mRCC previously treated with VEGF inhibitors and ICI.[23]

Macrophages

Experimental models have revealed that tumor cells could recruit and polarize macrophages to an M2-like phenotype, called tumor-associated macrophages (TAMs), through the secretion of specific chemokines such as the C–C motif chemokine family (CCL2, CCL3, and CCL5).[24–26] Then, these TAMs facilitate the acquisition of malignant traits by tumor cells, such as proliferation, angiogenesis, immune evasion, and metastasis.[27] Some studies have translated these experimental data to clinical settings, such as the observation of a statistically positive association of inferior survival with high tissue levels of CCL5 through microarray assay in patients with breast-localized phyllodes tumors.[26] Similar results were also demonstrated by Yang et al.,[24] who found a positive correlation between tumor colony-stimulating factor 1 receptor (CSF-1R), a tyrosine kinase transmembrane receptor involved in tissue macrophage maintenance, expression through immunohistochemistry, and more aggressive clinical and pathological tumoral features, besides inferior survival in a cohort of 268 patients with resected clear cell renal cell carcinoma.[28,29] Corroborating these findings, a meta-analysis involving 55 studies with a total of 8692 patients diagnosed with solid tumors also showed that an increase in the density of TAMs, identified through immunohistochemistry for CD68 in tumor samples, was associated with a decrease in overall survival (1.15-fold higher relative risk for mortality), which was more pronounced in breast, endometrial, prostate, bladder, ovary, and urothelial cancers.[30]

The previous analysis highlighted that the clinical benefit from some cytotoxic chemotherapy agents may derive in part from their actions on TAMs by reducing their tumoral population (e.g., structurally related marine-derived compounds trabectedin and lurbinectedin) or inducing phenotypic changes toward an M1 profile (e.g., the anti-metabolites gemcitabine and 5-fluorouracil).[31,32]

Aiming to deplete the TAM population, pexidartinib, a CSF1R inhibitor, was evaluated in a phase 2 dose extension study with a total of 23 patients harboring tenosynovial giant-cell tumors. From these, 12 had a partial response with a median duration of response above 8 months, and 7 had stable disease.[33] A posterior phase 3 trial has confirmed pexidartinib efficacy in this setting, resulting in its FDA approval. Although it was tolerable in most patients, a few cases of liver failure have resulted in a Boxed Warning with the FDA approval.[34] Other phase I trials have assessed CSF1R blocked (with antibodies or small molecules) in combination with cytotoxic agents or immunotherapy in different histologies, and efficacy studies are necessary.[35]

Blocking the chemokine-dependent recruitment of TAMs was assessed in phase 1 trials based on combination antibodies or small molecules with cytotoxic agents with modest tumor activity. Carlumab, an anti-CCL2 monoclonal antibody, presented mild efficacy (one partial response in 53 patients) in combination with cytotoxic standard chemotherapy in a phase 1b trial in individuals harboring solid tumors. Although a brief initial reduction in serum CCL2 was followed by its increase along chemotherapy treatments, non-distinct new adverse events regarding standard agents were related.[36] A CCL2 receptor blocker, PF-04136309, was analyzed for patients with metastatic ductal pancreatic cancer in combination with nab-paclitaxel and gemcitabine in the phase 1b study. Besides a non-increase in the objective response rate of 23.8%, a high incidence of pulmonary toxicity, one case with grade 4, was related.[37]

Table 2. Agents targeting the vasculature currently in clinical use

Signaling	Targets	Drug
VEGF signaling	VEGF	Bevacizumab
	VEGFR	IMC-1121B (Ramucirumab)
	VEGFR	Sunitinib (Sutent)
	VEGFR	Sorafenib (Nexavat)
	VEGFR	Pazopanib (Votrient)
FGF signaling	FGFR	BMS-582664 (Brivanib)
PDGF signaling	PDGF	SU6668
EGFR signaling	EGFR	Cetuximab (Erbitux)
	EGFR	Panitumumab
	EGFR	Erlotinib
	EGFR	Gefitinib
mTOR signaling	mTOR	Everolimus

EGFR: epidermal growth factor receptor; FGF: fibroblast growth factor; mTOR: mammalian target of rapamycin; PDGF: platelet-derived growth factor; VEGF: vascular endothelial growth factor.

Despite this biological rationale and preclinical evidence, the combination of motolimod, a TLR8 agonist, to the EXTREME regimen (combination therapy using cetuximab antibody and platinum-based chemotherapy) in a randomized phase 2, double-blinded, placebo-controlled trial with patients diagnosed with recurrent/metastatic squamous cell carcinoma of the head and neck did not demonstrate the gain in progression-free survival or overall survival. Otherwise, a statistical benefit in these two endpoints was demonstrated in patients with human papilloma virus (HPV)–positive oropharyngeal cancer. The addition of motolimod was associated with a higher incidence of adverse events related to local injection reactions, acneiform eruptions, chills, and pyrexia.[38] Promising results were achieved with the combination of pembrolizumab and intratumoral vidutolimod, a TLR9 agonist, in a phase 1b study involving 44 patients with advanced melanoma who had never responded to previous anti-programmed cell death 1 (PD-1) therapy. This combination resulted in an objective response rate of 25% and a satisfactory safety profile.[39]

In a distinct mechanism of pattern recognition TLR activation, CD40 signaling can induce an epigenetic reprogramming to the end of polarizing macrophages toward proinflammatory and anti-tumoral phenotypes experimentally.[40] These findings were corroborated in a phase 2 trial of sotigalimab, a CD40 agonistic antibody, in combination with nivolumab that achieved an overall response rate of 15%, most of them lasting more than 18 months, in 38 patients with metastatic melanoma who had previously progressed on anti-PD1 therapy. In this trial, sotigalimab-related grade 3 adverse events were evidenced in 13% and have been represented as systemic inflammatory reactions.[41]

Despite the description of many mechanisms in vivo and in vitro justifying the pivotal role of TAMs in many steps of the natural history of tumors, the clinical diffuse use of therapies targeting many aspects of the biology of these cells still needs more clinical evidence of benefit, which could be achieved upon appropriate patient selection based on biomarkers in large clinical trials.

T Cells

Immune checkpoint inhibitors

ICIs are a cancer treatment strategy that enhances T-cell responses within the TME. Certain molecules in the co-stimulatory pathway send inhibitory signals to activated T cells, regulating the strength of the immune response and functioning as "checkpoint" molecules.[42] The most recognized T-cell checkpoint molecules are cytotoxic T lymphocyte antigen 4 (CTLA4) and PD-1. Research advancements on these checkpoint molecules have led to the development of T-cell targeting antibodies that exhibit high efficacy across various cancers.[1] Two of the most evolving checkpoint inhibition approaches widely used in the past decade involve blocking the PD-1/PD-L1 and CTLA-4 pathways.[42] Other targets, including inhibitory receptors such as T-cell immunoglobulin and mucin 3 (Tim-3), V-domain immunoglobulin suppressor of T-cell activation (VISTA), T-cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT), and lymphocyte activation gene 3 (Lag-3), as well as activating molecules like OX40 (CD134) and glucocorticoid-induced TNFR-related protein (GITR), are currently under investigation.[42,43]

Cellular therapy

Tumor-infiltrating lymphocytes (TILs) are a specific type of immune cell known as T cells. These cells identify and attack antigens on the surface of cancer cells, penetrating solid tumors to destroy them. When a tumor is surgically removed from a patient, tissue samples are sent to a lab where TILs are extracted and cultivated over 3 weeks, allowing them to multiply into billions of cells. Before reinfusing the TILs into the patient, the individual undergoes chemotherapy and receives IL-2, an immune-stimulating chemical. This treatment temporarily depletes existing immune cells, creating space for the newly introduced TILs.[44]

Patients diagnosed with stage IIIC or IV melanoma, who had experienced disease progression following treatment with checkpoint inhibitors or BRAF/MEK targeted therapy (for those harboring BRAF V600 mutations), underwent extraction and ex vivo expansion of tumor tissue-derived T cells via a controlled manufacturing process (Lifileucel). Following nonmyeloablative lymphodepleting cytotoxic treatment, autologous reinjection of Lifileucel was administered, followed by up to six doses of IL-2. Encouraging outcomes from this single-arm trial, including an overall response rate of 31.5% (with a recommended dosing range of 7.5-72 $\times$ 10^9 viable cells) and a median duration of response not yet reached, led to its FDA-accelerated approval for

these refractory patients.[45] Also, the combination of TILs and ICIs is being evaluated in a phase I clinical trial (NCT05576077).

CAR T cell

The T-cell receptor (TCR) can be engineered to recognize a specific antigen and augment a targeted immune response. Building on this strategy, chimeric antigen receptor (CAR) T cells have been developed, revolutionizing the treatment landscape for certain refractory tumors.[46] Supported by compelling clinical trial data, CD19-targeted and B cell maturation antigen-targeted CAR T cells have received FDA approval for the treatment of patients with B cell lymphoma,[47] B cell acute lymphoblastic leukemia,[48] and multiple myeloma,[49] respectively, who have relapsed or progressed following prior therapies. These approaches have demonstrated elevated rates of complete and durable responses in either these or the other hematological malignancies.[50,51]

Regarding solid tumors, CAR T-cell trials have shown promising albeit less robust preliminary outcomes in neoplasms with historically poor prognoses and resistance to conventional treatments. A phase I/II clinical trial (NCT00902044) demonstrated encouraging outcomes using HER2 CAR T cells in the treatment of 19 patients with HER2-positive sarcomas, including 16 osteosarcomas, one primitive neuroectodermal tumor, one Ewing sarcoma, and one protofibroblastic small round cell tumor.[51]

IL-13Rα2 is significantly expressed in glioblastoma (GBM) tumor cells but rarely found in normal brain cells, making it a compelling target for CAR T-cell therapy in glioblastoma. In a study by Brown and colleagues[52] (NCT02208362), multi-dose treatment with IL-13Rα2-CAR T cells led to complete tumor regression for almost 8 months in a patient with disseminated glioblastoma. Another target in GBM tumors, epidermal growth factor receptor (EGFR), was examined in a phase I clinical trial in which 10 patients with recurrent EGFRvIII+ glioblastoma were treated with EGFRvIII engineered CAR T-cells (NCT02209376).[53] This study demonstrated an antitumor effect with a median overall survival (OS) of approximately 8 months in all patients. Other CAR T-cell targets in solid tumor include MUC1, CD133, MSLN, CEA, and GD2.[54] However, these CAR-T trials frequently report high incidences of inflammatory reactions, highlighting the need for comprehensive support from specialized teams to manage these cases.[55]

Recently, CAR natural killer (NK) cells and CAR-macrophages (CAR-M) have been introduced as alternatives or complements to CAR T-cell therapy for solid tumors. CAR NK cells might be a favorable substitute for CAR T cells because they do not require human leukocyte antigen (HLA) compatibility and have limited toxicity.[54] However, like CAR T cells, CAR NK cells face challenges such as migration to the tumor site, persistence in the immunosuppressive TME, and transduction.[54] Regarding CAR-M, several limitations are associated with their bioengineering, storage, expansion, persistence in the TME, and toxicity.[54]

Regulatory T cells

In the TME, regulatory T cells (Tregs) play multiple roles, particularly in suppressing T-cell activation.[56] Currently, there is no dedicated Treg-targeted therapy in oncology. However, research indicates that Tregs can also express the membrane receptors CTLA-4, PD-1, LAG3, and TIGIT,[56] which could be targetable with ICIs.[57]

Several strategies have been proposed to boost antitumor immunity, including depleting Tregs in the TME using kinase inhibitors, low-dose cyclophosphamide, and anti-CD25 antibodies. For example, sunitinib has shown effectiveness in renal cell carcinoma,[58] and metronomic cyclophosphamide has shown promise in patients with breast cancer.[59] In addition, targeting co-stimulatory signals like OX40, GITR, ICOS, and TNFR2, as well as blocking inhibitory cytokines derived from Tregs, such as IL-10, IL-35, and transforming growth factor (TGF)-β, can further diminish Treg suppressive functions within the TME.[60–64]

T-cell bispecific antibodies

A significant obstacle to the effectiveness of T-cell-based immunotherapy is the inadequate infiltration of T cells into the TME. To tackle this issue, T-cell engaging bispecific antibodies (bsAbs) have been developed. These bsAbs are engineered to bind simultaneously to an antigen on tumor cells and a surface molecule on T cells, thereby combining the specificity of two antibodies into a single molecule to efficiently redirect T cells to the tumor cells.[65]

One example involves targeting the CD3 chain of the TCR, due to its invariant nature, while the other arm targets tumor cell antigens like CD19, which is specifically expressed in hematologic malignancies. This approach led to the development and FDA approval of blinatumomab, a CD19/CD3 bsAb, for treating B-cell precursor acute lymphoblastic leukemia.[66] Currently, a more complex design of trispecific antibodies targeting CD3 and CD137 using a dual-specific Fab is also being tested among solid tumors expressing CLDN-6 (NCT05735366).

The bispecific T-cell engager (BiTE), a bsAb lacking an Fc domain, comprises variable regions from an antitumor cell antigen and an anti-CD3 antibody, connected by a short linker. The epidermal growth factor receptor variant III (EGFRvIII), often overexpressed in glioblastoma, is another target for BiTEs. An early clinical study involving EG-FRvIII-specific BiTEs, including AMG 596, has been conducted in patients with recurrent glioblastoma.[67]

Also, Tebentafusp is a distinctive BiTE. It connects an affinity-enhanced TCR that targets the glycoprotein-

100 (gp100)–HLA–A02 complex found on melanoma cells with an anti-CD3 chain. Tebentafusp showed a significant improvement in OS for patients with metastatic uveal melanoma, resulting in its approval in 2022.[68]

B Cells

Recent evidence indicates that tumor-infiltrating B lymphocytes (TIL-Bs), including B cells and plasma cells, are important and versatile players in antitumor immune responses.[69,70] In numerous cancers, TIL-Bs have demonstrated significant prognostic value and are emerging as crucial predictors of responses to ICIs.[69,70] In addition, TIL-Bs are involved in various other lympho-myeloid aggregates and engage in complex interactions with the tumor stroma, underscoring their multifaceted role in the TME.[69]

In a mice study, the development of immunosuppressive and effector B-cell responses within pancreatic ductal adenocarcinoma (PDAC) was examined using a multifaceted approach that included genetically engineered models, B-cell profiling, and functional assays.[70] The findings reveal that IL-35+ B cells inversely correlate with plasma cell frequency in PDAC. Through transcriptional profiling of naive B cells, it was discovered that IL-35 production by B cells induces a unique transcriptional state in naive B cells, inhibiting plasma cell differentiation by maintaining high levels of the B cell lineage-defining transcription factors Pax5 and Bcl6. Furthermore, targeting Bcl6 in naive B cells significantly increased the presence of intratumoral plasma cells and overcame resistance to immunotherapy, resulting in tumor growth control.[70] This study suggests that the transcriptional reprogramming of naive B cells can be a strategic target to modulate the balance between effector and regulatory B cell functions, enhancing tumor immunity in PDAC.[70]

In another study with PDAC, resistance to systemic treatment with stimulator of interferon gene (STING) agonists was partly due to the expansion of immunosuppressive B cells that hinder NK cell function.[71] Although previous research has shown that the STING-triggered IFN response is crucial for antitumor NK cell activity, this study presents a novel scenario in which systemic delivery of STING agonists suppresses NK cell-mediated antitumor responses via B cell-derived IL-35. This suppression mechanism explains why systemic delivery of STING agonists is less effective than intratumoral delivery.[71] By blocking B cell-specific IL-35 during 2′3′-cyclic GMP-AMP treatment, this negative regulatory circuit can be disrupted, offering a potential strategy to enhance tumor control. However, numerous challenges must be addressed when translating the immune capabilities of B cells into effective tumor immunotherapies.

Cancer-Associated Fibroblasts

Cancer-associated fibroblasts (CAFs) are a pivotal element within the TME, exerting control and influence on tumor behavior through comprehensive interactions with both tumor and stromal compartments.[72]

Transcriptomic analysis based on single-cell RNA-sequencing has revealed that a combination of biomarkers is capable of discriminating CAF subsets across different cancer types. CAFs were categorized using a presumed functional naming system such as myofibroblastic (myCAF), inflammatory (iCAF), antigen-presenting (apCAF), matrix, cycling (cCAF), or developmental (dCAF).[73,74] Importantly, CAF subsets are not invariable categories but can transition between each other through specific signaling pathways. This characteristic provides a rationale for inducing CAF phenotypic switching as a strategy in the development of anticancer therapy.

MyCAFs are found adjacent to tumor foci, where they are activated by direct contact with neoplastic cells.[75] They are distinguished by high expression of α-smooth muscle actin (αSMA) and low expression of IL-6. Conversely, iCAFs are found at a greater distance from tumor cells and are activated by cancer cell-derived factors such as IL-1 and TNF-α.[76] They are characterized by low expression of αSMA and high expression of IL-6. These two subtypes, myCAFs and iCAFs, are considered mutually exclusive.[77] There is evidence of conversion between iCAFs and myCAFs via TGF-β or IL-6 signaling pathways.[78]

Recently, apCAFs, a new subset of CAFs expressing major histocompatibility complex-II molecules was discovered proposing an immunomodulatory role of CAFs. In recent integrative analyses involving multiple single-cell RNA-sequencing studies and comprehensive lineage tracing assays, it has been identified that antigen-presenting CAFs (apCAFs) originate from mesothelial cells.[79] Throughout the progression of pancreatic cancer, mesothelial cells undergo a phenotypic transformation into apCAFs. This transformation involves a downregulation of mesothelial characteristics and an acquisition of fibroblastic features, a process driven by the cytokines IL-1 and TGF-β. Significantly, apCAFs have the capability to engage and reprogram naive CD4+ T cells into regulatory Tregs in a response that is specific to antigens.[79] Moreover, the application of a monoclonal antibody targeting the cell marker mesothelin has been shown to effectively inhibit the mesothelial-to-apCAF transition and the subsequent induction of Tregs by apCAFs.[80] Collectively, these findings highlight the potential role of mesothelial cells in promoting immune evasion during pancreatic cancer and offer valuable perspectives on potential strategies to enhance treatment efficacy.

Further insights into CAF dynamics were provided by tracking CAF subpopulations throughout breast tumor progression in mice.[81] A transcriptional shift from immunoregulatory activities toward functions related

to wound healing and antigen presentation as the tumor progresses was observed.[81] Comparable findings from melanoma models in mice showed that three specific CAF subclusters—S1, S2, and S3—vary in abundance through different stages of tumor growth, with the S3 subpopulation, characterized by high levels of Acta2, becoming dominant in later stages.[82] These findings, primarily derived from correlative analyses using single-cell RNA-sequencing and immunostaining, suggest that CAF subsets not only have distinct origins but also specialized functions. However, these observations are based on transcriptional profiles and in vitro studies, which may not fully capture their in vivo roles.[82]

Targeting CAFs has encountered significant challenges. One major issue is the absence of specific surface markers for CAFs, making their direct depletion difficult without harming normal tissue.[83] To address this, strategies involve targeting critical signals and effectors in CAFs, such as chemokine and growth factor pathways, to inhibit their activation and function. Molecules like all-*trans* retinoic acid (ATRA) or calcipotriol can normalize CAFs and induce an inactive phenotype. Additionally, CAF-based or mesenchymal stem cell (MSC)-based therapies can be used to deliver anticancer agents, including oncolytic adenoviruses, TNF-related apoptosis-inducing ligand (TRAIL), or type I IFN. Targeting CAF-derived extracellular matrix (ECM) proteins and associated signaling can induce stromal depletion. Direct depletion of CAFs can also be achieved using transgenic technologies or immunotherapies. Key targets and technologies include CAR, FAP (fibroblast activation protein), FGF2 (fibroblast growth factor 2), GPR77 (G protein-coupled receptor 77), IL-6, IL-6R (IL-6 receptor), mAb (monoclonal antibody), MMP (matrix metalloproteinase), MDSC (myeloid-derived suppressor cell), NK, PD-1, PDGFR, SDF1 (stromal-derived factor 1), SMO (smoothened), TAM, and Treg.[83]

Agents targeting FAP-α, CXCR4/CXCL12, HGF, PDGF, TGF-β, and hyaluronan signaling in CAFs are being studied in preclinical and clinical trials for breast cancer, chronic myelogenous leukemia, gastrointestinal stromal tumor, and melanoma.[84,85] Especially, TGF-R β is a promising target for pancreatic and colorectal tumors and is being evaluated in a myriad of phase I clinical trials (NCT06199466, NCT03436563, NCT05836324). Also, activating the Notch1 signaling pathway in CAFs inhibits melanoma cell growth in culture and in a xenograft mouse model.[86] In addition, vaccination against FAP-α, primarily expressed on CAFs, effectively suppressed B16/F10 melanoma development in mice.[87,88]

The multi-receptor somatostatin analogue pasireotide (SOM230; Novartis) has been used to inhibit the mTOR–4E-BP1 pathway responsible for protein synthesis in α-SMA+ CAFs, which highly express the somatostatin receptor SST1. In a murine xenograft model of PDAC, treatment with SOM230 reduced CAF-secreted molecules, including IL-6, thereby overcoming CAF-induced cancer cell resistance to chemotherapy (gemcitabine). Further research on the CAF-targeting effects of SOM230 could elucidate its anti-metastatic potential in pancreatic cancers.[89–91]

Extracellular Matrix

Beyond offering structural support, the ECM acts as a dynamic entity that influences cellular behaviors and undergoes remodeling, a common feature in tumors marked by increased collagen synthesis and deposition. This process, often accompanied by the expression of remodeling enzymes such as MMPs, lysyl oxidase (LOX), lysyl oxidase-like proteins (LOXLs), and WNT1-inducible signaling pathway proteins (WISPs), is crucial for the progression of cancer.[91]

Consequently, the ECM serves as a robust barrier that promotes tumor survival and progression. However, this barrier also presents vulnerabilities that can be exploited by anticancer therapies, thereby serving as a strategic entry point for therapeutic interventions. For instance, treatments that aim to normalize ECM stiffness have shown promise in various cancers. Techniques such as photothermal depletion of CAFs have been effective in reducing ECM stiffness in desmoplastic cholangiocarcinoma, thereby impacting the tumor's physical environment.[92] Similarly, therapies that target ECM stiffness to inhibit angiogenesis have been explored in liver metastasis.[93] These approaches indicate that reducing ECM stiffness is a promising approach for effective treatment. Conversely, strategies that increase ECM stiffness, such as cholesterol depletion treatments, have been found to enhance the effectiveness of T-cell immunotherapy by altering the tumor's biomechanical properties.[94]

There is also an application involving the ECM to enhance the effectiveness of CAR T therapy, facilitating easier penetration into solid tumors. This approach leverages modifications to the ECM, aiming to reduce its density or stiffness, thereby allowing CAR T cells to infiltrate and target cancer cells more effectively. To address this, reengineered CAR T cells that overexpress heparinase have been developed.[95] Heparinase can break down ECM components and enhance T-cell infiltration into the tumor, significantly impeding tumor growth. Additionally, a recent therapeutic approach combines an oncolytic adenovirus that delivers decorin with CAR T cells targeting carbonic anhydrase IX (CAIX).[96] This strategy has been shown to remodel the ECM and boost immune responses in cancer, demonstrating the potential for innovative combination therapies to overcome challenges in treating solid tumors.[96]

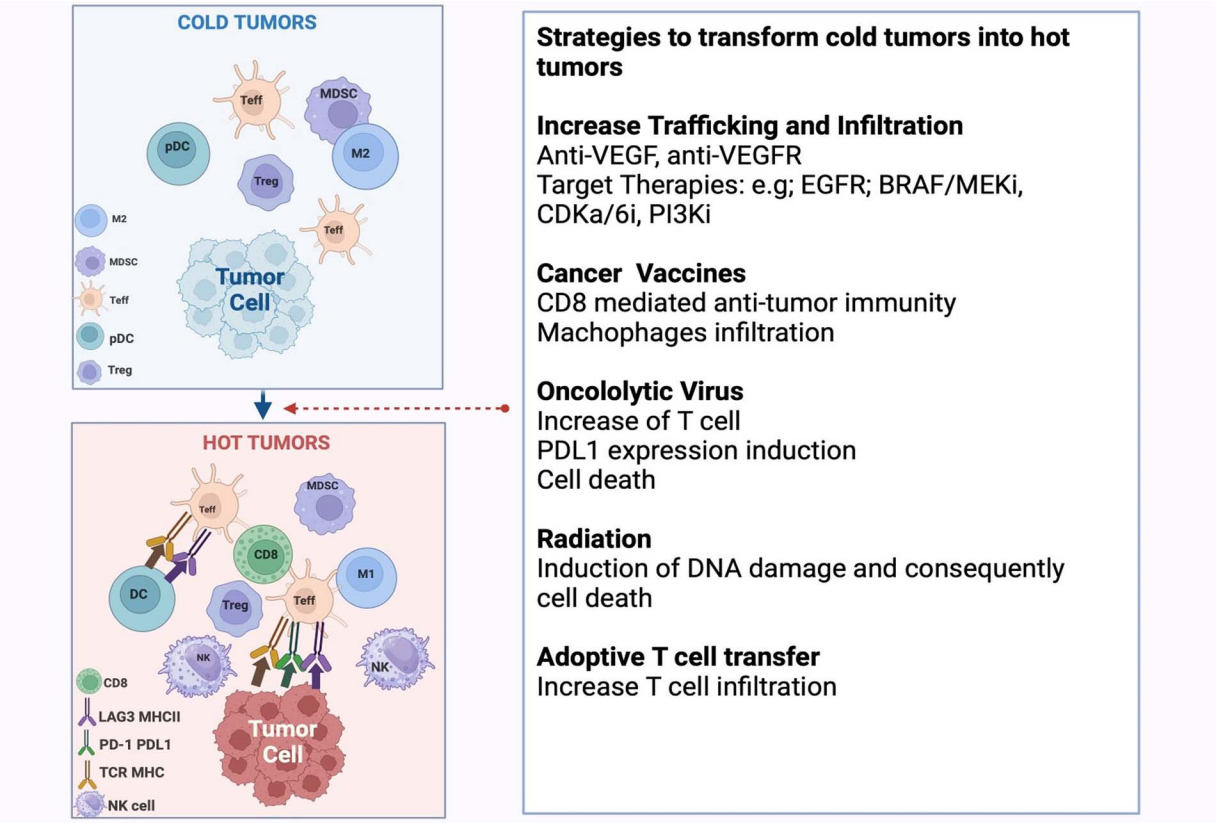

Figure 2. Cold and hot tumor environment and strategies to turn cold tumors into hot tumors. The primary cellular components and molecular interactions influencing the cold tumor phenotype and the hot tumor phenotype are delineated below. Key abbreviations include the following: NK (natural killer cells), DCs (dendritic cells), pDC (plasmacytoid dendritic cells), M2 (type 2 macrophages), MDSC (myeloid-derived suppressor cells), T eff (effector T cells), T reg (regulatory T cells), TCR (T-cell receptor), MHC (major histocompatibility complex), CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), LAG3 (lymphocyte activation gene-3), PD-1 (programmed cell death-1), and PD-L1 (programmed cell death-ligand 1). On the second table of the figures, we can see an overview of potential strategies to transform cold tumors into hot ones, along with their mechanisms of action, enhancing therapeutic outcomes when combined with immunotherapy.

STRATEGIES: TRANSFORMING "COLD" TUMORS INTO "HOT"

"Hot tumors" are characterized by a TME rich in TILs, PD-L1 overexpression, genomic instability, and preexisting antitumor immune responses.[97] "Cold tumors" lack inflammation and exhibit a deficiency in T cells both within the tumor and along its periphery, resulting in a low immunoscore.[97] They also show inadequate T-cell priming, characterized by low tumor mutational burden, impaired antigen presentation, and inherent resistance to T-cell–mediated killing[97] (Figure 2). Variable tumors are tumors in a variable state between cold and hot ones.

It is generally accepted that checkpoint inhibitors alone are more effective against "hot tumors" whereas having no benefit in treating "cold tumors" or "variable" tumors, which require a combination of other therapies to recruit immune cells to the tumor tissue to warm it up. Using combination therapies with immunotherapy approach stands as a pivotal method in addressing cold tumors such as chemotherapy, targeted therapy, radiation therapy, dual immunotherapy, oncolytic viruses, cancer vaccines, cytokines, cytotoxic chemotherapy, modulation of microbiome, radiation therapy, and other clinically viable combination techniques[98] (Figure 3). We discuss some of these therapies in the following sections.

Therapeutic Vaccines

The idea of a vaccine is to use tumor-specific antigens to activate antigen-specific T cells and establish antitumor immune memory.[99] This would induce influx of cytokine-producing CD8+ T cells and of intratumoral macrophages. Neoantigen-targeted vaccines show promise in activating long-lasting T-cell responses. Neoantigen quality is determined by various factors, including its dissimilarity to the wild-type amino acid sequence, its distribution among subclonal populations, its ability to be processed and presented on MHC molecules, and the affinity of the TCR for the neoantigen, among others.[100]

T-cell Receptor-Based Therapies and Adoptive T-cell Therapies

TCR-based therapy treatments are another evolving immunotherapy approach for inducing higher-quality T cells and directing them to the TME in immune-

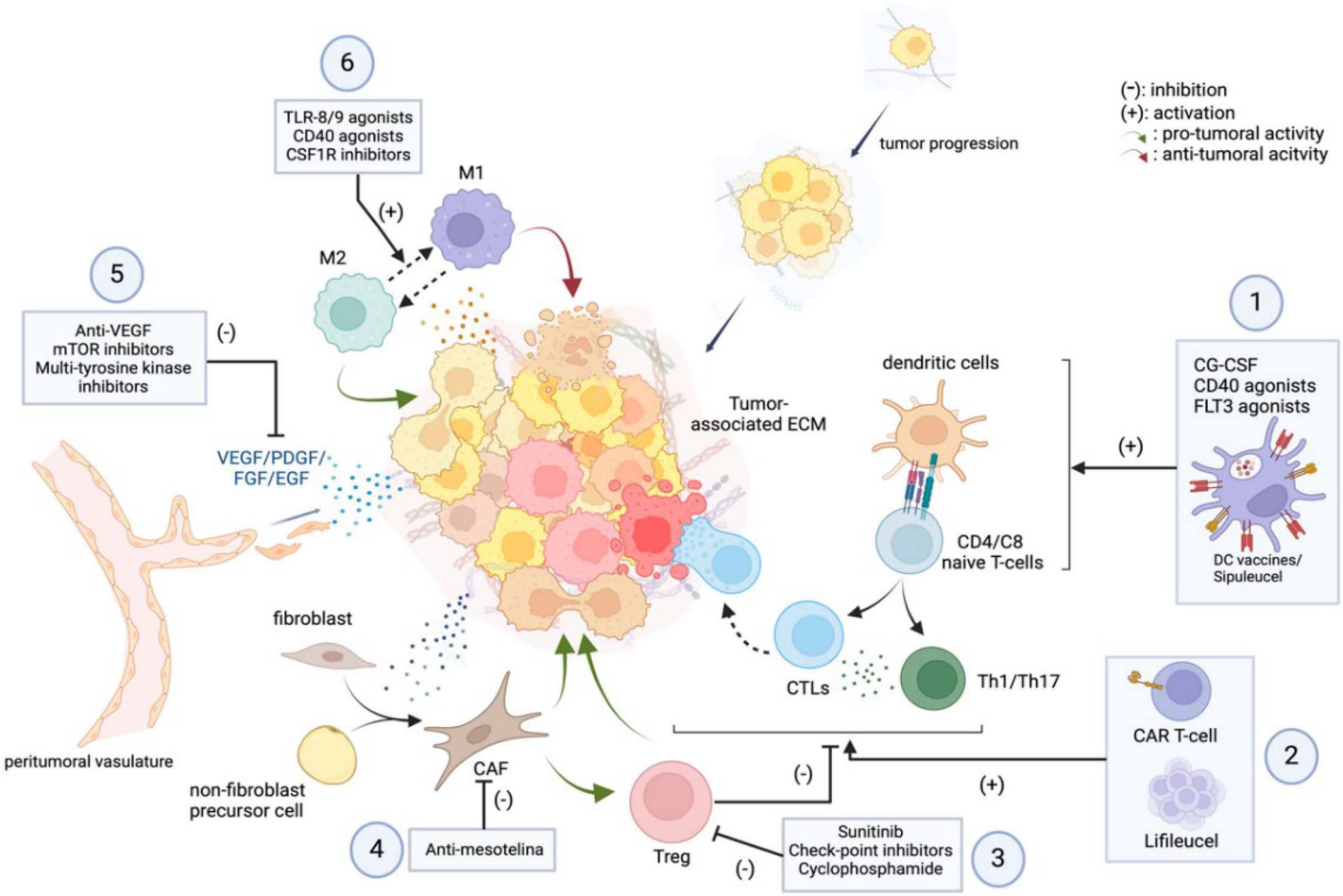

Figure 3. Mechanisms of action of therapies in TME. Summary of experimental and clinical interventions targeting tumoral stromal components. Tumor development is associated with intrinsic extracellular matrix modifications and cellular components that foster neoplastic progression. Tumor cells can induce pro-tumoral phenotypic changes in macrophages, fibroblasts, and lymphocytes by releasing soluble mediators (depicted as small colored circles). Tumors also release pro-angiogenic factors to guide the migration of vascular cells that form new branched vessels. Several strategies have been developed to disrupt tumor-stromal interactions and enhance immune cell-mediated tumoral attack:

[1]Activating DCs with CG-CSF, CD40, and FLT3 agonists to enhance antigen presentation. Another strategy involves administering DC vaccines or a pool of autologous mononuclear cells presenting antigens extracted from tumors and enhanced in vitro (Sipuleucel).

[2]Augment anti-tumoral cytotoxic lymphocyte activities by introducing CAR T cells or an autologous pool of polyclonal lymphocytes extracted from tumors and enhanced in vitro (Lifileucel).

[3]Suppression of Treg cell activities using sunitinib, checkpoint inhibitors, or cyclo-phosphamide treatments.

[4]Inhibition of CAFs using mesothelin antibodies in experimental models.

[5]Inhibition of the angiogenic process at various stages by targeting soluble mediators with anti-VEGF agents, blocking their receptors with multi-tyrosine kinase inhibitors, or perturbing downstream signaling using mTOR inhibitors.

[6]Manipulate macrophage polarization toward a proinflammatory and anti-tumoral phenotype by administering TLR-8/9 agonists, CD40 agonists, or CSFR1 inhibitors.

CSFR-1: colony-stimulating factor-1 receptor; CAFs: cancer-associated fibroblasts; CAR T cell: chimeric antigen receptor T cells; DCs: dendritic cells; CG-CSF: granulocyte-macrophage colony-stimulating factor; CSF-1R: colony-stimulating factor-1 receptor; CTLs: CD8 positive cytotoxic T lymphocytes; EGF: epidermal growth factor; FGF: fibroblast growth factor;FLT3: FMS-like receptor tyrosine kinase-3; M1: macrophages type 1; M2: macrophages type 2; PDGF: platelet-derived growth factor; Treg: T regulatory cells; TLR-8/9: Toll-like receptors 8 and 9; VEGF: vascular endothelial growth factor.

resistant solid tumors. Bispecific antibodies and engineered T cells redirect endogenous T cells to recognize and kill cancer cells.[101] Adoptive cell therapy, including CAR T cells, harnesses a patient's T cells to target cancer-specific antigens.

Oncolytic Viruses

Oncolytic viruses (OVs) are specially engineered viruses that selectively replicate within tumor cells and are considered promising cancer treatments.[89] A key area of interest with OVs is their ability to cause tumor cells to burst and die through replication, a process called lytic cell death. This type of cell death is highly immunogenic and can transform immunologically inactive, or "cold," tumors into active, or "hot," ones.[102] Tumors treated with OVs have shown a rise in the infiltration of CD8+ T cells and an increase in tumor-specific CD8+ T cells throughout the body. The potential synergy between OVs and ICIs is as a possible new approach to treating stubborn, immunologically cold tumors.[102]

Radiation Therapy

Radiation therapy can modify the TME to trigger an anticancer immune response by inducing an immunologic form of cell death.[103] This type of cell death can activate the patient's immune system to attack cancer cells even outside the radiation field, leading to what is known as the abscopal effect—where tumors distant from the irradiated area also regress.[104] Incorporating radiation into ICI regimens is an area of active investigation, with mixed results across different cancer types. For instance, the S1806 trial is evaluating the addition of atezolizumab to chemoradiation in patients with muscle-invasive bladder cancer, aiming to determine if this combination can enhance outcomes.[105] Similarly, the NIVES and RADVAX trials explored the use of radiotherapy combined with nivolumab alone or combined with ipilimumab in mRCC, offering insights into the potential benefits and challenges of this approach.[106,107]

Cytotoxic Chemotherapy

The effectiveness of cytotoxic chemotherapy in combating tumors partially relies on the immune system. It can reconfigure immune tolerance by eliminating immunosuppressive cells such as Tregs within the TME.[108] In addition, chemotherapy can induce cellular necrosis, a type of cell death that is more immunogenic than apoptosis. This process leads to the release of inflammatory signals like IL-8, TNF-α, and High Mobility Group Box Protein 1 (HMGB1), which further stimulate the immune response against the tumor.[109]

Targeted Therapies

VEGF is known to regulate the growth of vascular endothelial cells and contribute to immunosuppression. Research indicates that blocking VEGF pathways can enhance immune responses. Clinical studies have demonstrated that combining PD-L1 monoclonal antibodies with VEGF inhibitors yields synergistic effects, showing promise in treating various types of tumors.[110] Specifically, using PD-L1 inhibitors together with VEGFR2 inhibitors has been effective in reducing PD-1 and PD-L1 expression levels, increasing TILs, reducing Tregs and MDSCs, and inhibiting tumor growth.[110,111]

FUTURE PERSPECTIVES—DECODING THE TME WITH NEW TECHNOLOGIES

Spatial Technologies and Artificial Intelligence

Some available artificial intelligence (AI) approaches, such as deep/machine learning algorithms, could be configured to predict genomic and/or transcriptomic profiles based on digitized hematoxylin and eosin (H&E)-stained tumor slides after a previous training step with a certain accuracy. In a similar manner, the stroma components and their spatial distribution could be characterized from digitized slides, and this information would be used for prognostic and predictive purposes.[112] Lim et al.[113] demonstrated that the Lunit SCOPE IO, a deep learning machine tool based on convolutional neural network architecture, effectively distinguished between individuals experiencing disease recurrence and those without evidence of disease. This discrimination was based on lower stromal TIL density (mean of 630.2/mm^2) for the former and higher stromal TIL density (mean of 1021.3/mm^3) for the latter, in a retrospective analysis of 289 patients with stage II-III colon cancer who had undergone surgery followed by adjuvant therapy.[113]

Another retrospective study assessed the prognostic value of a trained deep-learning convolutional neural network–based algorithm for determining the tumor-stroma ratio (TRS) in H&E-stained slides (representing the most invasive element of the tumor) from patients with localized colorectal cancer.[114] This AI approach could predict differences in OS according to low (TRS $<$ 48.8%) and high (TRS $\geq$ 48.8%) cutoffs. In the discovery cohort (499 patients), the median OS was 72 and 67 months (unadjusted hazard ratio of 1.79; 95% CI, 1.30–2.47; log-rank test $P < 0.0010$) for low and high-TRS patients, respectively. Similar findings were described in the validation cohort, with a median OS of 49 and 46 months (unadjusted HR of 2.21; 95% CI, 1.35–3.63; $P = 0.002$) for low and high-TRS patients, respectively. Additionally, the high-TRS cutoff was still an independent prognostic for inferior survival in a multivariate analysis in both the discovery (HR 1.72; 95% CI, 1.24–2.37; $P = 0.001$) and validation (2.08; 1.26–3.42; $P = 0.004$) cohorts.[114]

AI methodologies can also analyze cell-to-cell interactions from H&E-stained tumor slides to infer possible correlations with clinical features, as evidenced in a retrospective analysis of 2231 luminal human localized breast cancer slides through a supervised deep learning model. In this study, the authors evidenced a positive

statistical association of adverse clinical features (e.g., positive lymph node status, Ki-67 ≥20%, tumor size ≥2 cm, and higher tumor grade) with a higher presence of TILs close to other stroma or tumor cells.[115]

Despite the emerging potential of AI tools for oncology research purposes in characterizing the TME regarding both component description and spatial distribution, most of the evidence in recent years has been focused on prognostic outcomes. Nevertheless, further studies are needed to assess their role in treatment prediction. Additionally, these strategies must undergo validation in other distinct scenarios, necessitating collaboration with multi-institutional datasets.

Nanotechnology

Nanoparticles (NPs), defined as materials smaller than 100 nm, exhibit unique properties due to their surface characteristics and small size. The key application of these nanomaterials is serving as a drug delivery system (DDS) that enables the precise delivery of therapeutic agents to specific cells and tissue environments. These DDSs enhance the effectiveness of the drug, minimize side effects, and improve both pharmacokinetics and bioavailability.[116] As a result, nanomaterial-based DDSs are emerging as innovative therapeutic modalities for a variety of cancers, owing to their ability to navigate biological barriers and optimize drug distribution. For example, in the treatment of melanoma, various nanomaterials such as liposomes, nanostructured lipid carriers (NLCs), solid lipid nanoparticles (SLNs), hydrogels, nanoemulsions, polymer micelles, and inorganic nanoparticles have been used to create nano-DDSs. These platforms offer several significant benefits, including targeted delivery to TAMs, modulation of T-cell responses, and enhancement of other immune responses.[116]

CONCLUSIONS

The complex development of tumors mirrors dynamic changes in the TME, which are pivotal in promoting tumor growth and metastasis. Although targeting specific components of the TME—such as the ECM, vasculature, and both non-immune and immune cells—presents valuable therapeutic opportunities, focusing on these elements individually may not yield comprehensive and lasting therapeutic outcomes.

The success of immunotherapy in treating cancer stems from advancements in understanding the critical mechanisms of T-cell activation and suppression. Emerging therapies, including CAR T, CAR NK, and CAR-M cells, show promise in treating solid tumors. However, clinical trials often struggle to demonstrate effective results due to both innate and acquired resistance in patients.

Exploring TME targets, such as the modulation of tumor vasculature in combination with immunotherapies, could help overcome these therapeutic challenges. A deeper understanding of the essential elements of the TME may drive the discovery and development of innovative treatments. Moreover, incorporating nanomedicine and AI into cancer research could offer novel approaches to targeting the TME.

Given the significant variability within the TME, integrating biomarker-driven patient selection in clinical trials is crucial. This approach is essential for effectively transitioning these strategies from the lab to clinical practice and ensuring their efficacy in cancer treatment.

References

1. Bejarano L, Jordão MJC, Joyce JA. Therapeutic targeting of the tumor microenvironment. *Cancer Discov*. 2021;11:933–959.
2. Wang Q, Shao X, Zhang Y, et al. Role of tumor microenvironment in cancer progression and therapeutic strategy. *Cancer Med*. 2023;12:11149–11165.
3. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Med*. 2013;19:1423–1437.
4. Salmon H, Remark R, Gnjatic S, Mérad M. Host tissue determinants of tumour immunity. *Nat Rev Cancer*. 2019;19:215–227.
5. Salmon H, Idoyaga J, Rahman A, et al. Expansion and activation of CD103+ dendritic cell progenitors at the tumor site enhances tumor responses to therapeutic PD-L1 and BRAF inhibition. *Immunity*. 2016;44:924–938.
6. Anandasabapathy N, Breton G, Hurley A, et al. Efficacy and safety of CDX-301, recombinant human Flt3L, at expanding dendritic cells and hematopoietic stem cells in healthy human volunteers. Bone Marrow Transplant. 2015;50:924–930.
7. Kumar A, Khani AT, Ortiz AS, Swaminathan S. GM-CSF: a Double-Edged Sword in cancer immunotherapy. *Front Immunol*. 2022;13. https://doi.org/10.3389/fimmu.2022.901277
8. Bol KF, Schreibelt G, Gerritsen WR, et al. Dendritic Cell–Based Immunotherapy: State of the art and beyond. *Clin Cancer Res*. 2016;22:1897–1906.
9. Lee J, Choi S, Jung N, et al. The effect of the tumor microenvironment and tumor-derived metabolites on dendritic cell function. *J Cancer*. 2020,11:769–775.
10. Sheng L, Chen X, Wang Q, et al. Interferon-α2b enhances survival and modulates transcriptional profiles and the immune response in melanoma patients treated with dendritic cell vaccines. *Biomed Pharmacother*. 2020;125:109966.
11. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med*. 2010;363(5):411–422.
12. Munn LL, Jain RK. Vascular regulation of antitumor immunity. *Science*. 2019;365:544–545.
13. Suwa T, Kobayashi M, Nam J, Harada H. Tumor microenvironment and radioresistance. *Exp Mol Med*. 2021;53:1029–1035.
14. Huang Y, Goel S, Duda DG, et al. Vascular normalization as an emerging strategy to enhance cancer immunotherapy. *Cancer Res*. 2013;73(10), 2943–2948.
15. Winkler F, Kozin SV, Tong RT, et al. Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation. *Cancer Cell*. 2004;6:553–563.

16. Huang Y, Kim BY, Chan C, et al. Improving immune–vascular crosstalk for cancer immunotherapy. *Nat Rev Immunol*. 2018;18:195–203.
17. Khan K, Kerbel RS. Improving immunotherapy outcomes with anti-angiogenic treatments and vice versa. *Nat Rev Clin Oncol*. 2018;15:310–324.
18. Choueiri TK, Kaelin WG. Targeting the HIF2–VEGF axis in renal cell carcinoma. *Nat Med*. 2020;26:1519–1530.
19. Jain RK. Normalizing tumor microenvironment to treat cancer: bench to bedside to biomarkers. *J Clin Oncol*. 2013;31:2205–2218.
20. Huang Y, Yuan J, Righi E, et al. Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. *Proc Natl Acad Sci U S A*. 2012;109:17561–17566.
21. Zhou J, Wang L, Cheng P, Fu P. Co-targeting tumor angiogenesis and immunosuppressive tumor microenvironment: a perspective in ethnopharmacology. *Front Pharmacol*. 2022;13. DOI: 10.3389/fphar.2022.886198
22. Wang S, Lu J, You Q, et al. The mTOR/AP-1/VEGF signaling pathway regulates vascular endothelial cell growth. *Oncotarget*. 2016;7:53269–53276.
23. Albiges L, Rini BI, Peltola K, et al. LBA88 Belzutifan versus everolimus in participants (pts) with previously treated advanced clear cell renal cell carcinoma (ccRCC): randomized open-label phase III LITESPARK-005 study. *Ann Oncol*. 2023;34:S1329–S1330.
24. Yang H, Zhang Q, Xu M, et al. CCL2- CCR2 axis recruits tumor associated macrophages to induce immune evasion through PD-1 signaling in esophageal carcinogenesis. Mol Cancer. 2020;19:41.
25. Qin R, Ren W, Ya G, et al. Role of chemokines in the crosstalk between tumor and tumor-associated macrophages. *Clin Exp Med*. 2023;23:1359–1373.
26. Nie Y, Huang H, Guo M, et al. Breast phyllodes tumors recruit and repolarize tumor-associated macrophages via secreting CCL5 to promote malignant progression, which can be inhibited by CCR5 inhibition therapy. *Clin Cancer Res*. 2019;25:3873–3886.
27. Zhu S, Yi M, Wu Y, et al. Roles of tumor-associated macrophages in tumor progression: implications on therapeutic strategies. *Exp Hematol Oncol*. 2021;10:60. Erratum in: *Exp Hematol Oncol*. 2022;11:4.
28. Pollard JW, Stanley ER. Pleiotropic roles for CSF-1 in development defined by the mouse mutation osteopetrotic (op). *Adv Dev Biochem*. 1996;4:153–193.
29. Chen D, Xiong L, Zhang L, et al. CSF1R is a prognostic biomarker and correlated with immune cell infiltration in the gastric cancer microenvironment. *Pharmgenomics Pers Med*. 2021;14:445–457.
30. Zhang QW, Liu L, Gong CY, et al. Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature. *PLoS One* 2012;7:e50946.
31. Germano G, Frapolli R, Belgiovine C, et al. Role of macrophage targeting in the antitumor activity of trabectedin. *Cancer Cell*. 2013;23:249–262.
32. Belgiovine C, Bello E, Liguori M, et al. Lurbinectedin reduces tumour-associated macrophages and the inflammatory tumour microenvironment in preclinical models. *Br J Cancer*. 2017;117:628–638.
33. Tap WD, Wainberg ZA, Anthony SP, et al. Structure-guided blockade of CSF1R kinase in tenosynovial giant-cell tumor. *N Engl J Med*. 2015;373:428–437.
34. Daiichi Sankyo Presents New Research in Breast Cancer at 2022 San Antonio Breast Cancer Symposium. www.daiichisankyo.com/media/press_release/detail/index_3177.html. Accessed Sep 12, 2024.
35. Nasir I, McGuinness C, Poh AR, et al. Tumor macrophage functional heterogeneity can inform the development of novel cancer therapies. *Trends Immunol*. 2023;44:971–985.
36. Brana I, Calles A, LoRusso PM, et al. Carlumab, an anti-C-C chemokine ligand 2 monoclonal antibody, in combination with four chemotherapy regimens for the treatment of patients with solid tumors: an open-label, multicenter phase 1b study. *Target Oncol*. 2015;10:111–123.
37. Noel M, O'Reilly EM, Wolpin BM, et al. Phase 1b study of a small molecule antagonist of human chemokine (C-C motif) receptor 2 (PF-04136309) in combination with nab-paclitaxel/gemcitabine in first-line treatment of metastatic pancreatic ductal adenocarcinoma. *Invest New Drugs*. 2020;38:800–811.
38. Ferris RL, Saba NF, Gitlitz BJ, et al. Effect of adding motolimod to standard combination chemotherapy and cetuximab treatment of patients with squamous cell carcinoma of the head and neck: the Active8 Randomized Clinical Trial. *JAMA Oncol*. 2018;4:1583–1588.
39. Ribas A, Medina T, Kirkwood JM, et al. Overcoming PD-1 blockade resistance with CpG-A Toll-like receptor 9 agonist vidutolimod in patients with metastatic melanoma. *Cancer Discov*. 2021;11:2998–3007.
40. Liu PS, Chen YT, Li X, et al. CD40 signal rewires fatty acid and glutamine metabolism for stimulating macrophage anti-tumorigenic functions. *Nat Immunol*. 2023;24:452–462.
41. Weiss SA, Sznol M, Shaheen M, et al. A phase II trial of the CD40 agonistic antibody sotigalimab (APX005M) in combination with nivolumab in subjects with metastatic melanoma with confirmed disease progression on anti-PD-1 therapy. *Clin Cancer Res*. 2024;30:74–81.
42. Esfahani K, Roudaia L, Buhlaiga N, et al. A review of cancer immunotherapy: from the past, to the present, to the future. *Curr Oncol*. 2020;27:87–97.
43. Chauvin J-M, Zarour HM. TIGIT in cancer immunotherapy. *J Immunother Cancer*. 2020;8:2.
44. Reardon S. First cell therapy for solid tumours heads to the clinic: what it means for cancer treatment. *Nature*. 2024. DOI: 10.1038/d41586-024-00673-w
45. Chesney J, Puzanov I, Collichio F, et al. Randomized, open-label phase II study of pembrolizumab with or without talimogene laherparepvec in unresectable stage III-IV melanoma. *J Immunother Cancer*. 2022;10:e005755.
46. June CH, Sadelain M. Chimeric antigen receptor therapy. *N Engl J Med*. 2018;379:64–73.
47. Schuster SJ, Bishop M, Tam CS, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-Cell lymphoma. *N Engl J Med*. 2019;380:45–56.
48. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med*. 2018;378:439–448.
49. Raje N, Berdeja JG, Lin Y, et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. *N Engl J Med*. 2019;380:1726–1737.
50. Wang M, Muñoz J, Goy A, et al. KTE-X19 CAR T-cell therapy in relapsed or refractory mantle-cell lymphoma. *N Engl J Med*. 2020;382:1331–1342.

51. Cappell KM, Sherry RM, Yang JC, et al. Long-term follow-up of anti-CD19 chimeric antigen receptor T-cell therapy. *J Clin Oncol*. 2020;38:3805–3815.
52. Brown CE, Alizadeh D, Starr R, et al. Regression of glioblastoma after chimeric antigen receptor T-cell therapy. *N Engl J Med*. 2016;375:2561–2569.
53. Choi BD, Gerstner ER, Frigault MJ, et al. Intraventricular CARV3-TEAM-E T cells in recurrent glioblastoma. *N Engl J Med*. 2024;390:1290–1298.
54. Maalej KM, Merhi M, Inchakalody VP, et al. CAR-cell therapy in the era of solid tumor treatment: current challenges and emerging therapeutic advances. *Mol Cancer*. 2023;22.
55. Brudno JN, Kochenderfer JN. Recent advances in CAR T-cell toxicity: mechanisms, manifestations and management. *Blood Rev*. 2019;34:45–55.
56. Li Q, Lu J, Li J, et al. Antibody-based cancer immunotherapy by targeting regulatory T cells. *Front Oncol*. 2023;13.
57. Guan X, Hu R, Choi Y, et al. Anti-TIGIT antibody improves PD-L1 blockade through myeloid and Treg cells. *Nature*. 2024;627:646–655.
58. Adotévi O, Péré H, Ravel P, et al. A decrease of regulatory T cells correlates with overall survival after sunitinib-based antiangiogenic therapy in metastatic renal cancer patients. *J Immunother*. 2010;33:991–998.
59. Ge Y, Domschke C, Stoiber N, et al. Metronomic cyclophosphamide treatment in metastasized breast cancer patients: immunological effects and clinical outcome. *Cancer Immunol Immunother*. 2011;61:353–362.
60. Hirschhorn-Cymerman D, Rizzuto GA, Merghoub T, et al. OX40 engagement and chemotherapy combination provides potent antitumor immunity with concomitant regulatory T cell apoptosis. *J Exp Med*. 2009;206:1103–1116.
61. Zappasodi R, Sirard C, Li Y, et al. Rational design of anti-GITR-based combination immunotherapy. *Nat Med*. 2019;25:759–766.
62. Hanson A, Elpek K, Duong E, et al. ICOS agonism by JTX-2011 (vopratelimab) requires initial T cell priming and Fc cross-linking for optimal T cell activation and anti-tumor immunity in preclinical models. *PLoS One*. 2020;15:e0239595.
63. Chen X, Oppenheim JJ. Resolving the identity myth: Key markers of functional CD4+FoxP3+ regulatory T cells. *Int Immunopharmacol*. 2011;11:1489–1496.
64. Vignali DaA, Collison LW, Workman CJ. How regulatory T cells work. *Nat Rev Immunol*. 2008;8:523–532.
65. Kontermann RE, Brinkmann U. Bispecific antibodies. *Drug Discovery Today*. 2015;20:838–847.
66. Hilal T, Prasad V. Eliminating MRD—FDA approval of blinatumomab for B-ALL in complete remission. *Nat Rev Clin Oncol*. 2018;15:727–728.
67. Rosenthal MA, Balana C, van Linde ME, et al. ATIM-49 (LTBK-01). AMG 596, a novel anti-EGFRvIII bispecific T cell engager (BITE®) molecule for the treatment of glioblastoma (GBM): planned interim analysis in recurrent GBM (RGBM). *Neuro-Oncol*. 2019;21:vi283.
68. Nathan P, Hassel JC, Rutkowski P, et al. Overall survival benefit with tebentafusp in metastatic uveal melanoma. *N Engl J Med*. 2021;385:1196–1206.
69. Zhang E, Ding C, Li S, et al. Roles and mechanisms of tumour-infiltrating B cells in human cancer: a new force in immunotherapy. *Biomarker Res*. 2023;11.
70. Laumont CM, Nelson BH. B cells in the tumor microenvironment: multi-faceted organizers, regulators, and effectors of anti-tumor immunity. *Cancer Cell*. 2023;41:466–489.
71. Li S, Mirlekar B, Johnson BM, et al. STING-induced regulatory B cells compromise NK function in cancer immunity. *Nature*. 2022;610:373–380.
72. Yang D, Liu J, Xu W, Zhuang Q. Cancer-associated fibroblasts: from basic science to anticancer therapy. *Exp Mol Med*. 2023;55:1322–1332.
73. Sebastian A, Hum NR, Martin KA, et al. Single-cell transcriptomic analysis of tumor-derived fibroblasts and normal Tissue-Resident fibroblasts reveals fibro-blast heterogeneity in breast cancer. *Cancers*. 2020;12:1307.
74. Kim D, Kim JS, Cheon I, et al. Identification and characterization of cancer-associated fibroblast subpopulations in lung adenocarcinoma. *Cancers*. 2022;14:3486.
75. Öhlund D, Handly-Santana A, Biffi G, et al. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. *J Exp Med*. 2017;214:579–596.
76. Zhang X, Zheng S, Hu C, et al. Cancer-associated fibroblast-induced lncRNA UPK1A-AS1 confers platinum resistance in pancreatic cancer via efficient double-strand break repair. *Oncogene*. 2022;41:2372–2389.
77. Biffi G, Oni TE, Spielman B, et al. IL1-Induced JAK/STAT signaling is antagonized by TGFB to shape CAF heterogeneity in pancreatic ductal adenocarcinoma. *Cancer Discov*. 2019;9:282–301.
78. Huang H, Wang Z, Zhang Y, et al. Mesothelial cell-derived antigen-presenting cancer-associated fibroblasts induce expansion of regulatory T cells in pancreatic cancer. *Cancer Cell*. 2022;40:656–673.e7.
79. Tsai J, Sinha R, Seita J, et al. Surgical adhesions in mice are derived from mesothelial cells and can be targeted by antibodies against mesothelial markers. *Sci Transl Med*. 2018;10.
80. Friedman G, Levi-Galibov O, David E, et al. Cancer-associated fibroblast compositions change with breast cancer progression linking the ratio of S100A4+ and PDPN+ CAFs to clinical outcome. *Nat Cancer*. 2020;1:692–708.
81. Davidson S, Efremova M, Riedel A, et al. Single-cell RNA sequencing reveals a dynamic stromal niche that supports tumor growth. *Cell Rep*. 2020;31:107628.
82. Chen X, Song E. Turning foes to friends: targeting cancer-associated fibroblasts. *Nat Rev Drug Discov*. 2018;18:99–115.
83. Gonda TA, Varro A, Wang TC, Tycko B. Molecular biology of cancer-associated fibroblasts: can these cells be targeted in anti-cancer therapy? *Semin Cell Dev Biol*. 2010;21:2–10.
84. Togo S, Polanska UM, Horimoto Y, Orimo A. Carcinoma-associated fibroblasts are a promising therapeutic target. *Cancers*. 2013;5:149–169.
85. Shao H, Cai L, Grichnik JM, et al. Activation of Notch1 signaling in stromal fibroblasts inhibits melanoma growth by upregulating WISP-1. *Oncogene*. 2011;30:4316–4326.
86. Lee J, Fassnacht M, Nair S, et al. Tumor immunotherapy targeting fibroblast activation protein, a product expressed in tumor-associated fibroblasts. *Cancer Res*. 2005;65:11156–11163.
87. Zhou L, Yang K, Andl T, et al. Perspective of targeting cancer-associated fibroblasts in melanoma. *J Cancer*. 2015;6:717–726.

88. Duluc C, Moatassim-Billah S, Chalabi-Dchar M, et al. Pharmacological targeting of the protein synthesis mTOR/4E- BP 1 pathway in cancer-associated fibroblasts abrogates pancreatic tumour chemoresistance. *EMBO Mol Med*. 2015;7:735–753.
89. Tschumperlin DJ, Lagares D. Mechano-therapeutics: targeting mechanical signaling in fibrosis and tumor stroma. *Pharmacol Ther*. 2020;212:107575.
90. Paolillo M, Schinelli S. Extracellular matrix alterations in metastatic processes. *Int J Mol Sci*. 2019;20:4947.
91. Yuan Z, Li Y, Zhang S, et al. Extracellular matrix remodeling in tumor progression and immune escape: from mechanisms to treatments. *Mol Cancer*. 2023;22.
92. Nicolás-Boluda A, Vaquero J, Laurent G, et al. Photothermal depletion of cancer-associated fibroblasts normalizes tumor stiffness in desmoplastic cholangiocarcinoma. *ACS Nano*. 2020;14:5738–5753.
93. Ying S, Wang X, Lu J, et al. Reduction of liver metastasis stiffness improves response to bevacizumab in metastatic colorectal cancer. *Cancer Cell*. 2020;37:800–817.e7.
94. Lei K, Kurum A, Kaynak M, et al. Cancer-cell stiffening via cholesterol depletion enhances adoptive T-cell immunotherapy. *Nat Biomed Eng*. 2021;5:1411–1425.
95. Caruana I, Savoldo B, Hoyos V, et al. Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes. *Nat Med*. 2015;21:524–529.
96. Zhang C, Fang L, Wang X, et al. Oncolytic adenovirus-mediated expression of decorin facilitates CAIX-targeting CAR-T therapy against renal cell carcinoma. *Mol Ther Oncolytics*. 2022;24:14–25.
97. Galon J, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. *Nat Rev Drug Discov*. 2019;18:197–218.
98. Wang L, Geng H, Liu Y, et al. Hot and cold tumors: immunological features and the therapeutic strategies. *MedComm*. 2023;4:e343.
99. Jou J, Harrington KJ, Zocca M, et al. The changing landscape of therapeutic cancer vaccines-novel platforms and neoantigen identification. *Clin Cancer Res*. 2021;27:689–703.
100. McGranahan N, Swanton C. Neoantigen quality, not quantity. *Sci Transl Med*. 2019;11.
101. Goebeler M, Bargou RC. T cell-engaging therapies - BiTEs and beyond. *Nat Rev Clin Oncol*. 2020;17:418–434.
102. Lovatt C, Parker AL. Oncolytic viruses and immune checkpoint inhibitors: the "Hot" new power couple. *Cancers*. 2023;15:4178.
103. Demaria S, Coleman CN, Formenti SC. Radiotherapy: changing the game in immunotherapy. *Trends Cancer*. 2016;2:286–294.
104. Janopaul-Naylor J, Shen Y, Qian DC, Buchwald ZS. The abscopal effect: a review of pre-clinical and clinical advances. *Int J Mol Sci*. 2021;22:11061.
105. Phase III randomized trial of concurrent chemoradiotherapy with or without atezolizumab in localized muscle invasive bladder cancer. SWOG Cancer Research Network. SWOG clinical trial number: S1806. Accessed Sep 12, 2024. www.swog.org/clinical-trials/s1806
106. Masini C, Iotti C, Ciammella P, et al. NIVES study: A phase II trial of nivolumab (NIVO) plus stereotactic body radiotherapy (SBRT) in II and III line of patients (pts) with metastatic renal cell carcinoma (mRCC). *J Clin Oncol*. 2018;36(15_suppl):TPS4602–TPS4602.
107. Hammers HJ, Vonmerveldt D, Ahn C, et al. Combination of dual immune checkpoint inhibition (ICI) with stereotactic radiation (SBRT) in metastatic renal cell carcinoma (mRCC) (RADVAX RCC). *J Clin Oncol*. 2020;38(6_suppl):614–614.
108. Zhao P, Zhu D, Zhang Z, et al. Gemcitabine treatment enhanced the anti-tumor effect of cytokine induced killer cells by depletion of CD4+CD25bri regulatory T cells. *Immunology Lett*. 2017;181:36–44.
109. Wang S, Zhang Y. HMGB1 in inflammation and cancer. *J Hematol Oncol*. 2020;13:116.
110. Gao F, Yang C. Anti-VEGF/VEGFR2 monoclonal antibodies and their combinations with PD-1/PD-L1 inhibitors in clinic. *Curr Cancer Drug Targets*. 2020;20:3–18.
111. Amin A, Plimack ER, Ernstoff MS, et al. Safety and efficacy of nivolumab in combination with sunitinib or pazopanib in advanced or metastatic renal cell carcinoma: the CheckMate 016 study. *J Immunother Cancer*. 2018;6.
112. Baxi V, Edwards R, Montalto M, Saha S. Digital pathology and artificial intelligence in translational medicine and clinical practice. *Mod Pathol*. 2022;35:23–32.
113. Lim Y, Choi S, Oh HJ, et al. Artificial intelligence-powered spatial analysis of tumor-infiltrating lymphocytes for prediction of prognosis in resected colon cancer. *npj Precis Oncol*. 2023;7:124.
114. Zhao K, Li Z, Yao S, et al. Artificial intelligence quantified tumour-stroma ratio is an independent predictor for overall survival in resectable colorectal cancer. *EBioMedicine*. 2020;61:103054.
115. Makhlouf S, Wahab N, Toss M, et al. Evaluation of tumour infiltrating lymphocytes in luminal breast cancer using artificial intelligence. *Br J Cancer*. 2023;129:1747–1758.
116. Xu M, Li S. Nano-drug delivery system targeting tumor microenvironment: A prospective strategy for melanoma treatment. *Cancer Lett*. 2023;574:216397.
117. Flt3L in treating patients with metastatic colorectal cancer. ClinicalTrials.gov identifier: NCT00003431. Accessed Aug 18, 2024. clinicaltrials.gov/study/NCT00003431
118. Sanborn R, Gabrail N, Carneiro B, et al. 596 Results from a phase 1 study of CDX-1140, a fully human anti-CD40 agonist monoclonal antibody (mAb), in combination with pembrolizumab [Abstract]. *J Immunother Cancer*. 2022:A624.
119. Nelson BE, Adashek JJ, Sheth AA, Subbiah V. Predicting the abscopal effect: associated tumor histologic subtypes and biomarkers. *Mol Cancer Ther*. 2023;22:706–716.
120. Bhardwaj N, Pavlick AC, Ernstoff MS, et al. A phase II randomized study of CDX-1401, a dendritic cell targeting NY-ESO-1 vaccine, in patients with malignant melanoma pre-treated with recombinant CDX-301, a recombinant human Flt3 ligand. *J Clin Oncol*. 2016;34(15_suppl):9589–9589.
121. Lowenstein P. First-in-human phase I trial of the combination of two adenoviral vectors expressing HSV1-TK and FLT3L for the treatment of newly diagnosed resectable malignant glioma: initial results from the therapeutic reprogramming of the brain immune system. *J Clin Oncol*. 2019;37(15_suppl).
122. flt3L with or without vaccine therapy in treating patients with metastatic melanoma or renal cell cancer. ClinicalTrials.gov identifier: NCT00019396. Accessed Aug 18, 2024. clinicaltrials.gov/study/NCT00019396

123. GM-CSF in treating patients with relapsed prostate cancer. ClinicalTrials.gov identifier: NCT00908141. Accessed Aug 2018, 2024. clinicaltrials.gov/study/NCT00908141
124. GM-CSF, sargramostim in women with recurrent ovarian cancer. ClinicalTrials.gov identifier: NCT00157573. Accessed Aug 18, 2024. clinicaltrials.gov/study/NCT00157573
125. Sargramostim in treating patients with kidney cancer that has spread to the lung. ClinicalTrials.gov identifier: NCT00006483. Accessed Aug 18, 2024. clinicaltrials.gov/study/NCT00006483
126. Martinez M, Ono N, Planutiene M, et al. Granulocyte-macrophage stimulating factor (GM-CSF) increases circulating dendritic cells but does not abrogate suppression of adaptive cellular immunity in patients with metastatic colorectal cancer receiving chemotherapy. *Cancer Cell Int*. 2012;12:2.
127. Small EJ. A prospective randomized phase II trial evaluating maintenance GM-CSF in an intermittent chemotherapy (chemo) regimen for metastatic castration-resistant prostate cancer (mCRPC): a DoD prostate cancer clinical trials consortium trial. Published June 20, 2011. Accessed Sep 12, 2024. meetings.asco.org/abstracts-presentations/67964
128. Phase II GM-CSF plus mitoxantrone in hormone refractory prostate cancer. ClinicalTrials.gov identifier: NCT00477087. Accessed Aug 18, 2024. clinicaltrials.gov/study/NCT00477087
129. Herceptin and GM-CSF for metastatic breast cancer. ClinicalTrials.gov identifier: NCT00429104. Accessed Aug 18, 2024. clinicaltrials.gov/study/NCT00429104
130. Monoclonal antibody therapy and colony- stimulating factor in treating patients with metastatic colorectal cancer. ClinicalTrials.gov identifier: NCT00002664. Accessed Aug 18, 2024. clinicaltrials.gov/study/NCT00002664
131. A phase II/III trial of nivolumab, ipilimumab, and GM-CSF in patients with advanced melanoma. ClinicalTrials.gov identifier: NCT 02339571. Accessed Aug 18, 2024. clinicaltrials.gov/study/NCT02339571
132. Daniels GA, McKinney M, Ongkeko W, et al. A phase 1 clinical trial of a PSA/IL-2/GM-CSF containing prostate cancer vaccine in PSA defined biochemical recurrent prostate cancer patients. *J Clin Oncol*. 2016;34(15_suppl):e14584.
133. Simons JW, Sacks N. Granulocyte-macrophage colony-stimulating factor–transduced allogeneic cancer cellular immunotherapy: the GVAX® vaccine for prostate cancer. *Urol Oncol Semin Orig Investig*. 2006;24:419–424.
134. Vaccination of patients with ovarian cancer with dendritic cell/tumor fusions with granulocyte macrophage colony-stimulating factor (GM-CSF) and imiquimod. ClinicalTrials.gov identifier: NCT00799110. Accessed Aug 18, 2024. clinicaltrials.gov/study/NCT00799110
135. Mittendorf EA, Lu B, Melisko M, et al. Efficacy and safety analysis of nelipepimut-S vaccine to prevent breast cancer recurrence: a randomized, multicenter, phase III clinical trial. *Clin Cancer Res*. 2019;25:4248–4254.
136. Amato RJ, Drury N, Naylor S, et al. Vaccination of prostate cancer patients with modified vaccinia ankara delivering the tumor antigen 5T4 (TroVax): a phase 2 trial. *J Immunother*. 2008;31:577–585.
137. HER-2/Neu vaccine plus GM-CSF in treating patients with stage III or stage IV breast, ovarian, or non-small cell lung cancer. ClinicalTrials.gov identifier: NCT00003002. Accessed Aug 18, 2024. clinicaltrials.gov/study/NCT00003002
138. Chen Z, Zhang S, Han N, et al. A neoantigen-based peptide vaccine for patients with advanced pancreatic cancer refractory to standard treatment. *Front Immunol*. 2021;12:691605.
139. Brunsvig PF, Guren TK, Nyakas M, et al. Long-term outcomes of a phase I study with UV1, a second generation telomerase based vaccine, in patients with advanced non-small cell lung cancer. *Front Immunol*. 2020;11:572172.
140. Lorigan P, Medina T, Nyakas M, et al. Ipilimumab and nivolumab plus UV1, an anticancer vaccination against telomerase, in advanced melanoma. *J Clin Oncol*. 2024;-42(17_suppl):LBA9519.
141. Agrawal P, Offin M, Ginsberg MS, et al. Combining a WT1 cancer vaccine (galinpepimut-S) with checkpoint inhibition (nivolumab) in patients with WT1-expressing diffuse pleural mesothelioma (DPM): A phase I study. *J Clin Oncol*. 2024;42(16_suppl):8083–8083.
142. Emamekhoo H, Kyriakopoulos C, Liu G, McNeel DG. Phase II trial of a DNA vaccine encoding prostatic acid phosphatase (pTVG-HP) and nivolumab (Nivo) in patients (pts) with nonmetastatic, PSA-recurrent prostate cancer (PCa). *J Clin Oncol*. 2020;38(6_suppl):TPS273.
143. Haakensen VD, Nowak AK, Ellingsen EB, et al. NIPU: a randomised, open-label, phase II study evaluating nivolumab and ipilimumab combined with UV1 vaccination as second line treatment in patients with malignant mesothelioma. *J Transl Med*. 2021;19:232.
144. Using a targeted cancer vaccine (galinpepimut-s) with immunotherapy (nivolumab) in mesothelioma. ClinicalTrials.gov identifier: NCT04040231. Accessed Aug 18, 2024. clinicaltrials.gov/study/NCT04040231
145. Scholz M, Yep S, Chancey M, et al. Phase I clinical trial of sipuleucel-T combined with escalating doses of ipilimumab in progressive metastatic castrate-resistant prostate cancer. *ImmunoTargets Ther*. 2017;6:11–16.
146. Zhang L, Sinha M, Subudhi SK, et al. The impact of prior radiation therapy on outcome in a phase 2 trial combining sipuleucel-T (SipT) and ipilimumab (Ipi) in patients (pts) with metastatic castration resistant prostate cancer (mCRPC). *J Clin Oncol*. 2021;39(15_suppl):5045–5045.
147. Pachynski RK, Morishima C, Szmulewitz R, et al. IL-7 expands lymphocyte populations and enhances immune responses to sipuleucel-T in patients with metastatic castration-resistant prostate cancer (mCRPC). *J Immunother Cancer*. 2021;9:e002903.
148. Dendritic cell activating scaffold in melanoma. ClinicalTrials.gov identifier: NCT01753089. Accessed Aug 18, 2024. clinicaltrials.gov/study/NCT01753089
149. Jansen Y, Kruse V, Corthals J, et al. A randomized controlled phase II clinical trial on mRNA electroporated autologous monocyte-derived dendritic cells (TriMixDC-MEL) as adjuvant treatment for stage III/IV melanoma patients who are disease-free following the resection of macrometastases. *Cancer Immunol Immunother*. 2020;69:2589–2598.
150. Besse B, Charrier M, Lapierre V, et al. Dendritic cell-derived exosomes as maintenance immunotherapy after first line chemotherapy in NSCLC. *OncoImmunology*. 2016;5:e1071008.