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REVIEW

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Harnessing the tumor microenvironment: targeted cancer therapies through modulation of epithelial-mesenchymal transition

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Abstract

The tumor microenvironment (TME) is integral to cancer progression, impacting metastasis and treatment response. It consists of diverse cell types, extracellular matrix components, and signaling molecules that interact to promote tumor growth and therapeutic resistance. Elucidating the intricate interactions between cancer cells and the TME is crucial in understanding cancer progression and therapeutic challenges. A critical process induced by TME signaling is the epithelial-mesenchymal transition (EMT), wherein epithelial cells acquire mesenchymal traits, which enhance their motility and invasiveness and promote metastasis and cancer progression. By targeting various components of the TME, novel investigational strategies aim to disrupt the TME's contribution to the EMT, thereby improving treatment efficacy, addressing therapeutic resistance, and offering a nuanced approach to cancer therapy. This review scrutinizes the key players in the TME and the TME's contribution to the EMT, emphasizing avenues to therapeutically disrupt the interactions between the various TME components. Moreover, the article discusses the TME's implications for resistance mechanisms and highlights the current therapeutic strategies toward TME modulation along with potential caveats.

Keywords Cancer, Tumor microenvironment (TME), T-cells, B-cells, tumor-associated macrophages(TAMs), Natural killer (NK) cells, Myeloid-derived suppressor cells (MDSCs), Tumor-associated neutrophils (TANs), Dendritic cells (DCs), Cancer-associated fibroblasts (CAFs), Extracellular matrix (ECM), Chimeric antigen-receptor (CAR) T-cell therapy, T-cell receptor (TCR) therapy, Metastasis, Epithelial-mesenchymal transition (EMT), Theranostics

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Introduction

Significant development in the field of cancer therapy have taken place during the last decade, resulting in increased life expectancy. Nevertheless, the efficacy of these strategies often depends on the type of cancer, its genomic/molecular alterations, and patients [1]. Metastasis is a hallmark of cancer and the main contributor to the deaths of cancer patients [2, 3]. Hence, metastasis represents the final destination of an evolutionary journey, during which continuous and intricate interactions between cancer cells and their surrounding microenvironment result in alterations that enable these cells to deviate from their originally programmed behavior [4, 5]. Even though metastasis is responsible for the high mortality and morbidity of cancer, its underlying mechanisms are poorly understood. Epithelial-mesenchymal transition (EMT) is a biological process wherein polarized epithelial cells assume a mesenchymal phenotype through reversible changes in gene expression and morphology that enhance their migratory capacity and contributes to cancer metastasis [6, 7]. EMT leads to epithelial cells transitioning into cells with a more mesenchymal phenotype [6–9]. While EMT has important roles in normal embryonic development, tissue repair, and wound healing [10], it is also associated with tumorigenesis, metastasis, stemness, and therapeutic resistance [11–14]. The induction of EMT not only enhances motility and invasion of cancer cells, but also mediates resistance to senescence (15] and apoptosis [16]. In addition, EMT stimulation increases stem cell-like features, as well as the formation of self-renewing tumor-initiating cancer stem cells (CSCs) [17–20]. The shift of cells to a more mesenchymal-like phenotype is also critical for tumor cells to invade nearby tissues, and subsequently, enter the systemic circulation and metastasize to distant organs [21]. Furthermore, the reversal of EMT at the metastatic niche, known as mesenchymal-epithelial transition (MET), contributes to metastatic colonization of distant organs [22], disseminated tumor cell-induced metastases, and re-initiation of tumor growth [23–25]. Importantly, while the process of MET is not well understood [26, 27], the reversibility of EMT and the phenotypic plasticity suggest that EMT is a dynamic process controlled by either intrinsic and/or extrinsic signals. Originally, EMT has been viewed as a simple binary model encompassing two extreme phenotypes, EMT (fully mesenchymal) and MET (fully epithelial). More recently, however, it is being considered a dynamic process with intermediate transition states between these two extremes [28]. When undergoing EMT in a physiological context, e.g., a lineage-labeled mouse model of pancreatic ductal adenocarcinoma (PDAC) to study EMT in vivo, carcinoma cells can lose their epithelial phenotype through different molecular mechanisms associated with distinct modes of epithelial marker loss and invasive activity. In states of "partial EMT" (pEMT), the cell expresses both epithelial and mesenchymal markers to a varying extent [29, 30]. Of note, pEMT involves mostly alterations at the protein level (as opposed to those at the transcriptional/RNA level) of cell surface-associated epithelial proteins, such as internalization and relocalization to intracellular stores. Moreover, while these cells migrate/invade in cell clusters rather than singly [28, 30], cells having undergone a "complete EMT" (cEMT) are characterized by transcriptional repression of epithelial markers and a single-cell migration mode [28, 30]. In PDAC and other cancers, the pEMT and cEMT programs are reflected in the various histopathological subtypes and their diverse clinical behaviors [31]. The actual EMT phenotypes are dynamically governed to a large extent by extracellular signals from the tumor microenvironment (TME) [32–34]. The TME is a complex, rich, multicellular, and unique ecosystem surrounding a tumor [35–37]. The consistent mutual interaction between different components of the TME and tumor cells support cancer growth and invasion of healthy tissues, which correlates with poor prognosis and tumor resistance to current treatments [38–40]. Accordingly, this complex bidirectional crosstalk between tumor cells and the TME has been reported to drive cancer growth and metastasis [22, 41] (Fig. 1). Interestingly, the cells of the TME can exert either a suppressive or a supporting role toward the tumor [38, 42], depending on the cancer stage and/or cancer site. These divergent functions are mainly determined by tumor type, education, and ontogeny of the cells present inside the neoplasm [42). The TME typically comprises various cell types such as stromal cells including cancer-associated fibroblasts (CAFs) [43–45], mesenchymal stromal cells (MSCs) [46, 47], and pericytes [48, 49]; immune cells including tumor-associated macrophages (TAMs) (50, 52], T-cells (53–56], B-cells [57, 58], natural killer (NK) cells [59, 60], myeloid-derived suppressor cells (MDSCs) [61, 62], tumor-associated neutrophils (TANs) [63, 64], and dendritic cells (DCs) [65, 66]; as well as extracellular matrix (ECM) [67, 68] and secreted molecules including extracellular vesicles (ECVs) [69, 70], growth factors [71, 72], hormones [71, 73], cytokines [74, 32], and chemokines [74, 75] (Fig. 2) (Table 1). Besides, the TME includes communicating lymphatic vascular and blood networks, as well as cancer subpopulations present in diverse locations within a tumor [76, 50]. Given that EMT can be induced not only by signals originating within malignant cells but also by multiple components within the TME [77], therapeutic strategies aimed at targeting EMT must comprehensively target these diverse and potent sources of EMT-inducing cues to achieve efficacy. This review thus first seeks to equip readers with essential knowledge of the EMT process and its associated signaling pathways. Following this, we explore the TME components that have been shown to induce

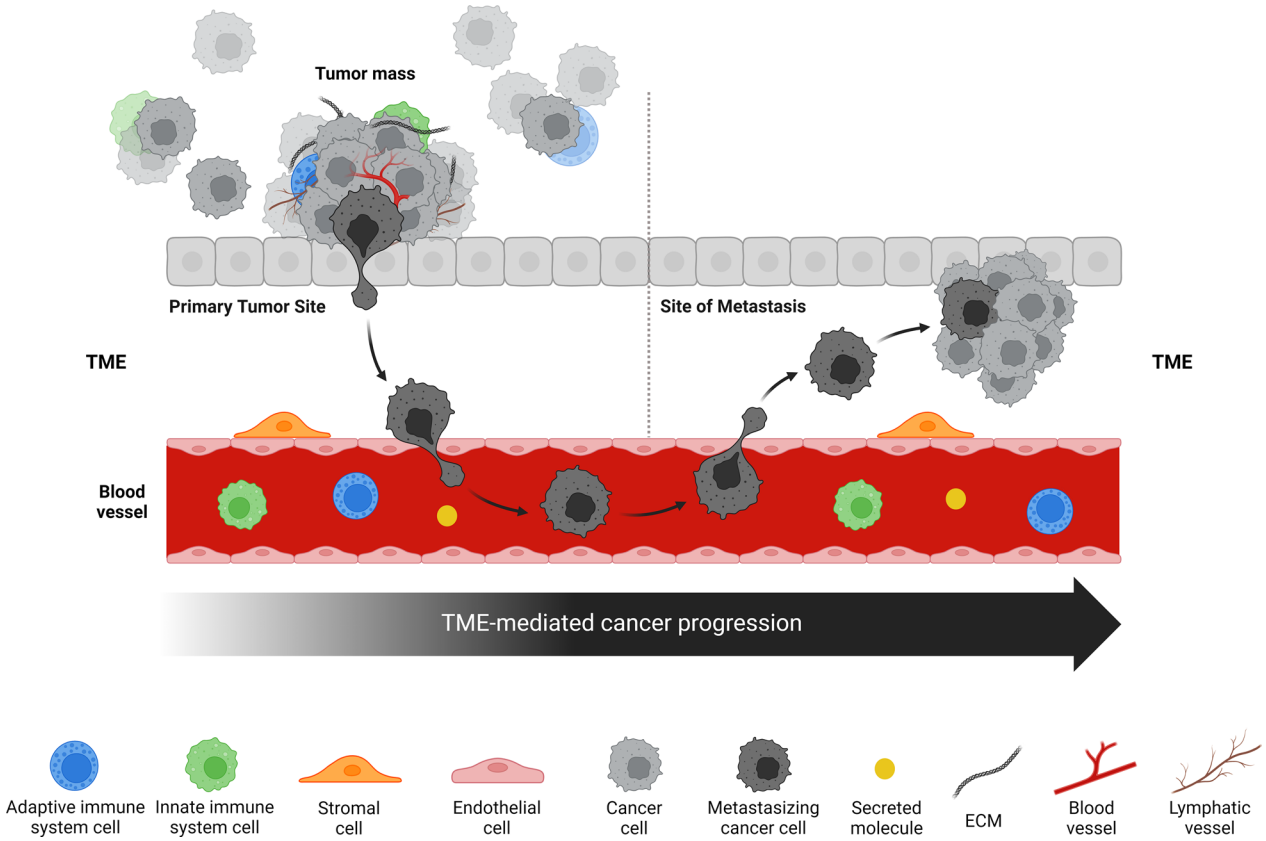

Fig. 1 Metastasis is responsible for the diffusion of tumor cells to distant regions of the body leading to increased drug resistance, therapy failure, and mortality. The plasticity of EMT suggests that metastasis is regulated by extracellular signals from the TME, a complex multicellular and unique tumor-surrounding ecosystem, which denotes the non-malignant cells and their released molecules present in the tumor via epigenetic modifications in cancer cells. The TME comprises various cell types such as immune cells from the adaptive immune system, immune cells from the innate immune system, and stromal cells, as well as blood/lymphatic vascular network, ECM, and secreted molecules; all of which communicate to signals modulating EMT. The constant interdependent interaction between cancer cells and their TME, as well as the heterogeneity of TME, represent the major contributors toward metastasis, cancer progression, and reduced therapeutic response. TME: Tumor microenvironment; ECM: Extracellular matrix. This figure has been created with BioRender.com

EMT and the mechanisms behind these interactions, aiming to identify key TME cell types for therapeutic targeting. Finally, we examine current TME-targeting strategies in both pre-clinical and clinical studies, highlighting research gaps and challenges faced, and prioritizing critical areas for future studies. Together, we hope that this review will facilitate the development of more effective and precise EMT-targeting therapies, ultimately mitigating cancer invasion and metastasis.

TME-induced EMT

In cancer, the TME includes stromal cells secreting several cytokines and/or chemokines acting via paracrine signaling on nearby tumor cells. These paracrine cellular communication signals can trigger an EMT program in tumor cells, encouraging cancer progression and metastasis. This section of the review discusses how stromal cells in the TME can contribute to activating EMT.

TME-mediated signaling pathways activating EMT

The cellular changes that occur during EMT are driven by EMT-inducing transcription factors (EMT-TFs), which comprise basic helix-loop-helix (bHLH) transcription factors twist-related protein 1 (TWIST1) and twist-related protein 2 (TWIST2), zinc finger E-box binding homeobox factors 1 and 2 (ZEB1/2), zinc finger protein SNAI2 (Slug), and zinc finger protein SNAI1 (Snail) [78–80]. Different signaling pathways control the expression and activation of these master regulators, which are in turn influenced by signals from the TME [11]. One of the key pathways is the leukocytes-, stromal cell-, and platelet-released pleiotropic cytokine transforming growth

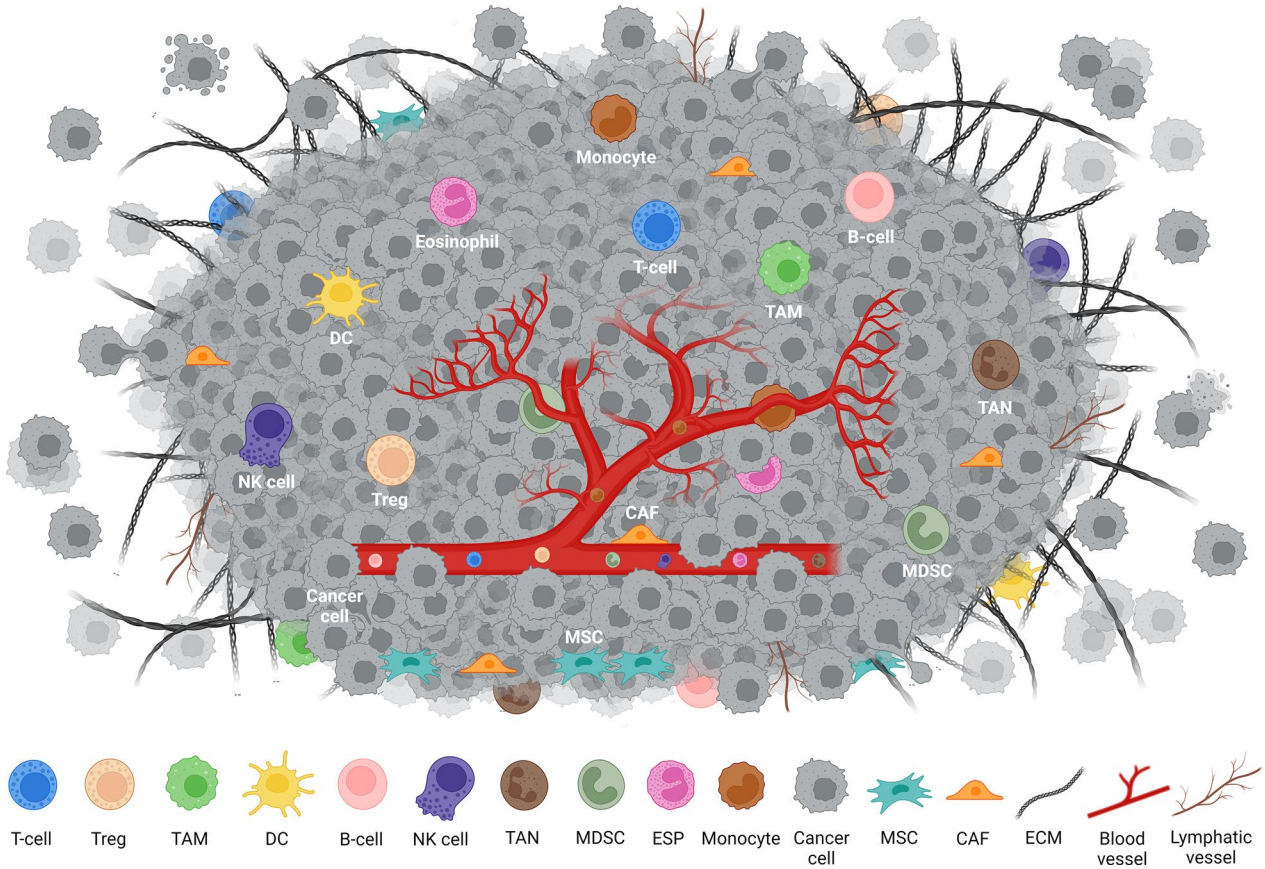

Fig. 2 TME is composed of different cell types, cell structures, and secreted factors. The TME is populated by heterogeneous cancer cells and various cell types including immune cells such as T-cells, B-cells, NK cells, DCs, TAMs, MDSCs, neutrophils, monocytes, and eosinophils; stromal cells including CAFs and MSCs; as well as blood and lymphatic vascular networks. CAF: Cancer-associated fibroblast; DC: Dendritic cell; ECM: Extracellular matrix; ESP: Eosinophil; MDSC: Myeloid-derived suppressor cells; MSC: Mesenchymal stromal cells; NK cell: Natural killer cell; TAM: Tumor-associated macrophages; TAN: Tumor-associated neutrophil; Treg: Regulatory T-cells. These cells secrete ECM components, cytokines, growth factors, and ECVs important for signaling among different cell types in the TME. This figure has been created with BioRender.com

factor-β (TGF-β) [81], whose signaling exerts regulation of EMT-TFs through SMAD-dependent/independent pathways [82]. Other pathways that regulate the expression of EMT-TFs include the Wnt/β-catenin pathway [83, 84], the Notch signaling [85], and various growth factors such as epidermal growth factor (EGF) [86], insulin-like growth factors 1 (IGF-1) [87], and hepatocyte growth factor (HGF) [88].

Hypoxia-inducible factor 1α (HIF-1α) signaling induces EMT and mediates cellular response to hypoxia

The HIF-1α signaling also induces EMT [89] and mediates cellular response to hypoxia, a characteristic microenvironment hallmark of solid tumors arising from an imbalance between the heightened oxygen requirement of rapidly proliferating cells and an inadequate oxygen supply in the tumor [90–92]. In fact, extensive cancer cell proliferation distances cells from the vasculature, causing insufficient blood-carrying oxygen in the local environment [93]. This response to low oxygen concentration is primarily regulated by HIF-1α, which acts as a master transcriptional regulator and whose stability, nuclear localization, and activity are altered by hypoxic conditions [90]. HIF-1α mediates EMT through the regulation of EMT-TFs and reduction of E-cadherin expression [89–94]. HIF-1α binds directly to hypoxia-responsive elements (HREs) in the *Twist* proximal promoter in hypopharyngeal and breast cancer cells [95], as well as the *ZEB1* proximal promoter in colorectal cancer (CRC) cells, resulting in increased cell migration and invasion [96]. In addition, Choi et al. (2017) have identified a hypoxia-induced deubiquitinating enzyme, USP47, which promotes the stabilization of Snail to enhance EMT and cancer cell metastasis [89]. Hypoxia also shares several inter-related signaling pathways with EMT, including the critical EMT-inducing TGF-β pathway. HIF-1α promotes the TGF-β signaling by upregulating TGF-β1/β2 and phosphorylating Smads [97–99].

Table 1 Major components (stromal cells, immune cells, ECM and secreted molecules) of the tumor microenvironment

Major components of the tumor microenvironment		
TME component	**Description**	**R**
Stromal cells		
CAFs	CAFs are a highly heterogeneous cell population in both origin and functionality. Even though the majority of CAFs result from the induction and expansion of local tissue-resident fibroblasts, various studies have shown that CAFs originate from pericytes, adipocytes, endothelial cells, and bone marrow-derived mesenchymal stem cells. CAFs, which are the most abundant stromal cells in the TME, can promote tumorigenic by secreting cytokines and initiate the remodelling of the ECM. CAFs can also stimulate angiogenesis, tumor formation and metastasis. Hence CAFs themselves and downstream effectors are potential targets for improving the sensitivity of antitumor therapies. Expression pattern of some CAFs surface markers include α-SMA, SPARC, and PDGFβ	43–45
MSCs	MSCs are a subset of heterogeneous non-hematopoietic fibroblast-like multipotent progenitor cells with immuno-suppressive properties. MSCs possess a high capacity for self-renewal while maintaining their multipotency. MSCs can differentiate into several types of cells, such as osteoblasts, chondrocytes, myocytes, and adipocytes. MSCs are found in nearly all tissues but are mostly located in perivascular niches, playing a remarkable role in tissue repair and regeneration. MSCs are found within most tumors and influence the formation and function of the TME. MSCs support cancer growth by differentiating into other pro-tumorigenic stromal components, enhancing the EMT, augmenting cancer cell survival, promotion cancer metastasis, endorsing angiogenesis, and suppressing the immune response	46–47
Pericytes	Pericytes are mural cells located between the endothelial cells of capillaries and the basement membrane, playing a crucial role in maintaining vascular function and blood flow. Their secretome, in addition to pro-inflammatory cytokines, angiogenic growth factors, and ECM, has strong impact on the formation, stabilization, and remodeling of vasculature. Their capacity for differentiation further contributes to vascular remodeling in different manners. Pericytes have several interactions with different components of the TME, such as composing the pre-metastatic niche, endorsing cancer cells growth, enhancing drug resistance through paracrine activity, and activating M2 macrophage polarization	48–49
Immune cells		
TAMs	Macrophages are myeloid lineage cells of the innate immune system arising from bone marrow-derived monocytic progenitor cells that differentiate into tissue macrophages, bone resorbing osteoclasts, and antigen-presenting dendritic cells. Macrophages are critical to maintain tissue homeostasis and protection against infectious agents through phagocytosis, cell engulfment, and clearance of cellular debris. In cancer cells these functions are frequently inhibited and the TAM population consists of tissue-resident macrophages as well as monocyte-derived cells, which are recruited from the circulation to the TME. TAMs are present in high numbers in the TME and exert an immuno-modulatory effect by secreting diverse factors such as cytokines and chemokines	50–52
T-Cells	T-cells play a central role in the adaptive immune response and present a T-cell receptor (TCR) on their cell surface. T-cells produce cytokines to regulate other types of immune cells. Their immune-mediated cell death is exerted by two major subtypes: CD4 + helper T-cells, which function by activating memory B cells and cytotoxic T cells, resulting in a larger immune response; and CD8 + killer T cells, which are cytotoxic and therefore are able to directly kill cancer cells. Other types of T-cells include regulatory T-cells (Tregs), which provide the critical mechanism of tolerance whereby immune cells are able to distinguish invading cells from "self"; and γδ T-cells, which regulate immunosuppressive functions of IELs and also play roles in development of tolerance	53–56
B-Cells	B-cells function in the humoral immunity component of the adaptive immune system and secrete antibody molecules. Antigen-activated memory B-cells proliferates and differentiates into an antibody-secreting effector cell called plasma cell or plasmablast. B-cells present antigens and secrete cytokines. B-cells maturation occur in the bone marrow. There is increasing evidence that tumour-infiltrating B-cells and plasma cells, jointly referred to as tumour-infiltrating B-lymphocytes (TIL-Bs), play a critical synergistic role in cancer control. TIL-Bs endorse anticancer immunity through their antigen presentation to T-cells, and their role in assembling and perpetuating immunologically "hot" TMEs involving T-cells, NK cells, and myeloid cells	57–58
NK Cells	NK cells, which are defined as CD3- CD56 + cells in humans, are cytotoxic lymphocyte belong to the innate immune system and protect the host by killing stressed, infected, or transformed cells. NK cells orchestrate anticancer immune responses via cellular cross-talk. NK cells are a plastic and heterogenous population allowing them to gain diverse phenotypes dependent on the signaling cues or tissue context to which they are exposed. Differently from T-cells, NK cells require no tumour-specific recognition and are not limited by MHC inhibition. The widespread anticancer effects and relative therapeutic safety of NK cells, which directly detect and destroy cancer cells, make them promising candidates for cancer immunotherapy	59–60
MDSCs	MDSCs are heterogeneous activated immature cells from the myeloid lineage and are an important component of immunosuppressive networks. MDSCs can interact with T-cells, NK cells, macrophages, and DCs to regulate their functions. MDSCs potently inhibit T-cell activity contributing to the immune escape of cancer. Immature MDSCs with remarkable immunosuppressive activity accumulate during tumor development and endorse tumor progression through supporting cell survival, invasion, metastases and angiogenesis. High levels of MDSC in the TME correlate with lower survival of patients with solid tumors. Targeting MDSCs may be a promising strategy for immunotherapy, modifying the immunosuppressive microenvironment and augmenting the efficiency of tumor immunotherapy	61–62

Table 1 (continued)

Major components of the tumor microenvironment		
TME component	**Description**	**R**
TANs	Neutrophils are myeloid derived white circulating cells in blood and are primarily involved in the human innate immunity against pathogens. TANs promote cancer progression and metastasis through communication with other immune cells, multiple growth factors, inflammatory factors, and chemokines, which together establish an immunosuppressive TME. The function of TANs in tumor has been the subject of contradicting reports pointing toward a dual role played by them in cancer progression. Indeed, upon cytokine stimulation, TANs acquire the potentiality to polarize to antitumor (N1) or pro-tumor (N2) phenotype: N1 TANs are characterized by high levels of an TNFα, ICAM-1, CCL3, and low levels of Arginase axis, whereas N2 TANs are defined by upregulation of chemokines CCL2-CCL4, CCL8, CCL12, CCL17, CXCL1, CXCL2, CXCL8 and CXCL16	63–64
DCs	DCs orchestrate anticancer immune responses and are impaired in tumor patients. DCs continuously scan and protect the environment for danger signals in an immature state. DCs become activated, mature, and trigger anticancer immune responses in presence of tumor antigens and danger signals. Thus, DCs possess the unique capacity to act as messengers between the innate and the adaptive immune systems by cross-presenting antigens and priming T-cells. DCs become regressed into an immature state, compromising their ability to activate T-cells, resulting in T-cell anergy, Treg recruitment, and thus fostering cancer tolerance, in an immunosuppressive tumor environment. Dysfunctional DCs are implicated in immune evasion, cancer growth, metastasis initiation, and cancer treatment resistance	65–66
ECM		
ECM	The ECM is one of the main components of cancer exerting important functions such as modulating the microenvironment, providing mechanical support, and serving as a source of signaling molecules. The quantity of ECM components are primary factors determining tissue stiffness. During carcinogenesis, the interplay between tumor cells and the TME frequently leads to the stiffness of the ECM, resulting in aberrant mechanotransduction and further malignant transformation. In cancer, several components of the ECM are subject to alterations which are mainly due to increased or reduced quantity of the ECM components, as well as changes in the function of ECM molecules. These alterations can be induced either indirectly by TME cells with CAFs being of particular interest in this regard, or directly by the cancer cells	67–68
Secreted Molecules		
ECVs	ECVs are secreted by all types of cells, are protected by a lipid bilayer, and contain proteins, lipids, and/or RNAs. ECVs play a critical role in intercellular communications. ECVs can induce angiogenesis and ECM remodeling, impact on tumor cell proliferation, establish pre-metastatic niches, endorse cancer metastasis, and inhibit immune response. ECVs can contribute to the crosstalk among tumor, immune, stromal, and endothelial cells to provide TME diversity. ECV components can be locally delivered to the TME and/or transferred to distant sites to direct cancer behaviour. Thus, ECVs as carriers possess the important capacity to shuttle regulatory molecules between tumor cells and multiple stromal cells, producing significant phenotypic alterations in the TME	69–70
GFs	GFs act as cellular signaling factors to regulate numerous processes such as cell growth, function, differentiation, and metabolism. GFs play a key role in regulating important processes in healthy cells, and affect tumor growth and progression in cancer cells. The primary communication between tumor cells and their microenvironment is through GFs and receptors for these molecules. A growth factor binds to its cell-surface receptor and initiates intracellular signal cascades that results in the modulation of gene expression. Both epithelial and mesenchymal cells produce growth factor into the microenvironment. Hence, abnormal cellular responses to GFs are underly malignant transformation. The most common GFs in the TME are EGFs, PDGFs, IGFs, FGFs, VEGFs, and TGF-β	71–72
Hormones	Hormones act as cellular signaling factors to regulate several processes such as cell growth, function, differentiation, function, and metabolism in healthy cells. Hormones exert their functions by binding to specific receptors on target cells to induce a downstream signal transduction pathway that typically activates gene transcription, leading to increased expression of target proteins, which can enhance or suppress the aforementioned processes. Hormones also mediate the interplay between tumor cells, their interaction with the ECM, and other cells of surrounding tissues. These complex interactions remarkably affect tumor growth, tumor progression, and angiogenesis in cancer cells. Hormone-induced modulation affects several cell types within the TME, including CAFs and TILs, which interplay with cancer cells	71, 73
Cytokines	Cytokines are small proteins important in cell signaling. Cytokines are produced by a broad range of cells and include chemokines, interferons, interleukins, lymphokines, and tumour necrosis factors. Cytokines are responsible for the pleiotropic actions in tumor such as growth, EMT, angiogenesis, leukocyte infiltration, and therapy resistance. The TME directly affects tumor progression and invasion by synthesizing different cytokines. Several pro-inflammatory cytokines, including IFN-γ, TNFα, TGF-β, and ILs contribute to the initiation, progression, and metastasis in cancer. Cytokines present in the TME can have a dual role, since they can show both a pro-inflammatory and anti-inflammatory potential, driving infiltration and inflammation, and also endorsing evasion of immune system and pro-tumoral effects	74, 32
Chemokines	Chemokines are a family of small cytokines or signaling proteins secreted by cells that activate directional movement of leukocytes and other cells. Chemokines are important for biological processes such as morphogenesis, wound healing and cancers. Chemokines are responsible for the pleiotropic actions in tumor such as growth, EMT, angiogenesis, leukocyte infiltration, and therapy resistance. Chronic inflammation is also an instructive process of tumor progression, where chemokines are spatio-temporally secreted by cancerous cells and leukocyte subtypes that trigger cell trafficking into the TME. Chemokines present in the TME can have a dual role, since they can display both a pro-inflammatory and anti-inflammatory potential, driving infiltration and inflammation, and also supporting evasion of immune system and pro-tumoral effects	74–75

TME: Tumor microenvironment. R: References. CAFs: Cancer-associated fibroblasts; ECM: Extracellular matrix; MSCs: Mesenchymal stromal cells; TAMs: Tumor-associated macrophages; NK cells: Natural killer cells; MDSCs: Myeloid-derived suppressor cells; TANs: Tumor associated neutrophils; DCs: Dendritic cells; ECVs: Extracellular vesicles; GFs: Growth factors; TIMCs: Tumor-infiltrating myeloid cells; TILs: Tumor-infiltrating lymphocytes

Moreover, TGF-β suppresses the expression of PHD2, a negative regulator of HIF-1α, thereby impairing the degradation of HIF-1α and increasing its stability to form a positive feedback loop [100]. Furthermore, HIF-1α synergizes with the Notch coactivator MAML1 to stimulate the expression of Notch target genes, in turn regulating the expression of EMT-TFs Slug and Snail to augment the migration and invasion of breast cancer cells [101]. In addition, HIF-1α can also promote EMT by regulating hypoxia-responsive non-coding RNAs (HRNs) [102]. One example is the lncRNA-UCA1 that is contained in exosomes derived from hypoxic bladder cancer cells, endorsing proliferation, migration and invasion of tumor cells through EMT induction [103]. Finally, overexpression of HIF-1α under hypoxic condition also induces upregulation of miR-210, leading to an increased expression of EMT mesenchymal markers and enhanced migration and invasive capabilities of pancreatic cancer cells [104]. The referenced studies reveal the multi-faceted direct and indirect roles of a hypoxic TME that can play in triggering EMT in cancer cells.

CAF-induced activation of EMT

The EMT can also be activated by CAFs, the most abundant non-tumor cell type residing in the tumor stroma and an important component of the TME [105, 106]. While fibroblasts in normal tissues are generally quiescent and can be activated to facilitate tissue repair and wound healing, CAFs are metabolically active and enhance tumor development by promoting cell proliferation, angiogenesis, ECM remodeling and immunosuppression [107, 108]. Accordingly, CAFs have been related to ECM remodeling and deposition, reciprocal nutrient exchange, molecular interactions, and signaling with adjacent cells in the TME [109, 110]. Numerous studies have also provided evidence that CAFs can induce EMT, mainly through their secretome and paracrine signaling effects [111–115].

Soluble CAF-derived factors

The TGF-β is one of the most widely studied cytokines released by CAFs with EMT-inducing capabilities [114, 116]. Breast cancer cells co-cultured with CAF-conditioned medium have shown enhanced migration and invasion, reduced E-cadherin expression, and enhanced EMT-TFs, vimentin, fibronectin, matrix metalloproteinase 2 (MMP-2), and matrix metalloproteinase 9 (MMP-9) expression [117]. This phenotype can be reversed using a TGF-β-neutralizing antibody, highlighting the critical role of TGF-β in CAF-induced EMT. TGF-β1 secretion by CAFs stimulates the expression of the lncRNA HOTAIR in breast cancer cells, promoting EMT and metastasis [118]. Similarly, Wang et al. (2019) have reported that CAF-induced EMT can be reversed using a TGF-β receptor kinase I inhibitor in colon cancer cells [119]. TGF-β in CAF-conditioned media induces EMT in urinary bladder cancer cells through the regulation of ZEB2, both at the transcriptional level and post-transcriptional level, by upregulating the expression of the lncRNA ZEB2NAT, a natural antisense transcript of *ZEB2* inducing the translation of the ZEB2 protein [120]. Notably, TGF-β signaling determines regulation of EMT-TFs through SMAD-dependent and SMAD-independent pathways [82]. The CAF-secreted factor pro-inflammatory cytokine interleukin 6 (IL-6), which induces JAK2/STAT3 pathway activation-mediated EMT in lung [121], hepatocellular [122], and bladder cancer [123] cells, can also enhance TGF-β signaling and TGF-β-induced EMT by increasing SMAD2 phosphorylation and membrane localization of TGF-β type I receptor [124]. Thus, the cross-talk between IL-6 and TGF-β appears to form a vicious cycle that augments malignant features such as EMT, invasion, metastasis and chemoresistance [125]. Though TGF-β is a notorious major CAF-released cytokine with high EMT-inducing capability, its therapeutic blocking in the clinic still remains a challenge due to its dual role in cancer, acting as early-stage tumor suppressor and late-stage tumor promoter [126–129]. Moreover, CAFs also induce EMT through the secretion of other growth factors. For instance, higher levels of EGF, hepatocyte growth factor (HGF), and fibroblast growth factor 2 (FGF-2) in CAF-derived conditioned medium, which induces EMT in endometrial cancer cells and lung metastasis in vivo [130]. The CAF-derived HGF induces interleukin 6 receptor (IL-6R) expression and thus enhances IL-6 signaling in gastric cancer cells, while CAF-derived IL-6 increases the expression of the HGF receptor c-Met in gastric cancer cells, demonstrating the complicated crosstalk and signaling loops between CAFs and tumor cells [131]. Besides, the CAF-induced fibroblast growth factor 1 (FGF-1) upregulates Snail and MMP-3 expression while activating the MEK/ERK pathway to induce EMT in ovarian cancer cells [132]. CAF-secreted periostin (*POSTN*) promotes cancer progression and drug resistance in non-small cell lung cancer (NSCLC) by enhancing cell proliferation, migration, and EMT via ERK pathway activation [133]. In pancreatic cancer, CAF-mediated EMT regulation involves the hedgehog signaling pathway, and inhibiting the hedgehog signaling in these CAFs reverses EMT and reduce the migratory and invasive capacities of cancer cells [134].

CAFs in ECM remodeling

Under normal physiological conditions, the activated fibroblasts are responsible for producing a number of ECM components and ECM-remodeling enzymes to

maintain ECM homeostasis and respond to tissue injury as part of the wound healing process [135–138]. Importantly, CAFs are directly involved in synthesizing ECM components [139], including collagens, fibronectin and proteoglycans, thereby contributing to increased matrix stiffening [111]. Interestingly, the matrix stiffening promotes EMT through various mechano-transduction pathways that respond to environmental signals, such as the Hippo pathway and ROCK signaling [140–143]. CAFs also mediate ECM remodeling by producing the enzymes lysyl oxidases (LOX), which induces collagen cross-linking resulting in matrix stiffening and cancer progression [144], as well as matrix metalloproteinases (MMPs), which degrade the ECM and facilitate cancer cell invasion [145]. Thus, CAFs respond to ECM stiffness in a LOX/MMP-dependent manner [146], to further fine-tuning the interactions between CAFs and the ECM [135]. Investigations of the interplay between CAFs and the immune microenvironment has revealing how CAFs participate in ECM remodeling and influence the effectiveness of immunotherapy [105]. Studies have shown that EMT is associated with epigenetic alterations in genes involved in ECM remodeling, including *ADAM19*, a gene coding for a protein involved in ECM degradation [147]. Recent advancements in biomimetic culture systems that utilize 3D manufacturing and novel material technologies that mimic the mechanical properties of the ECM are providing more physiologically relevant models for studying cancer cell behavior and EMT [148].

CAFs are a heterogeneous population within the TME

The heterogeneity of CAFs can be linked to the origin of the precursor cells, as well as their phenotypic and functional diversity. The main subsets of CAFs are: *1)* myofibroblast-like CAFs (myCAFs), marked by SMA^{high}, $IL6^{low}$, $Ly6c^{neg}$, and $MHCII^{-}$, *2)* inflammatory CAFs (iCAFs), marked via SMA^{low}, IL^{high}, $Ly6c^{+}$, and $MHCII^{-}$, and *3)* antigen-presenting CAFs (apCAFs), uniquely marked by $MHCII^{+}$, which have been mainly studied in pancreatic cancer. Indeed, myCAFs and iCAFs were first identified via PDAC organoids and murine stellate cells [149] followed by validation in pancreatic cancer patients [150]. All three populations exhibit distinct biological features that in turn contribute differently to cancer progression. *1)* myCAFs present myofibroblastic features and are activated by direct contact with neoplastic cells, which ultimately places them adjacent to tumor cells [149]. They tend to have both a pro- and anti-tumor role depending on the stage of tumor and the other factors within the TME [151]. Specifically, they can contribute to ECM remodeling. *2)* iCAFs are induced by secreted factors from cancer cells i.e. interleukin-1α (IL-1α) and tumor necrosis factor alpha (TNF-α). They are generally considered tumor-promoting via inflammatory secretion of IL6, interleukin 11 (IL-11), leukaemia inhibitory factor (LIF), and several chemokines [152], which contributes to proliferation, metastases, and chemoresistance of tumor cells [153]. Finally, *3)* apCAFs can directly educate T-cells via major histocompatibility complex class II (MHC-II). Specifically, they support antigen presentation and induction of regulatory T-cells (Tregs), making them a contributor to the immunosuppressive axis of solid tumors [154, 155]. However, this may be solid tumor dependent as new studies in lung tumors suggest that apCAFs help direct anti-tumor T-cell immunity [156]. Of note, the plasticity of these populations is varying within different solid tumors, and more focused single cell and spatial transcriptomic and biologic studies will generate a more comprehensive roadmap of intratumoral CAFs. One example of focused transcriptomic differences of these three subsets of CAFs has been demonstrated in robust single cell analysis of pancreatic cancer [157].

ECM-induced activation of EMT

The ECM is a complex network of hydrated macromolecular proteins, proteoglycans, glycoproteins, elastin, fibronectin, and sugars that not only provide structural support, but also regulate various cell functions, including cell adhesion, migration and differentiation [158, 159]. Consequently, the interactions between the ECM components and tumor cells play a crucial role in cancer progression [160] and contribute to modifying numerous cancer cell functions, including EMT activation [161, 162].

Matrix stiffness

Solid tumors in multiple cancer types, including breast, liver, pancreatic, and lung cancer, have been shown to be stiffer than normal or adjacent tissues [163]. The main causes for this are increased matrix deposition, contraction, and cross-linking, which can lead to stimulation of intracellular signaling pathways that promote cancer cell survival and tumor growth [142, 163]. Increased secretion of stiffness-promoting matrix components such as collagen and fibronectin by myofibroblast-like CAFs and cancer cells themselves have been reported, while overexpression of the LOX family proteins by cancer cells can also lead to increased collagen cross-linking and further stiffening of tumor tissue [163]. In particular, the YAP and TAZ transcription factors are known to be regulated by matrix stiffness, as well as by changes in the actin cytoskeleton and cell shape, which leads to an increase in YAP nuclear localization and activity [142–165]. Aberrant activation of YAP/TAZ induces EMT in triple-negative breast cancer (TNBC) and it can be inhibited with luteolin,

which triggers degradation of the YAP/TAZ proteins [166]. By culturing pancreatic cancer cell lines on polyacrylamide gels of varying stiffness, Rice et al. (2017) demonstrated that stiffer substrata stimulate increased vimentin expression, nuclear localization of β-catenin and YAP/TAZ, and cell shape changes associated with a mesenchymal-phenotype [142]. Moreover, substratum stiffness was also found to induce chemoresistance of pancreatic cells to paclitaxel but not to gemcitabine. The EMT-inducing TFs ZEB1 and Snail/Slug have been shown to bind directly to YAP or form complexes with YAP/TAZ, respectively, regulating the transcription of downstream target genes [167, 168]. It has also been reported that high matrix stiffness promotes the nuclear translocation of TWIST1 by releasing it from its cytoplasmic binding partner, G3BP2, thereby driving EMT and promoting tumor invasion in breast cancer cells [141]. Another interesting mechanism by which matrix stiffness can impact EMT is by modulating a TGF-β1 response. Leight et al. (2012) found that decreasing the rigidity of polyacrylamide gels promotes apoptosis in normal mammary gland cells and kidney epithelial cells in response to TGF-β1 by inhibiting phosphatidylinositol 3-kinase (PI3K)/AKT activity. In contrast. increased rigidity led to increased expression of mesenchymal markers and EMT-TFs [169]. Another study similarly found that in TGF-β1-treated mammary epithelial cells, stiff matrices promote EMT while soft matrices promote apoptosis. This phenotypic switch is mediated by integrin-linked kinase (ILK) [168, 170]. Importantly, matrix stiffness not only promotes EMT, but also regulates other aspects of cancer, including initiation, proliferation, migration, stemness, and drug resistance [163]. However, these areas will not be discussed in detail as they are beyond the scope of this review. One important consideration for studies investigating matrix stiffness is that the observed effects may vary depending on whether a 2-dimensional (2D) or 3-dimensional (3D) culture model is used. For example, while multiple studies have demonstrated that cells grown on a stiff 2D matrix migrate more actively than those on a soft 2D matrix [171–173], 3D matrices with higher stiffness increase fibronectin deposition around tumor spheroids and restrain tumor cell invasion [174]. Additionally, one study showed that a stiff 2D matrix induces resistance to sorafenib and lapatinib in breast cancer cells, while this drug resistance is reduced in a stiff 3D matrix [175, 163]. These findings highlight the importance of using culture models that better mimic the in vivo conditions of tumor tissues. Such models are crucial for a complete understanding of the roles of the ECM in cancer progression [164].

ECM components and their contributions to EMT

Collagen Collagens are one of the most abundant structural proteins in the ECM and their increased deposition is the most common ECM alteration in cancer [176]. While the increased density and enzymatic cross-linking of collagens can promote EMT via matrix stiffening, EMT can also be induced by collagen through other mechanisms. Culturing pancreatic cancer cell lines on collagen type I- and type-III, but not on fibronectin or collagen type IV, led to a reduction in E-cadherin expression, decreased cell–cell adhesion, and increased proliferation and migration of cells in a Src-kinase-dependent manner [177]. Collagen I was also shown to disrupt the E-cadherin adhesion complex in pancreatic cancer cells by activating focal adhesion kinase (FAK) and enhancing β-catenin phosphorylation [178]. Shintani et al. (2008) demonstrated that collagen I promotes EMT in NSCLC cell lines by activating autocrine TGF-β3 signaling. Indeed, in highly fibrotic cancers like NSCLC, ECM molecules such as collagen triggered signals that endorse EMT. Collagen I-induced EMT in NSCLC cell lines was prevented by TGF-β3 signaling. Interestingly, collagen I-mediated EMT was impeded by PI3K and ERK inhibitors, which promoted transcription of TGF-β3 mRNA in these cells, suggesting that collagen I determined EMT in NSCLC cells by inducing autocrine TGF-β3 signaling [179] (Table 2). Similarly, *PI3KCA* was found to be a mediator of collagen I-induced down-regulation of E-cadherin in ovarian and prostate cancer cell lines [180]. In another study, the interaction of collagen I fibrils with α2β1 integrin caused ILK-induced phosphorylation of IκB, leading to subsequent release and nuclear translocation of active NF-κB, which in turn increased the expression of EMT-promoting Snail and LEF-1 transcription factors. ILK also determined inhibitory phosphorylation of GSK-3β, a kinase that hinders functional activation of both Snail and LEF-1. These transcription factors altered the expression of epithelial and mesenchymal markers to induce EMT, and promoted cell migration. These results indicate the mechanisms whereby collagen I triggers EMT, and serve as guidance to identify potential therapeutic targets for blocking this transition in cancer [181] (Table 2). Together, these data demonstrate that collagens, especially collagen I, are potent EMT-inducers which can promote EMT via multiple mechanisms and signaling pathways.

Laminins Laminins are a large group (at least 16 members) of secreted heterotrimeric glycoproteins that, together with collagen IV, are the key constituents of basement membranes which provide structural support to various tissues and are required for cell polarization, adhesion, and migration, especially during development [67–184]. In cancer, laminins can activate multiple sig-

Table 2 Major TME components (exosomes, CAFs, TANs, and TAMs) regulating different EMT-related signaling networks in several cancer types

Major TME components regulating different EMT-related signalings in several cancers			
Signaling network	**Cancer type**	**Remarks**	**R**
ECM			
Collagen I/PI3K/ERK/TGF-β3	NSCLC	Collagen I induces EMT in NSCLC cell lines by activating TGF-β3, which can be prevented using inhibitors of PI3K and ERK	[179]
Collagen I/α2β1 integrin/ILK/NF-κB/EMT	Pancreatic cancer, CRC	Cells grown on collagen I show ILK-induced phosphorylation of IκB, leading to increased NF-κB transcription and EMT induction	[181]
Collagen XVII/laminin-5/FAK/AKT/GSK3β/EMT	Lung cancer	Col XVII induced EMT via stabilization of laminin-5 and upregulation of Snail expression via the FAK/AKT/GSK3-β pathway	[188]
Fibronectin/Src/ERK/MAPK/EMT	Breast cancer	Fibronectin-induced EMT depends on Src kinase and ERK/MAP kinase signaling in mammary epithelial cells	[194]
Hyaluronan/CD44/LOX/Twist/EMT	Breast cancer	Extracellular hyaluronan causes nuclear translocation of CD44 which triggers LOX transcription, which in turn stimulates Twist transcription	[204]
Tenascin C/SRC/FAK/EMT	Breast cancer	TN-C addition to medium of MCF-7 breast cancer cells induces EMT-like changes associated with FAK phosphorylation by SRC	[213]
Tenascin C/PI3K/AKT/mTOR/EMT	NPC	TN-C promote nasopharyngeal cancer cell proliferation and EMT via activation of the mTOR signaling pathway	[215]
TANs			
IL-17a/JAK2/STAT3	Gastric cancer	TANs secrete IL-17a which stimulates JAK2/STAT3 axis in triggering EMT and increasing metastasis of cancer cells	[248]
TAMs			
CCL5/β-catenin/STAT3	Prostate cancer	TAMs secrete CCL5 to induce β-catenin/STAT3 axis, leading to EMT and enhanced metastasis of tumor cells	[280]
Exosomes			
miR-224-5p/AR/EMT	NSCLC	Exosomal miR-224-5p shows overexpression in NSCLC and promotes migration and invasion. miR-224-5p suppresses androgen receptor (AR) signaling to induce EMT mechanism, resulting in an increase in cancer metastasis	[359]
CAFs			
miR-92a-3p/Wnt/β-catenin/EMT	CRC	CAFs secrete exosomes to increase miR-92a-3p expression, leading to activation of Wnt signaling and subsequent induction of EMT to promote metastasis of cancer cells	[354]
miR-181d-5p/CDX2/HOXA5	Breast cancer	CAFs secrete exosomes containing miR-181d-5p which downregulates CDX2 and HOXA5, leading to subsequent EMT induction and increased metastasis of cancer cells	[360]
miR-34a-5p/AXL/EMT	Oral cancer	CAFs secrete exosomes containing miR-34a-5p to reduce AXL expression. Subsequent induction of AKT/GSK-3β/β-catenin/Snail signaling cascade stimulates EMT and promotes metastasis of cancer cells	[334]
IL-6/STAT3/LRG1	CRC	CAFs secrete IL-6 to induce STAT3 signaling, resulting in upregulation of LRG1 and increased metastasis of tumor cells through EMT induction	[361]
HMGB1/ NF-κB/EMT	NSCLC	CAFs secrete HMGB1 via autophagy to induce NF-κB signaling, leading to EMT induction and increased metastasis of cancer cells	[362]
*	Breast cancer	CAF-educated monocytes exhibit strong immune suppression and enhance the motility/invasion of cancer cells in addition to increasing the expressions of EMT-related genes	[363]

The ECM regulates EMT-related signaling networks including Collagen I/PI3K/ERK/TGF-β3 in NSCLC, Collagen I/α2β1 integrin/ILK/NF-κB/EMT in pancreatic cancer and CRC, Collagen XVII/laminin-5/FAK/AKT/GSK3β/EMT in lung cancer, Fibronectin/Src/ERK/MAPK/EMT in breast cancer, Hyaluronan/CD44/LOX/Twist/EMT in breast cancer, Tenascin C/SRC/FAK/EMT in breast cancer, and Tenascin C/PI3K/AKT/mTOR/EMT in nasopharyngeal cancer; TANs regulate EMT-related signaling networks such as IL-17a/JAK2/STAT3 in gastric cancer; TAMs regulate EMT-related signaling networks including CCL5/β-catenin/STAT3 in prostate cancer; the exosomes regulate EMT-related signaling networks such as miR-224-5p/AR/EMT in NSCLC; and CAFs regulate EMT-related signaling networks including miR-92a-3p/Wnt/β-catenin/EMT and IL-6/STAT3/LRG1 in CRC, miR-181d-5p/CDX2/HOXA5 in breast cancer, miR-34a-5p/AXL/EMT in oral cancer, and HMGB1/NF-κB/EMT in NSCLC. R: References. NSCLC: Non-Small Cell Lung Cancer; CRC: Colorectal Cancer; NPC: Nasopharyngeal cancer

nal transduction pathways by interacting with integrin and non-integrin receptors on cancer cells, promoting tumor invasion and metastasis [185]. Their roles in EMT have also been reported. For example, laminin γ2 (*LAMC2*) promoted migration and invasion of lung adenocarcinoma cells in an integrin β1- and ZEB1-depend-

ent manner. While *LAMC2* knockdown in mice attenuated metastasis, elevated *LAMC2* levels in patients with lung adenocarcinomas was associated with a significantly higher risk of recurrence or death [186]. In hepatocellular carcinoma (HCC) cells incubation with laminin-5 in media led to upregulated levels of Snail and Slug, while E-cadherin was downregulated. However, both laminin 5 and TGF-β1 were required to cooperatively stimulate the complete EMT process in "non-invasive" HCC cells. This effect was reversed by anti-α3 but not by anti-α6 integrin blocking antibody [187]. Another study, using microarray analysis to identify candidate genes responsible for EMT in spheroid/monolayer cultures of lung cancer cells, also demonstrated the EMT-inducing role of laminin 5 in lung CSCs. An increased expression of several adhesion molecules in CSCs was found. The adhesion molecule collagen XVII was required for maintenance of EMT phenotypes in lung CSCs, and stabilized laminin-5 to activate the FAK/AKT/GSK3β signaling pathway, leading to suppression of Snail ubiquitination-degradation. Accordingly, patients undergoing surgical resection for lung cancer, and displaying overexpression of both collagen XVII and laminin-5, showed the worst prognosis of all expression types. Besides, suppression of the collagen XVII/laminin-5 signaling axis decreased the EMT phenotypes of lung CSCs in vitro and reduced the potential of lung metastasis in vivo. This study suggests that targeting collagen XVII and laminin-5 may be a valuable therapeutic strategy for treating lung cancer patients [188] (Table 2). Interestingly, another study examined the effects of laminin-332 and laminin-411 on EMT in three CRC cell lines (HT-29, HCT-116, and RKO). Different effects on the expression of laminin α4 chain (*LAMA4*), *SNAI1*, and epithelial marker genes were observed for each cell line, suggesting that the contribution of laminin to EMT could also depend on the initial characteristics of the cells [184]. Lastly, the EMT itself can also affect the expression of laminins, and has been shown to induce a switch from laminin-511 to laminin-411 in oral squamous carcinoma cells. This switch could be attributed to a direct control by Snail. While cells could adhere strongly to laminin-511, adhesion to laminin-411 was minimal and could potentially be exploited by tumor cells to facilitate their invasion [189]. Given the critical roles laminins play in the EMT and cancer cell metastasis, they could be attractive targets for cancer therapeutics. One example is the antibody for laminin receptor precursor (LRP), IgG1-iS18, which significantly reduced the adhesion of various types of cancer cells (e.g. pancreatic, colorectal, melanoma) to laminins and blocked their invasion in vitro [190].

Fibronectin Fibronectin, though lower in abundance, is a large glycoprotein molecule that has diverse functions in the ECM [67]. It can interact with other ECM components and cell surface receptors including integrins, inducing profound effects on tumor cell proliferation, angiogenesis, EMT, invasion and metastasis [191–193]. In the mammary gland, the stromal ECM undergoes remarkable changes during development and in carcinogenesis. In fact, normal breast tissue is devoid of fibronectin, whereas high fibronectin levels are present in the stroma of breast cancers. During EMT, epithelial cell adhesion switches from cell–cell contacts to cell-ECM interactions, augmenting the probability that fibronectin can promote this transition. In a study, MCF-10A mammary epithelial cells exposed to exogenous fibronectin underwent EMT, and upregulated various EMT markers (e.g. fibronectin, Snail, N-cadherin, vimentin, MMP2) via the activation of the ERK/mitogen-activated protein kinase (MAPK) signaling axis. Fibronectin initiated EMT under serum-free conditions, and this response was partially reversed by a TGF-β-neutralizing antibody, suggesting that fibronectin increases the effect of endogenous TGFβ. These results showed that cells interacting with fibronectin are primed to respond to TGFβ. The ability of fibronectin to promote EMT displayed an active role for the stromal ECM in this process, supporting the notion that the enhanced levels of fibronectin detected in breast cancers contribute to facilitating carcinogenesis [194] (Table 2). Increased levels of fibronectin, which induces upregulation of Slug and promotes lung metastasis, is also found in renal cell carcinoma (RCC) and soft tissue sarcoma (STS) [195]. Breast cancer cells grown in the presence of fibronectin can potently induce EMT and upregulate N-cadherin as well as vimentin through the activation of FAK upon fibronectin binding to integrin receptors, promoting cell migration and invasion [196]. Interestingly, several mall-molecule inhibitors which block the ATP binding site of FAK have shown promising results in inhibiting cell migration and metastasis in vivo [197]. The FAK inhibitor PF-00562271 has already completed phase 1 trial, supporting further study into FAK as a promising therapeutic target [198, 199].

Hyaluronan Hyaluronan, a linear polysaccharide, is another key component of the ECM which plays a potent role in EMT induction [200–202]. Overexpression of hyaluronan synthase-3 (HAS3) in epithelial lung cancer cells leads to an EMT phenotype, increased invasion, and greater activity of MMP-2 and MMP-9 [203]. El-Haibi et al. (2012) showed that bone marrow-derived human MSCs stimulate de novo generation of LOX from human breast carcinoma cells, which was sufficient to augment the metastasis of weakened metastatic tumor cells to the bones and lungs. LOX was found to be an important component of the CD44-Twist signaling pathway, in which

extracellular hyaluronan led to nuclear translocation of CD44 in tumor cells, thereby promoting LOX transcription by associating with its promoter. In turn, enzymatically active LOX triggered Twist transcription, which mediated the MSC-induced EMT of carcinoma cells. Though promotion of EMT in breast cancer cells was tightly related to the production of CSCs, LOX did not contribute to the capability of MSCs to endorse CSC formation in the carcinoma cell populations [204] (Table 2). Additionally, TGF-β induces EMT via HAS2 upregulation and knockdown of HAS2 inhibits TGF-β-induced EMT by 50% [205], suggesting the important role of hyaluronan production in EMT. Interestingly, 4-MU, an inhibitor of hyaluronan synthesis clinically approved to treat biliary spasms, has been reported to inhibit growth, migration and metastasis of pancreatic ductal adenocarcinoma [206, 207], as well as invasiveness of lung cancer [208] in vitro/*vivo*. While further clinical results are still required to determine its efficacy for cancer treatment, these findings suggest the potential for 4-MU as a novel therapeutic agent for cancer.

Tenascins Tenascins (TNs) are a family of large glycoproteins composed of four members, tenascin (TN)-C, -R, -X, and -W [209]. TN-C, in particular, has been reported in multiple studies to display pro-tumoral effects by promoting cell proliferation, EMT, migration, and invasiveness [210]. While TN-C is expressed at low levels in normal adult tissues, it was found to be expressed abundantly in the tumor stroma of almost all analyzed cancer types [211]. In breast cancers, TN-C expression assessed using immunohistochemistry (IHC) significantly associated with vimentin gene expression and correlated with higher tumor grade and negative estrogen receptor (ER) status [212]. TN-C contribution to the EMT was evident in experiments whereby the addition of TN-C to the medium induced the EMT phenotype in breast cancer cells. This process was mediated by the binding of tenascin to αvβ6 and αvβ1 integrins, triggering SRC-induced FAK phosphorylation, thereby leading to the loss of cell–cell adhesion and enhanced cell migration. Indeed, the EMT phenotype was related to SRC activation through phosphorylation at Y418 and phosphorylation of FAK at Y861 and Y925 of SRC substrate sites. These proteins co-localized with αv integrin-positive adhesion plaques. A neutralizing antibody against αv or a SRC kinase inhibitor blocked EMT. Thus, TN-C was able to promote EMT-like change exhibiting loss of intercellular adhesion and increased migration in breast cancer cells through SRC-mediated FAK phosphorylation [213] (Table 2). Tenascin is also a key driver of CRC invasiveness. Gene expression microarrays performed in 86 laser micro-dissected CRC tissues revealed the enrichment of genes related to EMT and TGF-β signaling in samples with high TN-C expression. Moreover, high TN-C expression significantly correlated with higher rates of recurrence in CRC patients [214]. In a recent study, TN-C gene transcription/expression was found to be high in nasopharyngeal carcinoma tissues in comparison to normal tissues. TN-C knockdown inhibited nasopharyngeal carcinoma cell proliferation, migration, and invasion. Besides, TN-C knockdown suppressed cancer growth in mice. Importantly, TN-C knockdown inhibited EMT and reduced activity of the PI3K/AKT/mTOR pathway in nasopharyngeal carcinoma cells. These results suggest that TN-C functions as an oncogene, thereby endorsing cell proliferation, promoting EMT, and increasing activity of the PI3K/AKT/mTOR signaling pathway in nasopharyngeal carcinoma cells [215] (Table 2). Finally, tenascins were also shown to associate via their FBG-like domains with small latent TGF-β complex, which promotes activation of latent TGF-β and subsequent EMT [210]. Similar to other ECM components, TN-C has multiple pro-tumorigenic roles that extend beyond EMT, including the regulation of angiogenesis, tumor immunity, and immunosuppression, making it a potential anti-cancer target [216].

Matrix metalloproteinases (MMPs)

MMPs are the primary enzymes responsible for collagen and other protein degradation in ECM [217–219]. MMPs are endopeptidases that utilize zinc and calcium ions for their enzymatic activity, with 24 MMPs found in humans [220]. The MMP-2, matrix metalloproteinase 3 (MMP-3), matrix metalloproteinase 7 (MMP-7), matrix metalloproteinase (MMP-13), matrix metalloproteinase 14 (MMP-14), and matrix metalloproteinase 26 (MMP-26) have been shown to be critical effectors of Wnt-induced EMT [221–223]. In addition, suppression of MMP-9 in highly invasive cervical carcinoma A433 cells greatly reduced the expression levels of vimentin, fibronectin, and migration capability [224]. Knocking down of MMP-9 can inhibit Snail expression, indicating the existential loop between MMP-9 and Snail expression [220]. Furthermore, MMP-3 causes an increase in reactive oxygen species (ROS), upregulating Snail expression and inducing EMT [225]. Despite the critical role of MMPs in cancer progression, development of MMP inhibitors has not been clinically successful. This is largely due to the fact that MMPs have normal system-wide functions, leading to poorly-tolerated musculoskeletal pain induced by the majority of tested anti-MMP formulations [226, 227]. Current research is focusing on developing inhibitors with higher selectivity for specific MMPs and novel delivery methods to target only the affected tissues, potentially leading to improved clinical outcomes.

Cancer cell-immune cell crosstalk promotes EMT

A complex interplay and bi-directional communication exists between immune cells and cancer cells in the TME [228–230]. EMT-TFs expressed by tumors have been reported to recruit and activate immune cells, which in turn release EMT-inducing cytokines and chemokines. Notably, Snail promotes neutrophil infiltration, upregulating chemokine (C-X-C motif) ligand 2 (CXCL2) and inducing lung cancer progression [231]. In addition, Snail endorses the recruitment of TAMs through the transactivation of chemokine (C–C motif) ligand 2 (CCL2) and chemokine (C–C motif) ligand 5 (CCL5) [232]. Twist can also enhance the recruitment of macrophages through the modulation of the macrophage chemoattractant CCL2 [233]. Induction of these immune cells through the EMT activation can promote EMT maintenance and facilitate tumor dissemination [234].

Neutrophils and macrophages

Neutrophils and macrophages are immune cells of myelogenous origin that can drive tumor progression and metastasis by secreting of EMT-inducing cytokines [235, 236]. TANs comprise a variable proportion of immune cells in the TME across malignancies, and the interest in their immunomodulatory role and potential for targeting has prominently increased in recent years [237]. Similar to other immune cells, TANs can be classified based on their functional state or polarization into two main subtypes: *1)* N1 or anti-tumor TANs, and *2)* N2 or immunosuppressive TANs. However, there is limited information on how to unambiguously identify these neutrophil subpopulations using specific markers in human malignancies. TANs are expected to enter tumors from peripheral blood, have a short half-life of ~ 7–10 h in circulation, exert immunosuppressive functions, and notoriously associate with worse prognosis in cancer patients [238–240]. The mechanisms mediating the immunosuppressive role of TANs are not fully elucidated and include the local production of neutrophil extracellular traps (NETs) after IL-8 stimulation [241–243] and release of immunosuppressive signals such as TGF-β, Arginase-1 and ROS [244–247]. EMT contributes to gastric cancer progression and recurrence following therapy. Li et al. (2019) showed that TANs produce interleukin-17A (IL-17a), which in turn activated the JAK2/STAT3 pathway to promote EMT and enhance the migration and invasion of gastric cancer cells. Accordingly, the addition of an IL-17a-neutralizing antibody was able to reverse neutrophil-mediated activation of STAT3, leading to reduced cell migration and invasion. TANs were largely present in tissues of gastric patients and were enriched especially at the invasion margin. TAN levels at the invasion margin were an independent predictor of poor disease-free survival and disease-specific survival. This study suggests that IL-17a-targeted therapy may be used in future treatments of patients with gastric cancer [248] (Table 2). Moreover, TANs induce EMT in breast cancer through the secretion of TIMP-1 cytokine. TIMP-1 production is reinforced via a sustained CD90-mediated contact of breast cancer cells with neutrophils. This demonstrates the complex crosstalk and the existence of multiple paracrine loops between tumor cells and immune cells, resulting in profound effects on tumor progression [249]. Possible strategies to target TANs in cancer patients include the blockade of the interleukin 8 (IL-8)/C-X-C motif chemokine receptor 1 (CXCR1)/C-X-C motif chemokine receptor 2 (CXCR2) axis, and the targeting of TGF-β, vascular endothelial growth factor (VEGF) and/or granulocyte–macrophage colony-stimulating factor (GM-CSF) signaling pathways using monoclonal antibodies or small molecule inhibitors [250–253]. The majority of tissues comprise resident macrophages that regulate tissue homeostasis and immune defense. Upon cancer formation, the number of macrophages expands through in situ proliferation, and concurrently, monocyte-derived macrophages (MDMs) are recruited into the TME [254]. This results in a distinct spectrum of TAMs, which is further modified through the myriads of cellular interactions within the TME, giving rise to TAMs exhibiting functional heterogeneity among various cancer types [255–257]. Macrophages are an extremely plastic cell type within the TME, since they can differentiate according to signals present in their individual microenvironments (e.g. cytokines) [258–260]. In addition, macrophages can be divided into at least two subtypes based on their extreme polarization status: *1)* pro-inflammatory phenotype-featuring classically activated (M1-like) macrophages, or *2)* anti-inflammatory phenotype-featuring alternatively activated (M2-like) macrophages [261]. This intrinsic macrophage plasticity is often influenced by the disease stage and/or the affected tissue, which regulate whether TAMs suppress or endorse carcinogenesis [262]. In general the anti-inflammatory M2-like TAMs perform functions and dampen anti-tumor activity, secrete tumor promoting growth factors and facilitate metastasis [263]. However, mounting evidence from single cell omics studies illustrated molecular heterogeneity of TAMs with at least seven distinct populations preserved across over 25 human cancer types [264]. Functionally, TAMs promote cancer initiation by inflammation [265] and enhance consecutive cancer progression by escaping immune surveillance, augmenting immunosuppression, and increasing tumor cell invasion [266–269]. Moreover, TAM activity may contribute to tumor relapse following treatment with conventional therapeutic modalities [270].

For instance, augmented migration of MDMs to the persisting cancer is guided by the increased generation of colony-stimulating factor 1 (CSF1), a TAM-secreted cytokine [271]. MDMs can also promote bone metastasis outgrowth of breast cancer in an interleukin-4 receptor (IL4R) dependent manner [272]. The presence of TAMs has been increasingly associated with the upregulation of a mesenchymal marker vimentin, a reciprocal downregulation of E-cadherin, alongside enhanced cell migration and invasion in numerous types of cancer [273–276], which could be driven by TAM-associated secretion of IL-6 [277, 278] and EGF [274]. In line with this, Kuwada et al. (2018) demonstrated that TAM-induced EMT, can in turn, confer resistance to gemcitabine chemotherapy in a pancreatic cancer model [279]. Huang et al. (2020) showed that TAM-secreted CCL5 can endorse the migration, invasion, and EMT of prostate cancer cells, as well as the self-renewal of prostate cancer stem cells (PCSCs) in vitro. *STAT3* was validated as the most significant response gene in prostate cancer cells following CCL5 treatment. CCL5 was further revealed to promote PCSC self-renewal and prostate cancer metastasis via activation of the β-catenin/STAT3 signaling. Interestingly, knockdown of CCL5 in TAMs not only suppressed prostate cancer xenografts growth and bone metastasis, but also inhibited the self-renewal and tumorigenicity of PCSCs in vivo. Finally, high CCL5 expression was significantly associated with high Gleason grade, poor prognosis, metastasis, and enhanced PCSC activity in prostate cancer patients. These results suggest that TAMs/CCL5 can promote PCSC self-renewal and prostate cancer metastasis via activating β-catenin/STAT3 signaling, and provide a novel rationale for the development of TAMs/CCL5 as a potential molecular target for PCSC elimination [280] (Table 2).

Myeloid-derived suppressor cells (MDSCs)

MDSCs, which are typically undetectable under normal physiological conditions, derive from the same differentiation lineage as neutrophils and macrophages but arise in cases of chronic pathological conditions induced by prolonged stress signals [281]. MDSCs possess immunosuppressive properties, mainly through the production of Arginase 1 (*ARG1*) and inducible nitric oxide synthase (iNOS) enzymes, and are highly expressed in cancers [282]. MDSCs stimulate breast cancer cell EMT in vitro through IL-6 induction [283] and in melanoma cells through activation of the TGF-β-, EGF and HGF signaling pathways [284]. MDSCs also produce large quantities of MMPs, especially MMP-9, further facilitating tumor migration and metastasis [285]. In addition, the AMPK inhibitor-induced suppression of MDSCs decreased cell migration in vitro [286], while the depletion of MDSCs significantly reduced the number of lung metastasis in vivo [287]. Interestingly, treatment strategies targeting MDSCs have exhibited promising outcomes to efficiently diminish cancer spread in numerous preclinical studies and clinical trials when administrated singly or in combination with other anticancer drugs [288–292].

Dendritic cells (DCs)

DCs are specialized innate immune cells involved primarily in antigen presentation and T-cell modulation. DCs are considered as professional antigen-presenting cells (APCs) and they comprise a heterogeneous population of cells classified on their maturation state and functional features into multiple subsets including conventional dendritic cells (cDC1 and cDC2), plasmacytoid dendritic cells (pDCs), Langerhans cells and inflammatory DCs [293–295]. Though some DCs can display a tolerogenic effect, their main functions in cancer are immunostimulatory, and include the internalization of antigens that are released in the TME or presented to naïve CD4/CD8$^+$ T-cells via major histocompatibility complex class I (MHC-I)/MHC-II molecules (e.g. T-cell priming), the production of proinflammatory cytokines such as IL-6, interleukin 12 (IL-12), interleukin 15 (IL-15), and the interaction with other innate immune cells such NK cells, macrophages, or mastocytes. Several studies indicate that major DC subpopulations are reduced in cancer, which can compromise the innate-adaptive immune interactions and mediate tumor tolerance. Specifically, cross-presenting BATF3/XCR1-expressing cDC1 have been shown to be critical to mount productive anti-tumor responses in preclinical models and mediate tumor rejection after treatment with immune checkpoint inhibitors (ICIs). Furthermore, several studies have reported a positive correlation between intra-tumoral cDC1 density and patient outcomes across different solid tumor types [296–299]. The role of other cDC subsets in tumorigenesis, cancer progression, and anti-cancer treatments of human tumors remains less understood.

T-cells and natural killer (NK) cells

T-cells Contrary to the aforesaid myelogenous immune cells, T-cells are lymphocytes primarily implicated in immune-surveillance and antitumor activities [300–303]. T-cells are critical for targeting cancer cells by recognizing antigens presented by major histocompatibility complex (MHC) molecules on cancer or antigen-presenting cells. Identifying and targeting cancer-specific or overexpressed self-antigens is essential for redirecting T-cells against tumors [304, 305]. This involves identifying mutated or overexpressed self-proteins in cancer cells, which guide T-cell receptors in recognizing and attacking these cells. Hence, antigen-directed cytotoxicity mediated by T-lym-

phocytes has become a central focal point in the battle against cancer utilizing the immune system. Both $CD8^+$ and $CD4^+$ T-cells are crucial in combating tumors. $CD4^+$ cells prime $CD8^+$ cytotoxic lymphocytes for full activation, which is essential for effective immune responses against tumors [306]. T-cells scan for MHC-peptide complexes to activate tumor-specific responses, and studies show that reducing $CD4^+$ cells impair tumor rejection. However, $CD4^+$ Tregs can suppress immune responses and might hinder anti-tumor immunity, representing a challenge for effective cancer immunotherapy. Tregs suppress immune functions through various mechanisms, including production of immunosuppressive cytokines, generation of immunosuppressive metabolites cytotoxic T-lymphocyte-associated protein-4 (CTLA-4)-mediated suppression of APC function, as well as consumption of interleukin 2 (IL-2) [307].

Natural killer (NK) cells NK cells are a vital component of the innate immune system, capable of identifying and eliminating malignant cells [308–311]. They recognize aberrant or stressed surface molecules commonly found on cancerous cells, while their functional activity is inhibited by human leukocyte antigen (HLA) molecules displayed on these cells. Upon identifying a target, NK cells release cytotoxic granules containing perforin and granzymes, which induce apoptosis in the target cells. In addition, NK cells engage in antibody-dependent cell-mediated cytotoxicity (ADCC) by binding to cancer cells coated with antibodies, thereby facilitating their destruction [312]. Furthermore, NK cells secrete cytokines such as interferon-gamma (IFN-γ) and TNF-α, which play pivotal roles in bolstering anti-tumor immune responses by activating other immune cells and impeding cancer cell proliferation. The versatility of NK cells for cancer immunotherapy is demonstrated by their ability to recognize stressed cells broadly, regardless of neoantigen presentation, and their enhanced activity against tumors that have lost class I HLA expression due to acquired resistance mechanisms. This positions them as promising candidates for cancer therapy, particularly through adoptive transfer [313, 314].

NK cells and T-cells contribute to EMT Several studies have demonstrated that both NK cells and T-cells can promote EMT [315, 316]. NK cells generate IFN-γ and TNF-α that promote EMT in hepatocellular carcinoma in vivo [317] and in melanoma cells [318]. In pancreatic cancer, co-culturing with $CD4^+CD25^-$ T-effector cells lead to a phenotypic change towards the mesenchymal phenotype, with an associated reduction of E-cadherin and upregulation of vimentin and ZEB1 [319]. Particularly, the expression of CD147 glycoprotein on T-cells is crucial for the induction of EMT [320]. Due to these aforementioned dual properties of NK and T-cells, cytokine therapy may be more effective than directly targeting these immune cells [321]. Accordingly, recent research has shown that abrogating cytokines can result in a decrease of cancer cell dissemination, despite the presence of NK cells or T-cells [317, 322]. Several groups have also reported that $CD24^+$ $CD25^+$ Tregs in the TME can promote tumor growth, EMT and activate metastasis through TGF-β signaling [323, 324]. Besides, Tregs exposure induces TGF-β-mediated EMT in surrounding melanoma cells, which in turn, enhance their migration, invasion and metastatic spread [323]. Similarly, hepatocellular carcinoma patients with poor cancer-free and overall survival (OS) rates have displayed high infiltrations of Tregs, decreased E-cadherin expression, as well as enhanced vimentin and TGF-β1 expression in cancerous tissue compared to normal liver tissue. In line with this, the addition of a TGF-β1-neutralizing antibody in Treg-conditioned media has been found to impede the migratory and invasive capacities of murine hepatoma cells [325].

Tumor- and CAF-derived exosomes contribute to EMT

Apart from the stromal and immune cells, exosomes are also critical TME players [326, 327] since they function as important signaling molecules that augment communication between cancer cells and TME [328–332]. Exosomes are small lipid-bilayer-enclosed vesicles [333] mediating the horizontal transfer of biological molecules from the donor to recipient cells by endocytosis or systemic transport to distant sites [334]. Tumor-derived exosomes have been implicated in EMT and cancer progression by carrying oncogenic materials to adjacent cells, thereby transforming cells into a pro-metastatic EMT phenotype, and thus, leading to a sustained tumor growth [335–338]. For instance, exosomes isolated from chronic myelogenous leukemia contain glycoprotein amphiregulin, triggering epidermal growth factor receptor (EGFR) in bone marrow MSCs that in turn induces the expression of Snail and its EMT-related targets [339]. Apart from tumor-derived exosomes, CAFs also produce exosomes to directly transfer Snail as an EMT-TF to lung cancer cells; and interestingly, this EMT-inducing effect can be inhibited by treating CAFs with an inhibitor of exosomal release [340]. In addition, Wnt10b delivered by fibroblast exosomes has been reported to induce EMT [341], while exosomes obtained from stromal adipocytes activate Hippo pathway mediated-EMT in breast cancer cells [342].

Exosomal RNAs

Numerous studies have shown that exosomal microRNA (miRNA or miR) play a key role in promoting EMT in

various cancer types [343–332]. Indeed, the hypoxic glioblastoma cell-secreted exosomal miR-301a and the lung cancer cell-secreted exosomal miR-1260b activate Wnt signaling/β-catenin [350, 351]. Moreover, high expression of the exosomal miR-665 induces HCC cell proliferation, invasion, migration and EMT through regulating the Hippo signaling pathway [352]. In CAF-derived exosomes, miRNA sequencing has identified increased levels of miR-21, miR-143, and miR-378e, promoting the EMT phenotype in breast cancer cells [353]. High levels of miR-92a-3p have also been detected in CAF-derived exosomes, which are directly transferred to CRC cells and induce EMT through activation of Wnt/β-catenin pathway [354]. Notably, cancer stem cell-like cells secrete exosomes containing the lncRNA DOCK9-AS2 that can increase metastasis of papillary thyroid carcinoma cells. This exosomal lncRNA DOCK9-AS2 promotes the expression of CTNNB1 via the miRNA-1972 sponging, resulting in Wnt/β-catenin induction and increased metastasis of cancer cells. Furthermore, the exosomal lncRNA DOCK9-AS2 is upregulated in papillary thyroid carcinoma and can be considered as a potential therapeutic target for reversing EMT and impairing metastasis [355]. Interestingly, CAFs are capable of secreting the exosomal lncRNA LINC00659 promoting metastasis of CRC cells via EMT induction. Exosomes function as cell communicators and transfer LINC00659 from CAFs to CRC cells [356]. The exosomal LINC00659 decreases miRNA-342-3p expression to upregulate ANXA2, leading to EMT-mediated metastasis [357]. In addition, the exosomal circRNAs have also been reported to induce EMT thereby endorsing metastasis. However, further studies are necessary to better understand the exact function of exosomal circRNAs in EMT modulation in tumors [358]. A recent study showed that exosome-derived miR-224-5p is upregulated in NSCLC patient tissues and cell lines, and induces cell proliferation and metastasis in NSCLC and human lung cells. In addition, androgen receptor (AR) was characterized as a direct target of miR-224-5p. Importantly, tumor xenograft assay experiments demonstrated that overexpression of miR-224-5p drive NSCLC tumor growth via the inhibition of AR and the mediation of EMT. These data suggest that miR-224-5p-enriched exosomes trigger carcinogenesis by directly targeting AR in NSCLC, which can provide novel potential therapeutics for NSCLC [359] (Table 2). Recently, novel mechanisms underlying the pro-tumorigenic effects of CAFs have been identified in several cancers. Hu et al. (2019) reported that CAFs secrete exosomes to promote metastasis and chemotherapy resistance by augmenting cell stemness and EMT in CRC. CAFs exerted their roles by directly transferring exosomes to CRC cells, resulting in increased miR-92a-3p level in CRC cells. Augmented expression of miR-92a-3p activated the Wnt/β-catenin signaling pathway and suppressed mitochondrial apoptosis by direct inhibition of MOAP1 and FBXW7, thereby contributing to cell stemness, EMT, metastasis and 5-fluorouracile (5-FU)/oxaliplatin resistance in CRC. Clinically, miR-92a-3p expression enhanced in CRC tissues and negatively correlated with the MOAP1 and FBXW7 levels in CRC specimens. Besides, high expression of exosomal miR-92a-3p in serum was significantly associated with metastasis and chemotherapy resistance in CRC patients [354] (Table 2). A recent study demonstrated that microRNA-181d-5p-containing exosomes derived from CAFs promote EMT by downregulating CDX2/HOXA5 in breast cancer. Nude mice bearing xenografted MCF-7 cells, injected with CAF-derived exosomes, were evaluated for tumor formation. HOXA5 was expressed at low level in breast cancer tissues, and its overexpression delayed MCF-7 cell proliferation, invasion, migration, and EMT, and concurrently increased apoptosis in vitro. Coculture of CAFs and MCF-7 cells led to CAF-mediated prolonged proliferation, and antagonized apoptosis of MCF-7 cells via release of exosomes. Coculture of MCF-7 cells and CAF-derived exosomes identified miR-181d-5p as a mediator of the exosomal effects on MCF-7 cells, in part via downregulation of CDX2 and HOXA5. In addition, CAF-derived exosomes containing miR-181d-5p endorsed tumor growth of nude mice bearing xenografted MCF-7 cells. These results suggest that exosomal miR-181d-5p plays an important role in CAF-mediated effects on tumor environment in breast cancer via CDX2/HOXA5 [360] (Table 2). Li et al. (2018) reported that CAFs contribute to oral cancer cell proliferation and metastasis via exosome-mediated paracrine miR-34a-5p. The expression of miR-34a-5p in CAF-derived exosomes was found to be reduced, and fibroblasts were able to transfer exosomal miR-34a-5p to oral squamous cell carcinoma cells. In experiments using xenografts, miR-34a-5p overexpression in CAFs suppressed the carcinogenesis of oral squamous cell carcinoma cells. Moreover, miR-34a-5p bound to its direct downstream target AXL to inhibit oral squamous cell carcinoma cell proliferation and metastasis. Stable ectopic expression of AXL in miR-34a-5p-overexpressing oral squamous cell carcinoma cells restored proliferation and motility abrogated by the miRNA. Furthermore, the miR-34a-5p/AXL axis stimulated oral squamous cell carcinoma progression via the AKT/GSK-3β/β-catenin pathway, which induced EMT to promote cancer cells metastasis. The miR-34a-5p/AXL axis increased nuclear translocation of β-catenin, and then triggered transcriptional upregulation of SNAIL, which in turn induced both MMP-2 and MMP-9. These data emphasize that the miR-34a-5p/AXL

axis can confer aggressiveness in oral cancer cells through the AKT/GSK-3β/β-catenin/Snail signaling cascade, and thus, may represent a therapeutic target for oral squamous cell carcinoma [334] (Table 2). A recent study showed that CRC-associated fibroblasts promote metastasis by upregulating leucine rich alpha-2-glycoprotein 1 (LRG1) through stromal IL-6/STAT3 signaling. CAF-induced LRG1 endorsed CRC migration and invasion that was concomitant with the induction of EMT. CAF-secreted IL-6 was responsible for LRG1 upregulation in CRC, which occurred through a direct transactivation by STAT3 following JAK2 activation. In clinical CRC samples, LRG1 expression was positively correlated with the CAF-specific marker α-SMA, and a higher LRG1 expression predicted unfavorable clinical outcomes, supporting the significant role of LRG1 in CRC progression. This study provided novel insights into CAF-mediated metastasis in CRC, and suggests that therapeutic targeting of the CAF-mediated IL-6-STAT3-LRG1 axis may be a valuable approach to reduce metastasis in CRC [361] (Table 2). Ren et al. (2021) reported that autophagic secretion of high mobility group box 1 B1 (HMGB1) from CAFs promotes metastatic potential of NSCLC cells via the NFκB signaling. Blockade of CAF autophagy diminished their regulation on EMT and metastasis-related genes of NSCLC cells. CAF-secreted HMGB1 mediated the effect of CAFs on lung cancer cell invasion. In particular, the autophagy suppression of CAFs revealed that release of HMGB1 was dependent on autophagy. In addition, HMGB1 was partially responsible for autophagy activation of CAFs, suggesting that CAFs remain active through an autocrine HMGB1 loop. Moreover, HMGB1 increased lung cancer cell invasion by activation of the NFκB pathway. These results clarified an oncogenic function for secretory autophagy in lung cancer-associated CAFs that endorses metastasis potential, and suggests HMGB1 as a novel therapeutic target [362] (Table 2). Another study demonstrated that CAF-educated monocytes exhibit strong immune suppression and enhance the motility/invasion of breast cancer cells in addition to increasing the expressions of EMT-related genes. Recruitment of monocytes by CAFs was mediated by monocyte chemotactic protein-1 (MCP-1) and stromal cell-derived factor-1 (SDF-1) cytokines. CAFs differentiated the recruited monocytes into M2-like macrophages, which were able of determining their immunosuppressive roles via the programmed cell death protein 1 (PD-1) axis. CAF-educated M1 macrophages exhibited enhanced expression of M2 markers, and generation of anti-inflammatory cytokine interleukin 10 (IL-10), in contrast to reduced production of pro-inflammatory cytokine IL-12, compared to control M1 macrophages. This suggested that CAFs were also capable of inducing the trans-differentiation of M1 macrophages to M2 macrophages. Interestingly, high grade of CAFs was significantly related to the number of TAMs in human breast cancer tissue samples, and with increased Ki-67 proliferation index and higher tumor volume. These data emphasize that CAFs play key roles in shaping the tumor microenvironment in breast cancer, and therapeutic approaches to reverse the CAF-mediated immunosuppressive microenvironment ought to be considered in future studies [363] (Table 2).

Strategies to analyze the TME

Multiple strategies have been used to study the TME in both preclinical models and human specimens. Spatially resolved methods to map different cell types, ECM components, biological signals/receptors and therapeutic targets include a wide range of platforms such as single-marker chromogenic IHC, low-plex multiplexed immunofluorescence (mIF), high-plex metal conjugation and mass spectrometry-based methods (e.g. imaging mass cytometry, multiple ion beam imaging), high plex barcoding-based and/or cyclic staining methods (e.g. CODEX, CycIF), and more recently spatial transcriptomics approaches. The major advantage of these platforms is the use of intact tumor specimens and preservation of architectural context for data visualization and interpretation (e.g. cell location, distribution and cell–cell or cell-molecule interactions). Additional technologies prominently used to study the TME composition using disaggregated tumor specimens include flow cytometry, high-plex cytometry by time of flight (e.g. CyTOF), bulk mRNA sequencing with cell-type deconvolution (e.g. CYBERSORTx, TIMER, etc.) and single-cell RNA sequencing-based approaches (e.g. single-cell RNAseq with or without 5 prime T-cell receptor (TCR)/B-cell receptor (BCR) clonality analysis, CITE-seq, scATAC-seq, etc.) [364, 365]. The major advantages of these strategies are their single-cell resolution and relatively high throughput. In general, these methods are expensive, can display limited sensitivity and produce large amounts of data requiring labor-intensive data analysis. To date, most of these strategies have not been incorporated in the clinic.

Developments in TME-targeting strategies

Due to the growing evidence highlighting the role of the TME in EMT induction, cancer progression, and therapeutic outcomes, targeting the key players involved in the TME has emerged as an attractive therapeutic strategy to prevent EMT and metastasis in recent years [366–374]. Several TME-targeting approaches have been developed as potentially treatments for cancers characterized by the presence of these important TME players.

These therapeutic approaches mainly focus on targeting the cells of the adaptive immune system (e.g., T-cells or T-lymphocytes and B-cells or B-lymphocytes), the cells of the innate immune system (NK cells, TAMs, MDSCs, DCs, and neutrophils), stromal cells (CAFs), as well as tumor vasculature and the ECM (Fig. 3). From a pharmaceutical design perspective, various targeting agents can be employed in TME-targeting strategies, including small molecule inhibitors, peptides, antibodies, nanoparticles, and bifunctional systems that combine these agents with imaging contrast moieties or therapeutic (radioactive or chemoactive) payloads. A rigorous review of pharmaceutical design in this context is outside the scope of this review, but we refer the reader to the following excellent reviews on this topic: Zeglis and Lewis (2011) [375], Blower (2015) [376], Heinzmann et al. (2017) [377], Maurer et al. (2022) [378], and Xie et al. (2023) [379]. Below we describe how various immune cell populations in the TME can be exploited to improve their anticancer responses, particularly focusing on therapies that have either been FDA-approved or are being tested in the clinic (Tables 3–8) (Figs. 4, 5).

Targeting the adaptive immune system

In the TME immune cells are the predominant non-cancerous cell type, including various adaptive immune cells such as T-cells, and innate immune cells such as NK cells, TAMs, MDSCs, neutrophils, and APC DCs [380–383]. Below, we describe the key functions of the main adaptive immune cell populations comprising

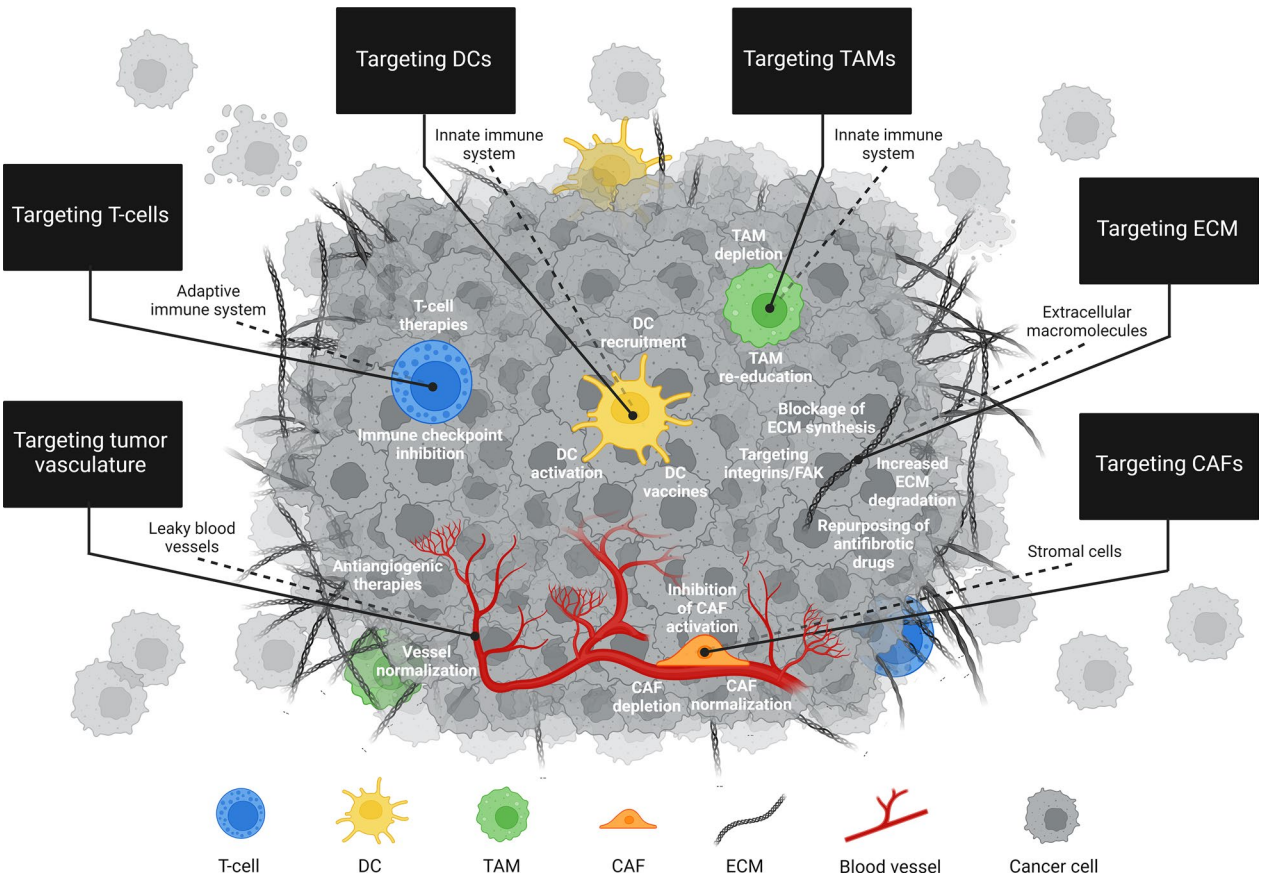

Fig. 3 Each cell type present in the TME can contribute to the regulation of cancer progression and therapeutic response individually and thus several TME-directed therapies have been developed. The major strategies, which either have been FDA-approved or are currently being under clinical investigation, principally focus on the targeting of T-cells, DCs, TAMs, CAFs, ECM, and tumor vasculature; and thus, are indicated in the figure (black boxes) and referenced in the review. Targeting T-cells includes immune checkpoint inhibition, and T-cell therapies; targeting DCs comprises DC activation, DC recruitment, and DC vaccines; targeting TAMs consists of TAM depletion, and TAM re-education; targeting CAFs includes CAF depletion, inhibition of CAF activation, and CAF normalization; targeting ECM comprises increased ECM degradation, blockage of ECM synthesis, repurposing of drugs with antifibrotic properties, and targeting integrins or the downstream effector FAK; and targeting tumor vasculature consists of antiangiogenic therapies, and vessel normalization. DCs: Dendritic cells; TAMs: Tumor-associated macrophages; CAFs: Cancer-associated fibroblasts; ECM: Extracellular matrix; FAK: Focal adhesion kinase. This figure has been created with BioRender.com

Table 3 Inhibitors and antibodies targeting the adaptive immune system in the TME for cancer therapy used in clinical trials or approved by the FDA. Data has been collected from http://www.fda.gov, and http://www.clinicaltrials.gov accessed in November 2023

Drugs targeting the adaptive immune system in the TME							
Targeting T-Cells							
Targeted Molecule	**Drug Name**	**Type of Agent/s**	**Mechanism of Action**	**Status**	**Cancer Type/s**	**NCTs**	**References**
PD-1	Spartalizumab	Neutralizing antibodies	Bind to PD-1 receptor and suppress its interaction with PD-L1/PD-L2, releasing PD-1 pathway-induced inhibition of anticancer response	Phase 2	ASTs	NCT02605967	PMID: 34,433,653
	Nivolumab			Approved	ASTs (CRC, EC, GC, HCC, HNSCC, MEL, MES, NSCLC, RCC, UC), HL	NCT01721772 (ASTs), NCT02181738 (HL)	PMID: 25,399,552 (AST), PMID: 28,438,889 (HL)
	Pembrolizumab			Approved	ASTs (BC, CC, EC, EMC, GC, MCC, MEL, HCC, HNSSC, NSCLC, RCC, SCC, UC), HL	NCT01866319 (ASTs), NCT02684292 (HL)	PMID: 25,891,173 (AST), PMID: 33,721,562 (HL)
	Cemiplimab			Approved	ASTs (BCC, cSSC, NSCLC)	NCT02760498	PMID: 29,863,979
PD-L1	Atezolizumab	Neutralizing antibodies	Bind to PD-L1 to block the interaction between PD-1 and PD-L1, restoring anticancer T-cell function	Approved	ASTs (ASPS, HCC, MEL, NSCLC, SCLC, TNBC, UC)	NCT02108652	PMID: 30,541,754
	Avelumab			Approved	ASTs (MCC, RCC, UC)	NCT02155647	PMID: 29,566,106
	Durvalumab			Approved	ASTs (BTC, HCC, NSCLC, SCLC)	NCT02125461	PMID: 28,885,881
CTLA4	Ipilimumab	Neutralizing antibody	Blocks the inhibitory signal of CTLA4, allowing cytotoxic T-cells to kill cancer cells	Approved	ASTs (CRC, HCC, MEL, MES, RCC, NSCLC)	NCT00094653	PMID: 20,525,992
TIGIT	BMS-986207	Blocking antibodies	Bind to TIGIT to prevent interaction with its ligands				
	Phase 2	ASTs	NCT02913313	PMID: 34,572,463			
	Tiragolumab			Phase 2	ASTs	NCT03563716	PMID: 35,576,957
	AB154			Phase 3	ASTs	NCT05568095	PMID: 38,724,599
	Vibostolimab			Phase 3	ASTs (MEL)	NCT05665595	PMID: 37,410,978
TIM3	Sym023	Antagonistic antibodies	Bind to TIM3 expressed on specific T-cells including TILs, thereby averting T-cell inhibition	Phase 1	ASTs	NCT03489343	PMID: 38,721,404
	LY3321367			Phase 1	ASTs	NCT03099109	PMID: 33,514,524
	BMS-986258			Phase 2	ASTs	NCT03446040	PMID: 33,407,992
	INCAGN02390			Phase 1	ASTs	NCT03652077	PMID: 37,670,328
	Cobolimab			Phases 3	NSCLC	NCT04655976	PMID: 38,730,635
	MBG453			Phase 3	LEU (CMML), MDS	NCT04266301	PMID: 37,083,373
LAG3 bispecific antibodies	FS118 Block MHC-II–LAG3	Blocking and antagonistic					

Table 3 (continued)

Drugs targeting the adaptive immune system in the TME							
Targeting T-Cells							
Targeted Molecule	**Drug Name**	**Type of Agent/s**	**Mechanism of Action**	**Status**	**Cancer Type/s**	**NCTs**	**References**
interaction	Phase 2	ASTs	NCT03440437	PMID: 36,342,102			
	LAG525			Phase 2	ASTs	NCT02460224	PMID: 35,217,575
	IMP321			Phase 1	ASTs	NCT03252938	PMID: 37,742,484
	Fianlimab			Phase 3	ASTs (MEL)	NCT05352672	PMID: 37,004,702
	Relatlimab			Approved	ASTs (MEL)	NCT03470922	PMID: 34,986,285
OX40	MOXR0916	Agonist antibody	Binds and activates OX40	Phase 1	ASTs	NCT02219724	PMID: 35,699,599
ICOS	Vopratelimab	Agonist antibody	Binds and activates ICOS	Phase 2	ASTs	NCT02904226	PMID: 35,511,938
VISTA	CA-170	Small-molecule VISTA/PD-L1 antagonist	Rescues the effector functions of T-cells inhibited by VISTA and PD-L1/PD-L2	Phase 1	ASTs	NCT02812875	PMID: 34,103,659
Targeting B-Cells							
Targeted Molecule	**Drug Name**	**Type of Agent/s**	**Mechanism of Action**	**Status**	**Cancer Type/s**	**NCTs**	**References**
BCR	Acalabrutinib	Small-molecule inhibitors	Inhibit the BTK preventing the activation of BCR signaling pathway, which in turn impedes B-cell activation and BTK-mediated induction of downstream survival pathways	Approved	LEU (CLL, SLL), MCL	NCT02477696 (LEU), NCT02213926 (MCL)	PMID: 34,310,172 (LEU), PMID: 29,241,979 (MCL)
	Ibrutinib			Approved	LEU (CLL, SLL), WM	NCT02477696 (LEU), NCT02165397 (WM)	PMID: 34,310,172 (LEU), PMID: 34,606,378 (WM)

Targeted Molecules: BCR: B-cell receptor; CTLA4: Cytotoxic T lymphocyte-associated protein-4; ICOS: Inducible T-cell co-stimulatory; LAG-3: Lymphocyte activation gene-3; OX40: OX40 receptor or tumor necrosis factor receptor superfamily, member 4 (TNFRSF4); PD-1: Programmed cell death protein 1; PD-L1: Programmed death-ligand 1; TIGIT: T-cell immunoreceptor with Ig and ITIM domains; TIM-3: T-cell immunoglobulin and mucin-domain containing-3; VISTA: V-domain Ig suppressor of T cell activation. *Cancer Types*: ASPS: Alveolar Soft Part Sarcoma; ASTs: Advanced Solid Tumors; BC: Breast Cancer; BCC: Basal Cell Carcinoma; BTC: Biliary Tract Cancer; CC: Cervical Cancer; CLL: Chronic Lymphocytic Leukemia; CMML: Chronic myelomonocytic leukemia; CRC: Colorectal Cancer; cSCC: Cutaneous Squamous Cell Carcinoma; EC: Esophageal Carcinoma; EMC: Endometrial Cancer; GC: Gastric Cancer; HCC: Hepatocellular Carcinoma; HL: Hodgkin's Lymphoma; HNSCC: Head and Neck Squamous Cell Carcinoma; LEU: Leukemia; MCC: Merkel Cell Carcinoma; MCL: Mantle Cell Lymphoma; MDS: Myelodysplastic syndrome; MEL: Melanoma; MES: Mesothelioma; NSCLC: Non-Small Cell Lung Cancer; RCC: Renal Cell Carcinoma; SCC: Squamous Cell Carcinoma; SCLC: Small Cell Lung Cancer; SLL: Small Lymphocytic Leukaemia; TNBC: Triple-Negative Breast Cancer; UC: Urothelial Carcinoma; WM: Waldenström's macroglobulinemia. In case drugs targeting the adaptive immune system in the TME for cancer therapy are being used in several ASTs, only a representative clinical trial (NCT) and its related reference (PMID) have been selected for (a) specific AST(s)

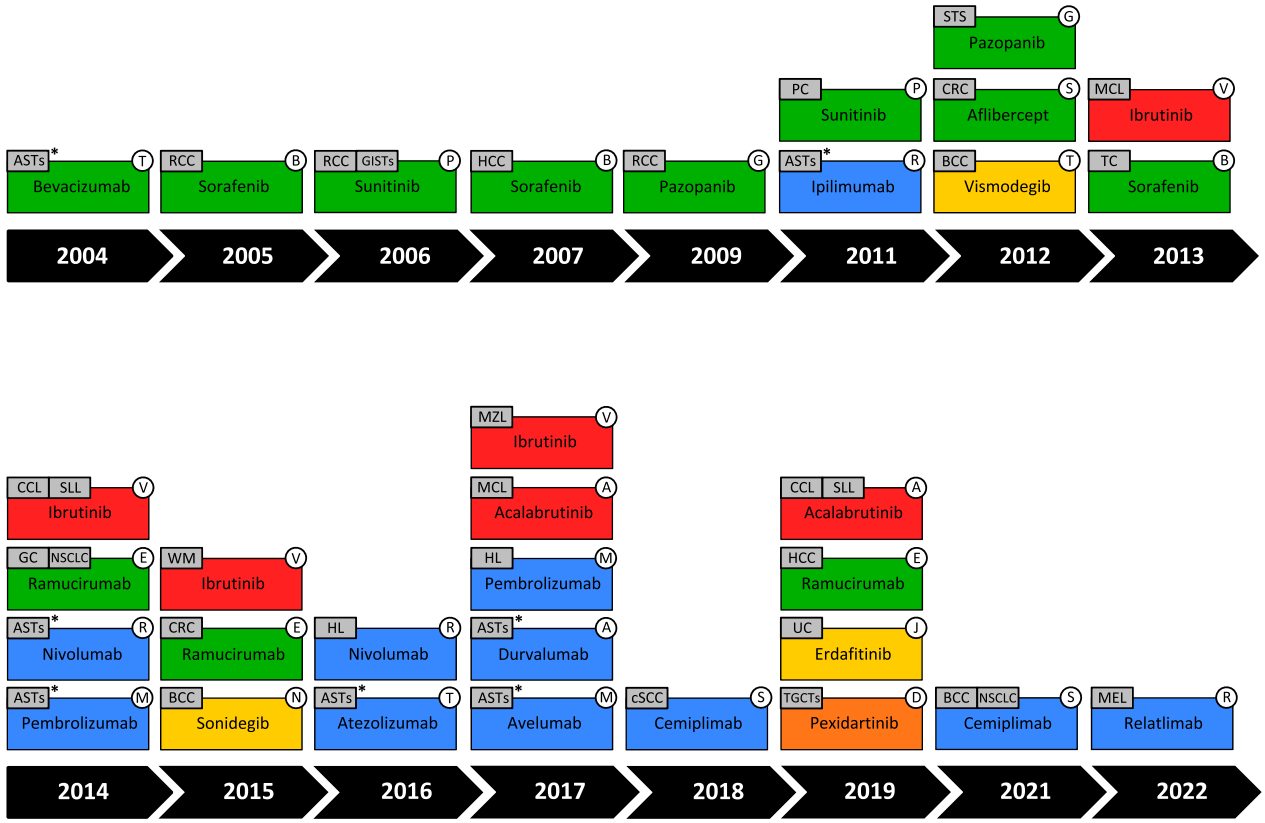

Fig. 4 Timeline of FDA approvals for ICIs targeting T-cells, B-cells, TAMs, CAFs, and tumor vasculature. Timeline of FDA approvals for ICIs targeting T-cells (blue rectangles), B-cells (red rectangles), TAMs (orange rectangle), CAFs (yellow rectangles), and tumor vasculature (green rectangles) is shown. Black arrows below the ICIs: years in which the first FDA approval occurred. Grey squares at the top-left of each inhibitor: cancer type/s related to the first FDA approval of the corresponding inhibitor. ASTs: Advanced solid tumors; BCC: Basal cell carcinoma; CLL: Chronic lymphocytic leukemia; CRC: Colorectal cancer; GC: Gastric cancer; GISTs: Gastrointestinal stromal tumors; HCC: Hepatocellular carcinoma; HL: Hodgkin lymphoma; MCL: Mantle cell lymphoma; MEL: Melanoma; MZL: Marginal zone lymphoma; NSCLC: Non-small cell lung cancer; PC: Pancreatic cancer; RCC: Renal cell carcinoma; SLL: Acute lymphocytic leukemia; STS: Soft tissue sarcoma; TC: Thyroid cancer; TGCTs: Tenosynovial giant cell tumors; UC: Urothelial cancer; WM: Waldenström macroglobulinemia. Asterisk (*) beside grey-squared ASTs: year in which the first (of a series of) AST FDA approval occurred for a particular ICI. Circled capital letters at the top-right of each inhibitor: biopharmaceutical companies related to the first FDA approval of inhibitors. Ⓐ: AstraZeneca; Ⓑ: Bayer AG; Ⓓ: Daiichi Sankyo Company; Ⓔ: Eli Lilly and Company; Ⓖ: GlaxoSmithKline (GSK); Ⓙ: Janssen Pharmaceuticals, Inc; Ⓜ: Merck Inc; Ⓝ: Novartis; Ⓟ: Pfizer Inc; Ⓡ: Bristol-Myers Squibb Company; Ⓢ: Sanofi SA; Ⓣ: Genentech Inc; Ⓥ: AbbVie Inc

the TME, emphasizing on ways to improve their functional responses against tumors. There is a current trend toward an increasing number of immunotherapeutic approaches being developed and explored. The most active and promising immunotherapies focus on immune checkpoints, adoptive cell transfer (e.g., tumor-infiltrating lymphocytes (TILs), TCRs, and chimeric antigen receptor (CAR) T-cell therapy, and cancer vaccines that intercept or treat cancer [384]. However, these therapies share several common challenges. Despite the impressive clinical success of several ICIs targeting programmed cell death-ligand protein 1 (PD-L1), CTLA-4, and lymphocyte activation gene-3 (LAG-3), only a minority of eligible tumors respond, with some tumor types not responding at all. Non-responsiveness is more common in immune-excluded and cold tumors, though inflamed tumors can also show resistance [385]. In addition, ICIs can cause immune-related toxicities, which may be chronic or even life-threatening [386]. Therefore, predictive biomarkers are urgently needed to better predict response and toxicity, ensuring optimal patient selection. CAR-T cells face challenges related to antigen specificity and they can also lead to serious side effects such as cytokine release syndrome (CRS), neurotoxicity, and chronic hypogammaglobulinemia. Expanding the success of CAR-T therapy from hematologic malignancies to solid tumors has proven difficult, largely due to tumor heterogeneity, inadequate T-cell trafficking, and the immunosuppressive TME [387]. Similarly, vaccines

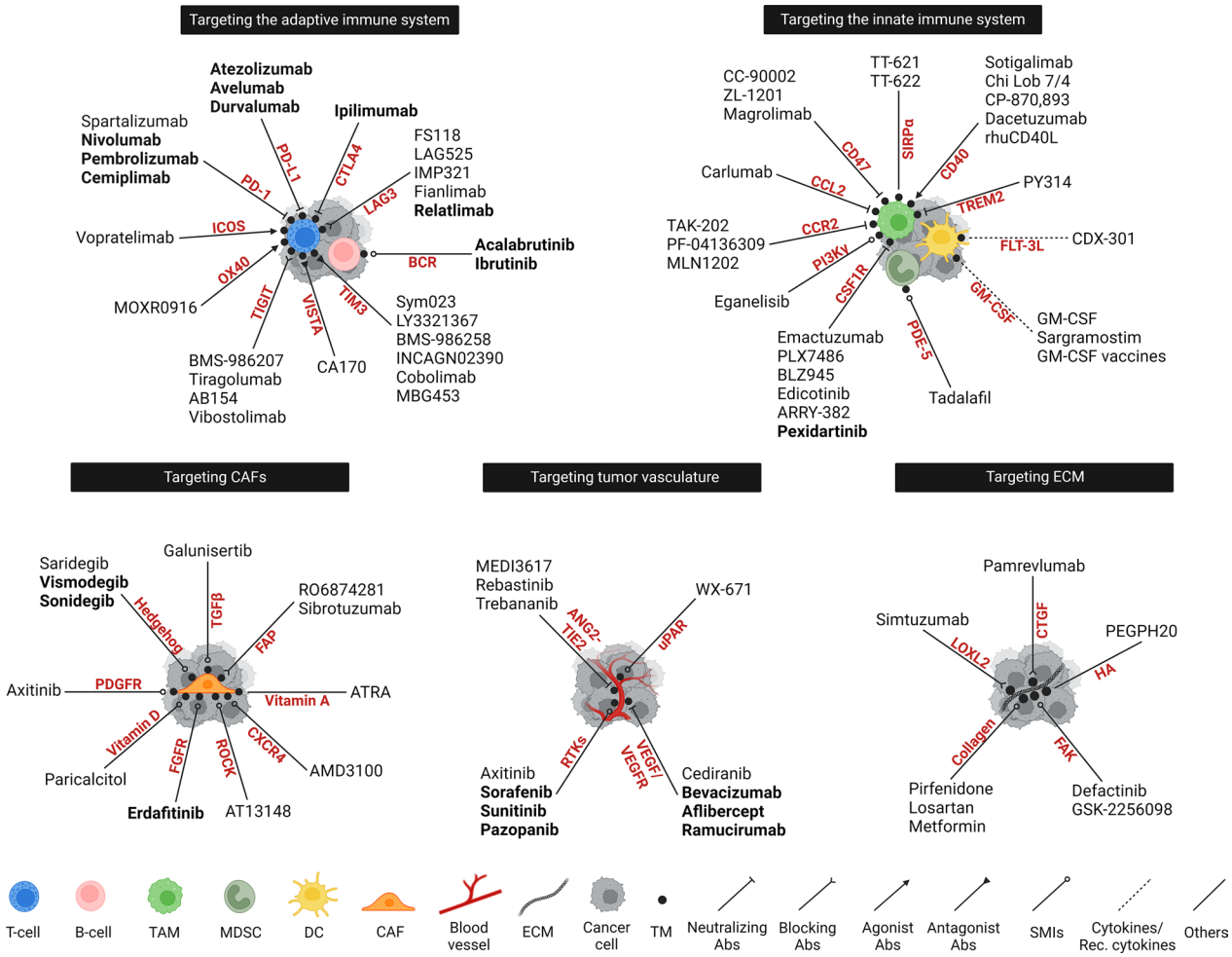

Fig. 5 Targeting of different TME components (adaptive immune system, innate immune system, CAFs, tumor vasculature, and ECM), for cancer therapy. Diverse agents are being/have been used to target different TME components such as adaptive immune system (T-cells and B-cells), innate immune system (TAMs, MDSCs, and DCs), CAFs, tumor vasculature, and ECM, for cancer therapy used in clinical trials or approved by the FDA. Targeted molecules (ANG2-TIE2, BCR, CCL2, CCR2, CD40, CD47, Collagen, CSF1R, CTGF, CTLA4, CXCR4, FAK, FAP, FGFR, FLT3L, GM-CSF, HA, Hedgehog, ICOS, LAG-3, LOXL2, OX40, PD-1, PDE5, PDGFR, PD-L1, PI3K, ROCK, RTK, SIRPα, TGF-β, TIGIT, TIM-3, TREM2, uPAR, VEGF, VEGFR, VISTA, Vitamin A, Vitamin D) are written (along the lines) in red. ANG2-TIE2: Angiopoietin-2-TIE2; BCR: B-cell receptor; CCL2: CC-motif chemokine ligand 2; CCR2: CC-chemokine receptor 2; CD40: Cluster of differentiation 40; CD47: Cluster of differentiation 47 or integrin associated protein (IAP); CSF1R: Colony-stimulating factor-1 receptor; CTGF: Connective tissue growth factor; CTLA4: Cytotoxic T lymphocyte-associated protein-4; CXCR4: C-X-C chemokine receptor type 4; FAK: Focal adhesion kinase; FAP: Fibroblast activation protein; FGFR: Fibroblast growth factor receptor; FLT3L: Fms-related tyrosine kinase 3 ligand; GM-CSF: Granulocyte–macrophage colony-stimulating factor; HA: Hyaluronan; HIF1α: Hypoxia-inducible factor 1α; ICOS: Inducible T-cell co-stimulatory; LAG-3: Lymphocyte activation gene-3; LOXL2: Lysyl oxidase like-2; OX40: OX40 receptor or tumor necrosis factor receptor superfamily, member 4 (TNFRSF4); PD-1: Programmed cell death protein 1; PDE5: Phosphodiesterase 5; PDGFR: platelet-derived growth factor receptor; PD-L1: Programmed death-ligand 1; PI3K: Phosphoinositide 3-kinase; ROCK: Rho-associated protein kinase; RTK: Receptor tyrosine kinase; SIRPα: Signal regulatory protein α; TGF-β: Transforming growth factor-β; TIGIT: T-cell immunoreceptor with Ig and ITIM domains; TIM-3: T-cell immunoglobulin and mucin-domain containing-3; TREM2: Triggering receptor expressed on myeloid cells 2; uPAR: urokinase-type plasminogen activator receptor; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor; VISTA: V-domain Ig suppressor of T-cell activation. TM: Targeted molecule; Abs: Antibodies; SMIs: Small-molecule inhibitors; Rec. cytokines: Recombinant cytokines; Others: Recombinant fragment fusion proteins, Vitamin A metabolite, and PEGylated enzyme. Drugs (written in bold): FDA-approved drugs

face the challenge of identifying the most specific and effective tumor-associated or disease-associated antigens to target, as well as determining the optimal patient populations. Lastly, practical challenges common to both CAR-T therapies and vaccines include

issues related to manufacturing, time, costs, and regulatory requirements [388].

Targeting T-cells

Presently, there are two major tumor immunotherapeutic strategies targeting T-cells: *1)* using ICIs to unleash the anti-cancer potency of T-cells; and *2)* boosting adaptive immunity by adoptive transfer using tumor-infiltrating T-cells or engineered T-cells furnished with TCRs, or CARs [389, 390] (Fig. 6).

Immune checkpoint inhibitors (ICIs) Elevated expression of checkpoint proteins results in the inactivation of T-cell immune responses [391–393]. ICIs are mostly effective in the TME characterized by highnumbers of exhausted T-cells expressing checkpoint proteins. Such tumors are usually deemed as immunologically "hot" and are most susceptible to the treatment with ICIs. Generally, the efficacy of ICIs is associated with cancer mutational burden, expression levels of checkpoint proteins/their ligands, and the presence of $CD8^+$ T-cells within a cancer [394–397]. In the majority of cancer patients, primary resistance can significantly reduce the potency of ICI-based therapies in the majority of tumor patients, and thus still represents the main problem related to this treatment [398–402]. T-cell activation is negatively regulated by several checkpoint molecules, exerting a precise control of the immune system by preventing its hyperactivation. Checkpoint molecules comprise: *1)* PD-1 [403, 404]; *2)* CTLA-4 [405, 406]; *3)* T-cell immunoglobulin and ITIM domain (TIGIT) [407, 408]; *4)* T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) [409, 410]; *5)* LAG-3 [411, 412] (Table 3) (Fig. 5).

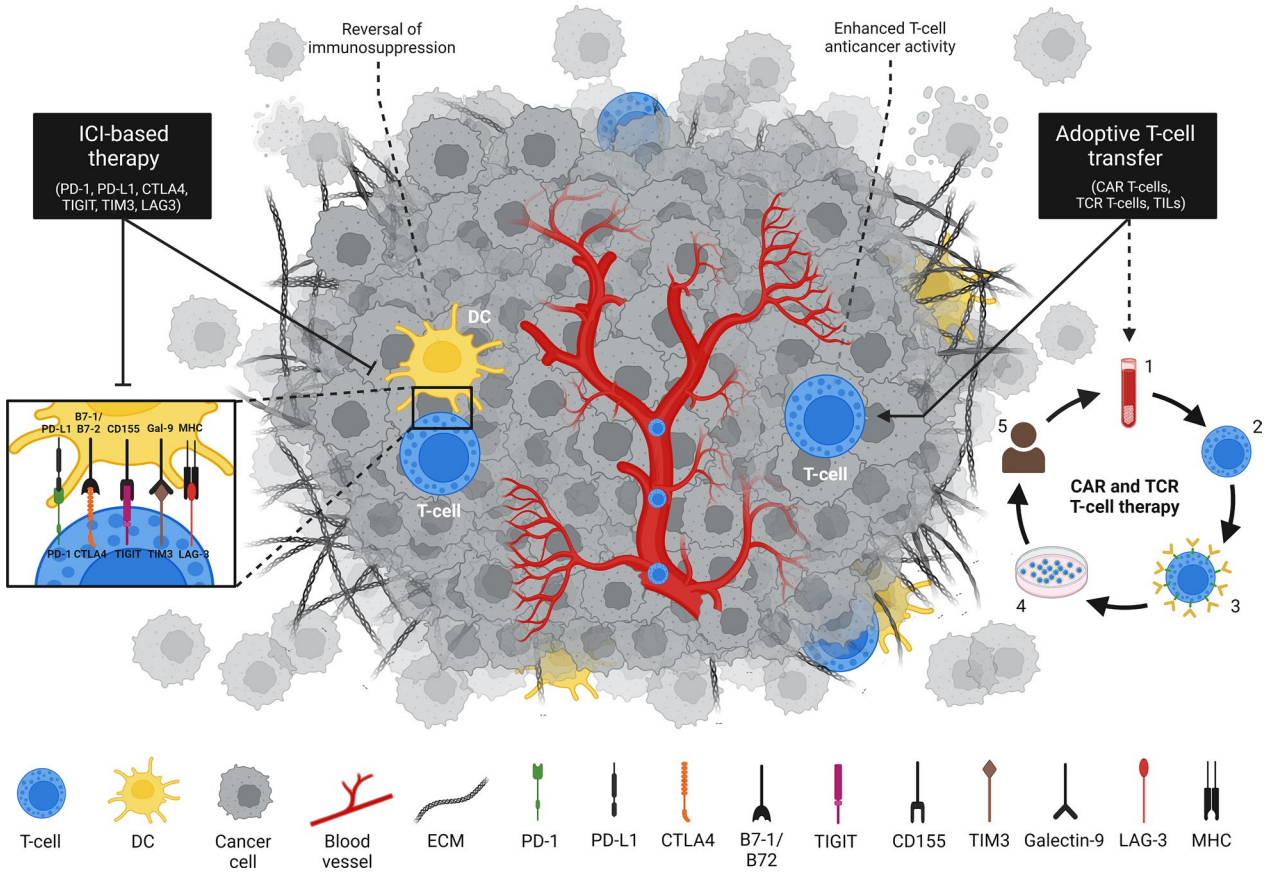

Fig. 6 Therapeutic targeting of T-cells to augment anti-cancer activity. T-cell antitumor activity can be increased through *1*) inhibition of several immune checkpoint molecules, or *2)* adoptive transfer of CAR T-cells, TCR T-cells, or tumor-infiltrating lymphocytes (TILs). The scheme on the bottom left of the figure displays five major protein/ligand interactions (e.g. PD-1/PD-L1). Dotted-lines select the square enlargement including protein/ligand interaction between a T-cell and a DC. The scheme on the bottom right of the figure shows the five main steps of CAR T-cell and TCR T-cell therapy: *1*) T-cell collection from blood patients; *2*) viral vector-mediated introduction of a CAR gene or a TCR gene; *3*) generation of CAR T-cells or TCR T-cells; *4*) *ex-vivo* expansion of engineered T-cells (CAR T-cells or TCR T-cells); *5*) infusion of CAR T-cells or TCR T-cells back into patients. This figure has been created with BioRender.com

- PD-1/PD-L1

Following TCR stimulation T-cells express PD-1, which binds to PD-L1 and programmed cell death-ligand protein 2 (PD-L2). Both ligands are present on APCs and are upregulated by proinflammatory cytokines [413]. PD-L1 expression on tumor cells is also induced by activated T-cell-secreted IFN-γ [414]. PD-1 regulates immune response by signaling-mediated inhibition of Tregs and effector T-cells; and thus, PD-1/PD-L1 signaling critically governs and checks the regular induction and division of these lymphocytes [306]. As such, the PD-1/PD-L1 axis has been a major target during the recent years, with promising results in several preclinical and clinical studies. PD-1-neutralizing antibodies such as nivolumab and pembrolizumab, administered as monotherapy or in co-treatment with other drugs, have exhibited significant clinical benefits in advanced solid tumor patients, as well as Hodgkin's lymphoma patients; and thus, have been Food and Drug Administration (FDA)-approved for use in these cohorts [50–416]. Notably, pembrolizumab has shown anticancer activity in advanced NSCLC patients, and expression of PD-L1 in at least 50% of cancer cells has correlated with improved potency of this PD-1-neutralizing antibody [417]. In addition, pembrolizumab has demonstrated a more substantial 6-month progression-free survival (PFS), greater OS benefit, and improved safety profile, in comparison to FDA-approved human CTLA-4-blocking antibody ipilimumab in melanoma [418]. Similarly, nivolumab has displayed a 72.9% OS in comparison to 42.1% OS, in chemotherapy-treated melanoma patients following one year of treatment [419]. More recently, based on a phase 2 trial (NCT02760498), cemiplimab determined a durable response in approximately 50% of locally advanced and metastatic cutaneous squamous cell carcinoma (cSCC) patients, and as a result has been FDA-approved for use in this cohort [420]. Moreover, cemiplimab has also received FDA approval for advanced basal cell carcinoma (BCC) patients (NCT03132636) [421], as well as first-line advanced NSCLC patients with PD-L1 expression of ≥ 50% as monotherapy (based on the EMPOWER-Lung 1 phase 3 trial NCT03088540), and first-line advanced NSCLC patients in combination with chemotherapy (based on the EMPOWER-Lung 3 phase 3 trial NCT03409614) [422]. Besides, anti-PD-1 antibody spartalizumab in combination with other drugs has also been used in phase 2 clinical trials in melanoma patients (NCT03484923), metastatic pancreatic ductal adenocarcinoma patients (NCT04390763), and recurrent/metastatic nasopharyngeal cancer patients (NCT02605967). In the latter study, spartalizumab displayed longer OS and duration of response (DOR) compared to chemotherapy, and a favorable safety profile, consistent with other anti-PD-1 antibodies [423]. APCs normally express PD-1 ligand PD-L1, which regulates differentiation and inhibitory activity of Tregs [424]. Nevertheless, TME components including cancer cells, DCs, and infiltrating myeloid cells, can upregulate PD-1 ligands to determine exhaustion of T-cells, thereby generating an immunosuppressive TME and promoting cancer progression [425]. PD-L1-neutralizing antibodies including atezolizumab, avelumab, and durvalumab, singly or in combination with other agents, have exhibited significant clinical benefits in advanced solid tumor patients, and therefore have also been FDA-approved for use in these cohorts [50]. Thus, analogous to PD-1, suppression of PD-L1 is also efficacious in treating a variety of cancers (Table 3) (Figs. 4, 5).

- CTLA4

The T-cell-expressed CTLA-4 binds to B7-1/B7-2 on APCs with more affinity than CD28, thus ending the co-activating interaction between B7-1/B7-2 and CD28 during antigen presentation [426]. Suppression of this molecule results in a T-cell activation that may be directed against cancer cells. Indeed, numerous preclinical studies have demonstrated that inhibition of CTLA-4 determines a durable immunologic memory in several tumors; but however, a significant impact has not been detected in cancers characterized by stronger anti-inflammatory TME, and/or in cancers with reduced immunogenicity [427, 50]. Over the last two decades, the CTLA-4-neutralizing antibody ipilimumab has been investigated in clinical trials for various types of cancer, with remarkably durable responses albeit in only a small subset of patients, especially in melanoma patients [428], leading to its FDA approval in 2011. Since the functions of PD-1 and CTLA-4 are non-overlapping, co-administration of these two agents also significantly increased the response rates in several advanced solid tumor patients, and consequently has also been FDA-approved for use in these cohorts [50]. For metastatic melanoma patients, the combination of ipilimumab + nivolumab is the most potent treatment with a median OS of more than 6 years. A variety of clinical trials are currently ongoing, often in combination with other agents to potentially allow a broad range of advanced solid tumor patients to benefit from this therapy. The downside of this combination is its highly toxicity with grade > 3 immunological adverse events occurring in about 60% of the patients. Accordingly, a low-dose co-treatment of ipilimumab and nivolumab has been evaluated in a multicenter phase 2 clinical trial (NCT02834013) in unresectable or metastatic metaplastic breast cancer. This combination has shown an overall response rate (ORR) of 18% at > 2 to

almost 3 years later, 2 months PFS, and 12 months OS. Hence, this co-treatment warrants further investigation, particularly because 65% of adverse events, and 47% of immune-related adverse events were observed in these metaplastic breast cancer patients [429] (Table 3) (Figs. 4, 5).

- TIGIT

The inhibitory receptor TIGIT is expressed by T-cells and NK cells [430–432]. CD155, which is expressed by both cancer cells and APCs, is the primary ligand of TIGIT, and mediates negative regulation of T-cell and NK-cell functions [407]. TIGIT-targeted cancer immunotherapy is considered a promising strategy since TIGIT notoriously suppresses numerous anti-cancer immunity mechanisms. Indeed, TIGIT is often upregulated in various cancers [433, 430], and its expression is frequently associated with poor clinical outcomes, particularly in acute myeloid leukemia (AML) [434] and melanoma [435]. These data suggest that TIGIT blockage can protect against several tumor types, resulting in the testing ofseveral monoclonal antibodies against human TIGIT in ongoing clinical trials [50]. Various clinical trials are currently investigating the efficacy and safety of several anti-TIGIT antibodies, either as monotherapy or in co-administration with other immunotherapies, including AB154 in phase 1 (NCT03628677) in advanced solid tumors (ASTs), in phase 3 (NCT05211895) (NCT05502237) in NSCLC, and in phase 3 (NCT05568095) in advanced upper gastrointestinal tract adenocarcinoma; vibostolimab plus anti-PD-1 receptor pembrolizumab *versus* pembrolizumab in a phase 3 trial of adjuvant therapy for resected stage IIB to IV melanoma patients (NCT05665595), BMS-986207 in phase 2 (NCT02913313) in ASTs, and in phase 2 (NCT05005273) in NSCLC; and tiragolumab in phase 2 (NCT04300647) in metastatic and/or recurrent PD-L1-positive cervical cancer. Due to significant data collected from the CITYSCAPE phase 2 clinical study (NCT03563716), tiragolumab has recently gained FDA Breakthrough Therapy Designation in co-treatment with the anti PD-L1 atezolizumab for PD-L1-positive recurrent or metastatic NSCLC patients. This combination has demonstrated significant clinical improvement in ORR and PFS compared to placebo plus atezolizumab, and a favorable safety profile, similar to that of atezolizumab singly [436]. However, in the SKYSCRAPER-01 phase 3 clinical trial (NCT04294810), this combination has improved neither PFS nor OS in newly diagnosed metastatic NSCLC with at least 50% PD-L1 expression compared to placebo plus atezolizumab. In addition, the ongoing SKYSCRAPER-02 phase 3 clinical trial (NCT04256421), using the same aforementioned co-administration, has failed to find OS or PFS benefit *versus* placebo plus atezolizumab in small cell lung cancer (SCLC) patients [437]. Furthermore, tiragolumab is presently being evaluated in phase 3 clinical trials in combination with atezolizumab in locally advanced esophageal squamous-cell carcinoma patients (NCT04543617) [438], and in a 4-drug combination with atezolizumab, carboplatin, and etoposide, in untreated extensive-stage SCLC patients (NCT04256421) [439] (Table 3) (Fig. 5).

- TIM3

TIM3 receptor is expressed in several types of cells, including T-cells, Tregs, B-cells, monocytes, DCs, NK cells, and macrophages [440–442]. Four ligands bind to TIM3: carcinoembryonic antigen cell adhesion molecule 1 (CEACAM1), high-mobility group protein B1 (HMGB1), phosphatidyl serine (PtdSer), and galectin-9. These four ligands bind to TIM3 causing a negative regulation of T-cells [443, 444]. Enhanced TIM3 expression is linked to poor prognosis in ASTs [445], as well as hematological malignancies [446]; and consequently, its suppression leads to significant anticancer activity, especially in co-treatment with anti-PD-1 antibodies [447, 448]. Over the last years, various antagonist antibodies of TIM3 have been produced and are currently being tested at early stage of clinical evaluation. Interestingly, in a phase 1 clinical trial (NCT02817633), cobolimab has demonstrated synergistic effects in co-administration with anti-PD-1 immunotherapy in AST patients, and in a phase 3 clinical trial (NCT04655976) is being tested in combination with dostarlimab and docetaxel in NSCLC patients progressing on previous anti-PD-L1 therapy and chemotherapy. In the STIMULUS-MDS2 phase 3 clinical trial (NCT04266301), MBG453 in combination with azacytidine aims to demonstrate the potential of this co-treatment to improve survival in leukemia and myelodysplastic syndromes (MDS) patients [449]. Several early phase clinical trials have begun to determine efficacy and safety of TIM3 inhibitors including BMS-986258 both singly and in combination with the anti-PD-1 nivolumab (NCT03446040), INCAGN02390 monotherapy (NCT03652077) [50], and novel, first-in-class LY3321367 in co-treatment with anti-PD-L1 antibodies (NCT03099109) [450], in AST patients. A phase 1 clinical trial (NCT03489343) is also investigating Sym023 in ASTs or lymphoma patients. Early data of these aforesaid agents have displayed only modest anticancer activity, with manageable safety profile, and favorable pharmacokinetics and pharmacodynamics. Therefore, TIM-3 blockade as potential cancer therapy warrants further investigation. It is worth

mentioning that recruitment related to three of eleven clinical trials using TIM-3 inhibitors in MDS and AML has been completed (NCT03946670, NCT04266301, and NCT03066648). In the American Society of Hematology (ASH) annual meeting 2022, first data from the STIMULUS-MDS1 phase 2 clinical study (NCT03946670), using combination of MBG453 and hypomethylating agent in MDS patients who were unsuitable for hematopoietic stem cell transplantation or intensive chemotherapy at screening, have been reported. Interestingly, the complete response (CR) is higher after MBG453 plus hypomethylating agent (21.5%) compared to placebo plus hypomethylating agent (17.7%); and the safety profile is favorable [451, 452]. Besides, using the same aforementioned co-treatment in MDS patients, an ongoing STIMULUS-MDS2 phase 3 clinical trial (NCT04266301) [449], and another STIMULUS MDS-US phase 2 clinical study (NCT04878432) are underway (Table 3) (Fig. 5).

- LAG-3

LAG-3 is expressed in activated $CD8^+$ T-cells, activated $CD4^+$ T-cells, Tregs, B-cells, pDCs, and distinct subgroups of NK cells [453–455]. LAG-3 binds to stable MHC-II, as CD4 does, and to other ligands including: fibrinogen-like protein 1 (FGL1), liver sinusoidal endothelial cell lectin (LSECtin), galectin-3, and α-synuclein fibrils. Its expression is induced after continuous exposure to antigen, as occurs during chronic infection or cancer, and is associated with T-cell exhaustion [440, 456]. The infiltration of $LAG3^+$ cells into the TME is related to poor prognosis and tumor progression in several cancers, such as NSCLC, RCC, and breast cancer [50]. In several preclinical mouse models, a robust anti-tumor effect has been observed after the co-blockade of LAG-3 and PD-1. This synergistic anti-tumor effect might result from the distinct mechanisms of action and expression profiles of these two molecules. It could also be due to the fact that single-agent checkpoint suppression in the TME can result in a counterbalanced upregulation of another/other different checkpoint molecule/s [457]. Hence, the combination of PD-1 blockade and LAG-3 suppression determines synergistic decrease of Tregs and augmentation of $CD8^+$ T-cell cytotoxicity, therefore leading to cancer growth inhibition [458]. These data emphasize that LAG-3, as well as PD-1, contribute to immune-escape mechanisms, supporting this co-treatment as a promising tumor immunotherapy [459, 460]. Based on preclinical studies, several agents have been developed to block LAG3 signaling, including relatlimab, fianlimab, LAG525, IMP321, and FS118. The potency of these drugs is presently under evaluation in various clinical studies, either singly or in co-treatment, usually employing anti-PD-1 and/or anti-PD-L1 neutralizing antibodies in advanced solid tumor patients (NCT03459222, NCT03642067, NCT05608291, NCT05352672, NCT03625323 and NCT03440437). In a recent phase 1/phase 2 clinical trial (NCT02460224), the combination of LAG525 and anti-PD-1 spartalizumab showed modest anticancer activity [2% CR, 8% partial response (PR), and 6.6% stable disease (SD) for 6 months] and favorable safety profile, in advanced malignancies patients [461]. These antibodies mainly suppress the LAG-3-MHC-II interaction [170], although this mechanism of action is not thoroughly understood. In the RELATIVITY-047 phase 2/phase 3 clinical trial (NCT03470922), the combination of the LAG-3 inhibitor relatlimab and the anti-PD-1 nivolumab has demonstrated higher benefit in term of PFS (47.7%) compared to PD-1 inhibition only (36%) in previously-untreated unresectable or metastatic melanoma patients. Though grade 3–4 treatment-related adverse events were more common among melanoma patients who received this combination than who received nivolumab singly, no new safety signals related to the co-treatment were observed, and thus the safety profile was favorable [462]. Besides, in the recent RELATIVITY-020 phase 1/phase 2clinicaltrial (NCT01968109), the same combination, has displayed durable clinical anticancer activity and a manageable safety profile in heavily pretreated advanced melanoma patients who had progressed on anti-PD-1/PD-L1-including regimens. Particularly, ORR were 12% and PFS is 2.1 months in advanced melanoma patients who progressed during/within three months of an anti-PD-1/PD-L1-including regimens; whereas ORR were 9.2% and PFS is 3.2 months in advanced melanoma patients who progressed during/within three months of ≥ 1 anti-PD-1/PD-L1-including regimens [463]. As a result, the LAG-3-blocking antibody relatlimab in combination with the anti-PD-1 nivolumab has demonstrated significant clinical benefits in unresectable or metastatic melanoma patients, and consequently has received its first FDA approval for use in this cohort [464] (Table 3) (Figs. 4, 5).

- VISTA

Besides, VISTA using small-molecule VISTA/PD-L1 antagonist CA-170 has demonstrated preclinical anticancer efficacy [465], and has been investigated in a phase 1 clinical trial (NCT02812875) in advanced solid tumor and lymphoma patients; however, no results have been reported (Table 1a).

- OX40 and ICOS

Other immune modulatory molecules are c0-stimulatory agents. The targets include OX40 using agonist antibody MOXR0916 (NCT02219724), and ICOS using agonist antibody vopratelimab (NCT02904226), which have shown favorable safety profile and evidence of tumor immune activation, in advanced solid tumor patients [466, 467] (Table 3) (Fig. 5). Overall, ICIs (targeting PD-1, CTLA-4, TIGIT, TIM-3, LAG-3, VISTA) and co-stimulatory agents (OX40, ICOS) have demonstrated substantial clinical efficacy, resulting in durable responses and improved survival in cancers such as melanoma, lung cancer, and renal cell carcinoma. However, challenges include a significant proportion of patients who do not respond, as well as severe immune-related adverse events (irAEs) such as colitis, hepatitis, and endocrinopathies. In addition, some of these agents despite showing therapeutic efficacy in preclinical models failed in clinical settings in part due to the clinical trial design and therapeutic regimens which have been tested in clinic. Future research should prioritize developing robust biomarkers to predict which patients would benefit, optimizing combination therapies with other modalities to enhance efficacy and overcome resistance, and devising better management strategies for irAEs.

- Adoptive T-cell transfer

In the adoptive T-cell transfer (ACT) mature T-cell subsets are infused into patients to augment immune system-mediated selection and elimination of cancer cells, thereby preventing disease recurrence [468–473]. The ACT is performed by first isolating autologous T-cells from patients or allogenic T-cells from donors, and then ex vivo expansion and injection into patients [474–476]. Due to its significant benefits, ACT is now considered a potential valuable therapy in several cancers [477–483]. Infusion of TILs in addition to lympho-conditioning and interleukins (ILs) have shown encouraging results in metastatic melanoma patients [484]. A recent randomized phase 3 study (NCT02278887) comparing TILs to ipilimumab has shown significant benefit in favor of TILs in metastatic melanoma patients [485]. Additionally, a recent phase 1 clinical study has displayed interesting data based on durable responses in NSCLC patients [486]. Nevertheless, these therapies present some caveats such as the time and cost related to ex vivo generation of T-lymphocytes, and the contrariety to envision which patients can display a response [478]. Two primary approaches have been developed to genetically modify T-cells: *1)* CAR T-cell therapy [487], and *2)* TCR therapy [488]. Both strategies are based on: *a)* manipulation of autologous ex vivo T-cells to promote expression of receptors recognizing tumor-specific antigens (TSAs), and *b)* reintroduction of these T-cells into the patient to augment T-cell-induced killing of tumor cell; although their mechanism for antigen recognition are different (Fig. 6).

- CAR T-cell therapy

CAR T-cell therapy produces engineered T-cells that express synthetic TCRs specific to tumor antigens [489–495]. Interestingly, an advantage of CAR T-cells is that they can directly recognize target molecules on the surface of cancer cells, without the requirement of recognizing antigen fragments presented by MHC/HLA molecules. This is critical due to the fact that MHC/HLA molecules are commonly downregulated in cancer [128, 496]. Notably, CAR T-cells have provided significant benefit in several hematological tumors [497–499]. Indeed, numerous clinical studies using CAR T-cell therapy have demonstrated favorable outcomes [477], leading to FDA and the European Commission approval of different agents between 2017 and 2021 [500]. Besides, various CAR T-cell therapies are currently clinically investigated to treat different subtypes of multiple myeloma (MM), lymphoma, and leukemia, as well as other types of cancer [501]. Additionally, new approaches are being evaluated for different markers on the tumor or in the TME, such as CAR T-cell therapy toward pan-cancer antigen B7-H3, which has already provided positive results in murine models of pediatric cancers [502]. Nonetheless, CAR T-cell therapies have not been significantly efficient for ASTs, such as neuroblastoma, since these cancers are notoriously heterogeneous, and their TME displays a higher complexity [503]. Engineering CAR T-cells can also be approached to operate on immune-suppressive TME and reinvigorate T-cells that are exhausted [504]. An example are the adjusted and updated second-generation armored CAR T-cells, expressing cytokines/ligands that significantly augment the overall efficacy [505–491]. In fact, these armored CAR T-cells, which are for example engineered to generate IL-12, overcome Treg- and myeloid cell-mediated immunosuppression in the TME, trigger recruitment of myeloid cell, enhance antigen presentation [507], and endorse $CD8^+$ T-cell cytolytic activity [508]. Even though using engineered T-cells is undoubtedly advantageous in various tumors [490], some obstacles need to be tackled in the near future, including selection of novel antigens, overcoming an immunosuppressive TME, and toxicity-related issues [509]. Indeed, disadvantages related to CAR T-cell therapy are mainly due to systemic inflammatory toxicity emerging in the host body [510–512]. However, more studies are focusing

on trying to tackle these problems associated with the use of CAR T-cell therapy. In addition, the development of novel imaging strategies to monitor the adoptively transferred T-cells in vivo may facilitate the development of better and safer treatments [513, 514] (Figure 6).

- TCR therapy

The target range of TCR T-cells is broad since they identify intra-cellular antigen fragments, as well as extracellular antigen fragments only displayed by MHC/HLA molecules; and thus, differently from CAR T-cell therapy, this is an impediment for engineered TCR T-cells [515]. Based on theHLA-restriction of TCR-T cell therapies, it is improbable that pharmaceutical companies will face the considerable expense oftesting this therapy in cancer, since CAR-T cell therapies have already shown encouraging results in terms of efficacy. Thus, TCR-T cells are usually tested in solid tumors where the lack of appropriate surface targets limit CAR-T cell therapy. Besides, it is important to predict potential on-target/off-tumor toxicity in TCR-T cells due to their higher antigen sensitivity in comparison to CAR-T cells [516–518]. Notwithstanding TCR-based therapy has been worthwhile in preclinical models and several clinical studies, there is no TCR T-cell therapy approved by the FDA yet [488, 492] (Fig. 6). Thus, ACT has achieved remarkable success especially in hematological malignancies by targeting specific tumor antigens, resulting in high response rates and long-term remissions. Despite this, complex manufacturing processes and severe toxicities like CRS pose significant challenges. In addition, the success in solid tumors has been limited probably due to the inability of T-cells to infiltrated tumors and the suppressive effect of the TME. Future research should continue to streamline production, identify new target antigens for solid tumors, overcome TME immune suppression, and develop strategies to mitigate toxicities.

- Cancer vaccines

The cancer vaccine-induced activation of host T-cells occurs by exposing them to tumor-specific neo-antigens. Cancer vaccine triggers anti-cancer immunity with tumor antigens, which can be mainly delivered as whole cells, peptides, and nucleic acids. Ideally, cancer vaccines overcome immune suppression in cancers, and concurrently, activate both cellular immunity and humoral immunity [519]. Nonetheless, the main limitation associated with this personalized therapeutic strategy is due to the identification of tumor-specific neoantigens. Indeed, tumor cells obtained from biopsies have not shown a proper activation of the host immunity mainly due to insufficiency of tumor-specific neo-antigens. However, recent new bioinformatics tools and next-generation sequencing have facilitated the discovery of tumor neoantigens, which arise from somatic mutations of the cancer, and thus, are tumor specific. Due to the diversity of tumor neoepitopes between different individuals, developing personalized cancer vaccines may be warranted in the upcoming years [520]. Along these lines, a recent phase 2 trial (NCT03897881) has shown relapse-free survival benefit of a personalized RNA-based vaccine combined with pembrolizumab *versus* pembrolizumab monotherapy in stage IIIB to IV resected melanoma patients. In this trial, RNA sequences coding for up to 39 neoantigens expressed by each patient's tumor were engineered in 6 to 8 weeks for 90% of the patients [521].

- Immune modulatory vaccines

The immune modulatory vaccines (IMVs) aim to reshape the TME to enhance the body's immune response against cancer cells [522]. Unlike traditional cancer vaccines described above that stimulate T-cells to directly attack the tumor, the IMVs activate anti-regulatory T-cells (anti-Tregs) that target the entirety of the TME [523, 524]. Anti-Tregs recognize tumor microenvironment antigens (TMAs) expressed by regulatory immune cells such as CAFs, Tregs, MDSCs, and TAMs in addition to tumor cells [305]. In the realm of cancer, the activation of anti-Tregs serves a dual purpose by instigating a direct assault on tumor cells in addition to orchestrating a transformation of the TME, rendering it immunocompetent and hostile to tumors. Unlike other clinical strategies focused on the reversion of the immunosuppressive environment, IMVs exert effects that encompass both the elimination of suppressive cells (through the direct cytotoxicity induced by activated cytotoxic T-cells) and the reprogramming of suppressive cells (by stimulating the release of pro-inflammatory cytokines), e.g., the conversion of M2-like TAMs into M1-like macrophages and the transformation of immunosuppressive CAFs into immunocompetent fibroblasts [525, 526]. Both in vitro and in vivo studies have substantiated that the activation of cytotoxic $CD8^+$ anti-Tregs can result in the direct eradication of target cells, including cancer and immune cells [525–529]. Moreover, the secretion of pro-inflammatory cytokines from $CD4^+$ anti-Tregs aim to transitioning the TME into an anti-tumor immune environment [530]. The significance of integrating both CD4 and CD8 T-cell epitopes in IMVs has been evidenced in animal models of cancer [525, 531]. The targeting of non-transformed cells with consistent HLA expression may in addition induce HLA expression in malignant cells; thus, the inflammation induced by IMVs may elevate surface HLA expression

on tumors. Consequently, IMVs can even have an impact on tumors with low HLA expression. IMVs trigger de novo T-cell activation and, consequently, combining IMVs with ICIs that boost T-cell immunity is an attractive approach. In a recent phase 2 trial (NCT03047928), the synergistic application of an IMV based on indoleamine 2,3-dioxygenase (IDO) and PD-L1 in conjunction with an anti-PD-1 antibody has demonstrated remarkable clinical efficacy as a first-line treatment for patients with metastatic melanoma [532]. Notably, the objective response rate reached 80%, and the complete response rate was recently updated to 50% [533]. This trial has further shown a correlation between the induction of a pro-inflammatory TME and the re-polarization of innate immune cells, as evidenced by a notable increase in class II HLA expression [532]. Recently, the subsequent phase 3 follow-up trial (NCT05155254) successfully completed recruitment involving 380 patients and is expected to reach PFS in the second half of 2025. Collectively, current studies of cancer vaccines have shown promising results in preclinical studies and early-phase clinical trials. However, their clinical success has been limited due to the heterogeneity of tumor antigens, immunosuppressive TME, and mechanisms of immune evasion by tumors. Future research should explore combination therapies to boost vaccine efficacy, develop personalized vaccines targeting neoantigens shared among patients, and investigate methods to counteract immune suppression and evasion mechanisms within the TME

Targeting B-cells

B-cells play critical roles in the TME of several cancers [534–539]. Bruton's tyrosine kinase (BTK) inhibitors are the most promising drugs targeting BCR signaling [540–543]. Acalabrutinib and ibrutinib are the two major small molecule inhibitors targeting BCR [544, 545]. Acalabrutinib monotherapy has shown significant results in chronic lymphocytic leukemia (CLL), small lymphocytic leukemia (SLL), and mantle cell lymphoma (MCL) patients. Besides, ibrutinib monotherapy or in combination with rituximab has shown significant clinical benefits in CLL, SLL, and Waldenström's macroglobulinemia (WM) patients. Consequently, both inhibitors have been FDA-approved for use in these cohorts [546–548] (Table 3) (Figs. 4, 5).

Targeting the innate immune system

Targeting NKcells

The CAR-NK immunotherapy has recently emerged as a valuable alternative to CAR T-cell therapy [549, 550]. In fact, CAR-NK cells are safer than CAR T-cell since they rarely elicit side effects including neurotoxicity and cytokine release syndrome [551, 552]. Besides, CAR-NK cells show no HLA-matching restriction and more availability from a variety of sources compared to CAR T-cells including the potential for off-the-shelf products readily available for immediate clinical use [553]. In addition, CAR-NK cells, which release diverse cytokines (e.g. INF-γ and GM-CSF) compared to those secreted by CAR T-cells [(e.g. interferon alpha (TNF-α), IL-2, and IL-6] [554], are able to exert neutralization of cancer cells both CAR-dependently as well as CAR-independently [553]. Interestingly, a recent clinical study (NCT03056339) using umbilical cord blood-derived CAR-NK cells showed a clinical response of 72.7% and a CR of 63.6% in CLL or non-Hodgkin's lymphoma (NHL) patients. Notably, administered CAR-NK cells caused no development of neurotoxicity, cytokine release syndrome, graft-versus-host disease, or major toxic effects [555]. Thus, CAR-NK cell therapy has great potential for future applications in cancer immunotherapy. However, clinical data are still limited, and challenges include the short lifespan and limited persistence of NK cells in the TME. Future research should focus on optimizing NK cell expansion and persistence, developing combination therapies to enhance NK cell activity, and conducting extensive clinical trials to validate their efficacy and safety across various cancers.

Targeting TAMs

Therapeutic strategies targeting TAMs in specific sites of organs have displayed promising results in preclinical models, although these cells are notoriously subject to tissue-specific imprinting [50, 556]. In fact, macrophage-targeted therapies can *a)* inhibit the ability of TAMs to support tumor cell survival, and *b)* augment cross-presentation to $CD8^+$ T-cells [262, 557] (Fig. 7).

Drug-mediated targeting of TAMs Several drugs have been in clinical evaluation to target TAM in the TME for different types of cancer including: *1)* CCL2 inhibitors and/or CC-chemokine receptor 2 (CCR2) inhibitors, which evade recruiting of TAM within TME; *2)* Colony-stimulating factor-1 receptor (CSF1R) inhibitors, to diminish TAMs in the TME; *3)* treatment with co-stimulatory molecules e.g. CD40, to increase the induction of T-cells; *4)* CD47/SIRPα complex antagonists, which augment TAM-induced tumor cell phagocytosis; *5)* triggering receptor expressed on myeloid cells 2 (TREM2) inhibitors; *6)* PI3Kγ inhibitors to re-engage TAMs toward an anti-cancer phenotype.

CCL2/CCR2 inhibitors

Chemokine release leads to augmentation of TAMs in the TME, resulting in the expansion of the tissue-resident macrophage pool, monocyte recruitment, and

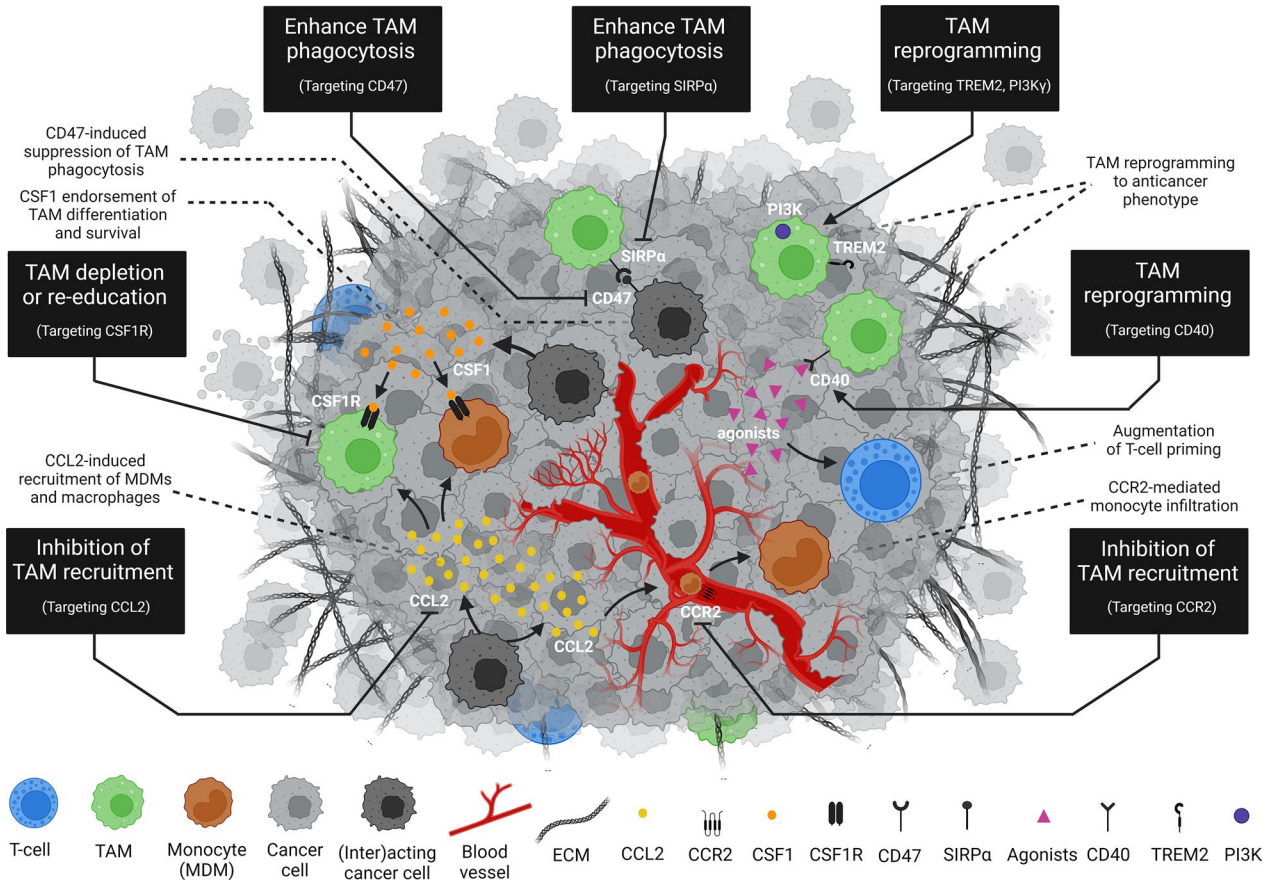

Fig. 7 Therapeutic targeting of TAMs to enhance anti-cancer activity. Several strategies have been and are being developed to determine TAM depletion and/or reprogramming to increase anti-cancer immune activity. The major approaches currently used or being evaluated and indicated in the black boxes and comprise: *1)* enhancing TAM-induced phagocytosis of tumor cells by inhibiting the "do not eat me" CD47/SIRPα pathway; *2)* reprogramming TAMs by augmenting their antigen presentation to T-cells via CD40 agonists, or by endorsing their re-education to anti-cancer phenotypes by inhibition of TREM2 or PI3Kγ; *3)* inhibiting TAM recruitment to the TME through suppression of CCL2 or CCR2; *4)* depleting or re-educating TAMs through inhibition of CSF1R signaling. This figure has been created with BioRender.com

MDM increase within the cancer [558–560]. The tumor cell-mediated release of CCL2 can recruit both CCR2-expressing Ly6C^{hi} monocytes and tissue-resident macrophages from the blood circulatory system extravasating into diverse cancer sites and then differentiating into TAMs [561]. The CCR2-dependent MDM recruitment is critical for breast cancer metastasis to lung and bone [562, 272]. In a phase 1b clinical trial (NCT01204996), the anti-CCL2 carlumab in combination with standard-of-care chemotherapy, showed favorable safety profile in advanced solid tumor patients although no long-term inhibition of serum CCL2 or significant cancer responses was detected [563]. The anti-CCR2 monoclonal antibodies PF-04136309, MLN1202, and TAK-202 have been used in the clinic. In a phase 1 clinical study (NCT01413022), PF-04136309 in co-administration with folinic acid + fluorouracil + irinotecan hydrochloride + oxaliplatin (FOLFIRINOX) chemotherapy led to 49% tumor response, and 96% local tumor control, in advanced pancreatic adenocarcinoma patients. Notably, PF-04136309 monotherapy does not show the same effect, emphasizing the requirement for rational combinatorial strategies in the clinic [564]. Besides, in a phase 2 clinical trial (NCT02732938), PF-04136309 in combination with nab-paclitaxel and gemcitabine displayed favorable safety profile, and 49% objective tumor response, in advanced pancreatic cancer patients [565]. Nevertheless, the effective advantage of targeting CCL2/CCR2 to treat cancer patients is still uncertain. In fact, there is still lack of significance in efficacy and unfavorable safety profile to date, mainly due to body-mediated capability to counteract and/or neutralize suppression of CCL2/CCR2 signaling pathway by enhancing compensatory mechanism-induced CCL2 systemic levels [566].

Table 4 Inhibitors, antibodies, recombinant fragment fusion proteins, recombinant cytokines, and cytokines targeting the innate immune system in the TME for cancer therapy used in clinical trials or approved by the FDA. Data has been collected from http://www.fda.gov, and http://www.clinicaltrials.gov accessed in November 2023

Drugs targeting the innate immune system in the TME							
Targeting TAMs							
Targeted Molecule	**Drug Name**	**Type of Agent/s**	**Mechanism of Action**	**Status**	**Cancer Type/s**	**NCTs**	**References**
CCL2	Carlumab	Neutralizing antibody	Limits monocyte and macrophage recruitment to the TME	Phase 1	ASTs	NCT01204996	PMID: 24928772
CCR2	TAK-202	Neutralizing antibodies	Limit monocyte recruitment and infiltration into the TME	Phase 1	ASTs (MEL)	NCT02723006	PMID: 37114134
	PF-04136309			Phase 2	ASTs (PC)	NCT02732938	PMID: 31297636
	MLN1202			Phase 2	ASTs	NCT01015560	PMID: 25688243
CSF1R	Emactuzumab	Neutralizing antibodies, small-molecule inhibitors	Reduce macrophage survival or lead to macrophage re-education	Phase 1	ASTs	NCT01494688	PMID: 31114846
	PLX7486			Phase 1	ASTs	NCT01804530	PMID: 28716061
	BLZ945			Phase 2	ASTs	NCT02829723	PMID: 34027092
	Edicotinib			Phase 2	LEU (AML)	NCT03557970	PMID: 33842370
	ARRY-382			Phase 2	ASTs	NCT02880371	PMID: 35302585
	Pexidartinib			Approved	ASTs (TGCT)	NCT02371369	PMID: 31229240
CD40	Sotigalimab	Agonist antibodies	Activate host APCs to induce significant clinical anticancer T-cell response	Phase 1	ASTs (PC)	NCT03214250	PMID: 33387490
	Chi Lob 7/4			Phase 1	ASTs	NCT01561911	PMID: 29637478
	CP-870,893			Phase 1	ASTs	NCT00607048	PMID: 23483678
	Dacetuzumab			Phase 2	DLBCL	NCT00435916	PMID: 24919462
	rhuCD40L			Phase 2	ASTs (HNSSC)	N/A	PMID: 11432896
CD47	CC-90002	Neutralizing antibodies	Interfere with recognition of CD47 by the SIRPα receptor on macrophages	Phase 1	LEU (AML), MDS	NCT02641002	PMID: 34981142
	ZL-1201			Phase 1	ASTs	NCT04257617	PMID: 35860564
	Magrolimab			Phase 1	MDS	NCT03248479	PMID: 36888930
SIRPα	TTI-621	Recombinant fragment fusion proteins	Bind to CD47 and preclude it from delivering an inhibitory "do not eat" signal to macrophages	Phase 2	DLBCL	NCT05507541	PMID: 36578079
	TTI-622			Phase 2	DLBCL	NCT05507541	PMID: 36578079
TREM2	PY314	Neutralizing antibody	Induces macrophage re-education into antitumoral phenotypes	Phase 1	ASTs	NCT04691375	PMID: 38372949
PI3Kγ	Eganelisib	Small-molecule inhibitor	Induces macrophage re-education into antitumoral phenotypes	Phase 2	ASTs	NCT02637531	PMID: 37000164

Table 4 (continued)

Targeting MDSCs							
PDE-5	Tadalafil	Small-molecule inhibitor	Inhibits MDSCs restoring T cell function and exerting anticancer effects	Phase 2	ASTs (HNSCC, MEL)	NCT00843635	PMID: 25320361
Targeting DCs							
GM-CSF	GM-CSF	Cytokines	Boost anticancer immunity by promoting differentiation of DCs	Phase 2	ASTs (MEL)	NCT00350597	PMID: 18591558
	Sargramostim			Phase 2	ASTs	NCT04703426	PMID: 19483646
	GM-CSF vaccines			Phase 2	ASTs (BC), MM	NCT00880464 (ASTs), NCT01349569 (MM)	PMID: 35482127 (ASTs), PMID: 34667029 (MM)
FLT3L	CDX-301	Recombinant cytokines	Expansion of DCs and infiltration in the TME	Phase 2	ASTs	NCT02839265	PMID: 29872569

Targeted Molecules: CCL2: CC-motif chemokine ligand 2; CCR2: CC-chemokine receptor 2; CD40: Cluster of differentiation 40; CD47: Cluster of differentiation 47 or integrin associated protein (IAP); CSF1R: Colony-stimulating factor-1 receptor; FLT3L: Fms-related tyrosine kinase 3 ligand; GM-CSF: Granulocyte-macrophage colony-stimulating factor; PDE5: Phosphodiesterase 5; PI3K: Phosphoinositide 3-kinase; SIRPα: Signal regulatory protein α; TREM2: Triggering receptor expressed on myeloid cells 2. *Cancer Types*: AML: Acute Myeloid Leukemia; ASTs: Advanced Solid Tumors; BC: Breast Cancer; DLBCL: Diffuse Large B-Cell Lymphoma; HNSCC: Head and Neck Squamous Cell Carcinoma; LEU: Leukemia; MDS: Myelodysplastic syndrome; MEL: Melanoma; MM: Multiple myeloma; PC: Pancreatic Cancer; TGCT: Tenosynovial Giant Cell Tumor. In case drugs targeting the innate immune system in the TME for cancer therapy are being used in several ASTs, only a representative clinical trial (NCT) and its related reference (PMID) have been selected for (a) specific AST(s)

Additionally, increased TAM cell division and angiogenesis can possibly weaken CCL2/CCR2 immunotherapy-mediated effects [567] (Table 4) (Fig. 5).

CSF1R inhibitors

Inhibition of CSF1R, which suppresses the receptor for the main survival and differentiation factor of TAM, is another valuable strategy to target TAMs [568–573]. The class III receptor tyrosine kinase CSF1R signaling occurs upon binding of its CSF1 and IL34 ligands, which are differently expressed in specific tissues [574, 575]. Neutralizing antibodies and small-molecule inhibitors against CSF1R either determine re-education of TAMs into a pro-tumoricidal phenotype [576] or reduce intra-tumoral TAMs [577]. This strategy has led to significant anticancer activity in glioblastoma, breast cancer, and pancreatic cancer, as well as decrease breast-to-lung metastasis in preclinical models [50]. In addition, CSF1R inhibition has been investigated in combination with other agents in preclinical studies [569–579]. Besides, suppression of CSF1R enhances the efficacy of tyrosine kinase inhibitors and radiotherapy in glioblastoma models, through TAM re-education [580, 581]. Also, the potency of paclitaxel is augmented by CSF1R inhibitor-induced TAM reduction in breast cancer models [582]. Several CSF1R-inhibiting drugs including emactuzumab, PLX7486, BLZ945, edicotinib, ARRY-382, and pexidartinib, have been tested in clinical studies, either singly or in combination with conventional therapies. Notably, numerous clinical trials have displayed contrasting results depending on the type of cancer. A phase 2 study (NCT01349036) using the tyrosine kinase inhibitor pexidartinib monotherapy showed penetration of the blood-tumor barrier and favorable safety profile in recurrent glioblastoma patients. Nevertheless, this treatment did not improve PFS, PR and CR, although TAMs were reduced in tumor biopsies of these patients [583]. Conversely, in a phase 3 study (NCT02371369), pexidartinib exhibited significant clinical benefit in advanced tenosynovial giant cell tumor (TGCT) patients defined by enhanced CSF1R and CSF1 expression [584]; and thus, has gained FDA approval as an oral medication for use in this cohort [585]. Also, pexidartinib is being investigated in several tumors as monotherapy or combination therapy. There are possibly three main explanations, listed below, for these diverse clinical results. *1)* CSF1R is remarkably enhanced in TAMs, but it is also expressed in other cells such as MDSCs, tumor cells, and neurons. Thence, variation in potency of CSF1R inhibition is likewise due to additive effects on different types of cells, in tumor-dependent manner and/or organ- dependent manner [50]. *2)* Acquired resistance to CSF1R inhibition can occur due to induced compensation of the IGF1R/PI3K/AKT/mTOR transduction pathway, which leads to cancer recurrence, as shown in

preclinical studies of glioblastoma [586–588]. In line with this, co-inhibition of CSF1R and these aforementioned signaling networks significantly augments survival in mice. Thus, these approaches may be considered in clinical trials, since the involvement of these signaling axes can also underlie CSF1R inhibitor resistance, especially in activated PI3K pathway-driven tumors [586]. *3)* The functional heterogeneity of TAMs detected in various TME [589, 590] accounts for cancer-dependent effects of CSF1R suppression, resulting in TAM reduction versus TAM re-education, and thus, causing divergent efficacy of therapy [576]. Therefore, environmental signal modulation including GM-CSF, IFNγ, and lipopolysaccharide, may be actuate to polarize TAMs to endorse an immune stimulation, thereby ultimately resulting in cancer regression [591] (Table 4) (Figs. 4, 5).

CD40 agonists

The TNF receptor superfamily member CD40 is expressed on APC (e.g. DCs and TAMs), and thus is essential for induction and proliferation of these specific cells [592, 593]. CD40 critically regulates T-cell-dependent anticancer immune response, and consequently interacts with $CD4^+$ T-cell-expressing CD40 ligand (CD40L) [594]. Induction of CD40/CD40L signaling pathway upregulates MHC molecules and generates pro-inflammatory cytokines important for priming T-cells [595, 596]. The agonistic antibodies toward CD40 promote generation of IFNγ, resulting in a tumoricidal phenotype reprogramming of TAMs, and leading to cancer regression in pancreatic cancer preclinical models and in pancreatic cancer patients [597]. Interestingly, this TAM reprogramming becomes more remarkable when a CD40 agonist and CSF1R inhibition are combined, before TAM depletion, resulting in a pro-inflammatory TME that bolsters a potent T-cell response [598]. CD40 monoclonal agonistic antibodies are being tested in various clinical trials of advanced solid tumor patients. In a phase 1 clinical trial (NCT00607048), CP-870,893 in combination with paclitaxel and carboplatin determined encouraging responses and a favorable safety profile in advanced solid tumor patients, providing a rationale for phase 2 studies [599]. Besides, in a recent phase 1b clinical trial (NCT03214250), the combination of CD40 antibody sotigalimab and chemotherapy (gemcitabine + nab-paclitaxel) with/without nivolumab, showed clinical activity and favorable safety profile in metastatic pancreatic adenocarcinoma patients. Thus, this co-treatment regimen may replace chemotherapy-only standard of care in these patients if successfully confirmed in later phase trials [600]. In addition, a phase 2 clinical trial of rhuCD40L exhibited efficacy, with one patient entering into long-term complete remission, several patients reaching PR, and others showing only mild responses, in advanced head and neck squamous cell carcinoma (HNSCC) patients [601]. Dacetuzumab and Chi Lob 7/4 were investigated in phase 2 for diffuse large B-cell lymphoma (DLBCL) patients (NCT00435916), and in phase 1 for ASTs (NCT01561911), respectively; however, they only displayed modest activity. Thence, overall CD40 agonistic antibodies have only shown moderate results as anticancer therapy (Table 4) (Fig. 5).

CD47 antagonists

TAMs normally exert cancer-endorsing effects. However, TAMs may also inhibit cancer growth through phagocytosis of tumor cells and activation of diverse immune responses [602–603]. The main mechanism whereby the phagocytic functions of TAMs can be subverted in the TME is via CD47-SIRPα interactions [604]. The immune checkpoint signaling receptor CD47 is a "do not-eat me signal". In normal cells, CD47 is constitutively expressed, whereas in tumor cells, CD47 is overexpressed, binding to myeloid inhibitory immunoreceptor SIRPα, primarily expressed in TAMs [605]. In macrophages, the CD47-SIRPα binding triggers signaling that suppresses their phagocytic capability [606]. Thus, blockage of the CD47-SIRPα interaction eliminates this repressive signal and enhances macrophage-induced tumor cell removal [607]. Interestingly, targeting the CD47-SIRPα signaling axis holds promise for cancer therapy toward innate immune checkpoints, as evidenced by significant data in preclinical models [608, 609]. Indeed, the anti-CD47 antibody magrolimab has displayed significant anti-cancer activity in mouse pediatric brain cancer [610]. Additionally, in a recent phase 1b clinical trial (NCT03248479), the combination of magrolimab and azacytidine, which increases expression of "eat-me signals", showed promising efficacy and favorable safety profile in untreated higher-risk MDS patients, including those with *TP53* mutations. Nevertheless, in a phase 1 clinical trial (NCT02641002), CC-90002 monotherapy displayed no objective responses, in high-risk MDS patients and relapsed/refractory AML patients [611]. Besides, the novel recombinant, humanized monoclonal antibody that specifically targets CD47, named ZL-1201, is presently being tested in a phase 1 dose escalation clinical study to assess its safety in advanced solid tumor patients [612]. TTI-621 and TTI-622, two recombinant SIRPα-crystallizable fusion proteins, in combination with pembrolizumab are also being investigated in a phase 2 clinical trial (NCT05507541), in relapse/refractory DLBCL. Results from ongoing trials with magrolimab in AML patients will plausibly provide important information on the therapeutic potential of CD47 neutralizing antibodies on cancer patients (Table 4) (Fig. 5).

TREM2 inhibitors

Suppression of TREM2 receptor is another approach whereby TAMs can be reprogrammed [613–616]. The Ig superfamily member TREM2 receptor interacts with adaptor proteins DNAX activation protein of 10 kDa (DAP10) and DNAX activation protein of 12 kDa (DAP12), along with other extracellular ligands [617, 618]. Combination of TREM2 deletion and PD-1 inhibition has been shown to decrease cancer growth in various animal models. Indeed, TREM2 deficiency shifts TAMs from an immunosuppressive phenotype to an anti-cancer phenotype, leading to reduced cancer progression in mice [619, 620]. Interestingly, TAMs have been reported to express TREM2 in at least 200 human tumor cases, and its elevated level correlates with negative results in breast cancer and CRC [90]. Consequently, humanized monoclonal antibody PY314 is being produced in order to diminish TREM2-expressing TAMs. In a recent phase 1 clinical study (NCT04691375), PY314 both as monotherapy or in combination with pembrolizumab, exhibited favorable safety profile in advanced solid tumor patients. A recommended dose for expansion has derived from these results, and enrollment in several prespecified tumors is ongoing [621] (Table 4) (Figure 5).

PI3K inhibitors

The PI3K family members exert critical effects on the immune system [622, 623]. Indeed, PI3Kγ is an important regulator of TAM-induced immunosuppression [624–626]. Suppression of PI3Kγ in TAMs reduces expression of IL10 and augments expression of MHC-II and IL-12, leading to immune cell recruitment, increased anti-cancer activity, and successive regression of cancer in mouse models [624]. Moreover, inhibition of PI3Kγ overcomes resistance to ICI, stimulating T-cell-induced regression of cancer [627]. Since PI3Kγ can switch immune suppression to immune stimulation, early phase clinical studies are presently testing the PI3Kγ inhibitor eganelisib either as monotherapy or in combination with ICI in several tumors. In the MARIO-1 phase 1/phase 1b clinical trial (NCT02637531), the combination of eganelisib and PD-1 inhibitor nivolumab, has shown anti-cancer activity in advanced solid tumor patients, including those who had previously progressed on PD-1 and/or PD-L1 inhibitors. Safety profile-based doses 30 mg/once daily and 40 mg/once daily of eganelisib, in co-treatment with PD-1 inhibitors and/or PD-L1 inhibitors, have been selected for phase 2 of this study [628]. In 2020 the FDA granted fast-track designation to a phase 2 clinical trial (NCT03961698) evaluating eganelisib in co-treatment with chemotherapy and ICI, for first-line treatment of advanced or metastatic TNBC patients (Table 4) (Fig. 5).

Collectively, targeting TAMs aims to reprogram these immunosuppressive cells within the TME to enhance anti-tumor immunity. Though preclinical studies related to CSF1R inhibition have shown potential, the results of clinical trials are conflicting and unsatisfactory, probably due to the complexity and heterogeneity of TAMs. Thus, future research should focus on identifying specific markers for different TAM subsets, developing more selective targeting approaches, selecting patients with high TAM infiltration, and combining TAM-targeted therapies with other treatments to improve overall efficacy.

Targeting MDSCs

In recent years, the heterogeneous population of immature MDSCs have also emerged as important cells to be targeted in cancer immunotherapy [292]. Notoriously, MDSCs endorse cancer growth by inhibiting the activity of NK-cells and T-cells, thence contributing to resistance to immunotherapy [629]. Indeed, numerous studies have shown significant correspondence between negative response to ICI therapies and high number of MDSCs [630–61]. MDSCs also contribute to EMT activation and can be targeted through inhibition of their immunosuppressive activity [288, 61]. Since overexpression of ARG1 and iNOS is one of the main mechanisms by which MDSCs induce anergy in NK cells and T-cells, PDE-5 inhibitors such as sildenafil and tadalafil are known to downregulate the expression and activity of these enzymes and subsequently restore NK and T-cell functions [632, 633]. Several mechanisms can target MDSCs [634]. Some of TAM-targeting agents currently under clinical investigation, including CSF1R inhibitors, can also contribute to interfering with MDSC recruitment into the tumor. Contrarily, the CSF1R inhibitor-related adaptive mechanisms of resistance engaging MDSCs can occur. Indeed, upon inhibition of CSF1R, CAFs release granulocyte-recruiting chemokines, resulting in enhanced cancer-stimulating MDSCs and enlargement of tumor in various animal models. Interestingly, CXCR2 inhibitors can decrease these adaptive effects, whereas adding anti-PD-1 agent-base co-treatments can further augment these adaptive effects [635]. Phase 2 clinical trials with PDE-5-targeting tadalafil in melanoma and HNSCC have demonstrated reduction in MDSCs and Tregs as well as an increase in $CD8^+$ T-cells, suggesting its ability to enhance anti-tumor immunity and improve clinical outcomes [636, 637]. The inhibition of MDSC migration to the tumor is another therapeutic strategy. Interestingly, the CXCR2 signaling has been found to be upregulated in human pancreatic cancer, particularly in MDSCs and neutrophils, but rarely in cancer cells. Since elevated levels of CCL2 and CCL5 in the TME recruit MDSCs through the chemokine receptor CXCR2, the use

of CXCR2-inhibiting peptides can promote T-cell infiltration, reduce metastasis and prolong survival in pancreatic cancer mouse models. Thus, the CXCR2 signaling can be considered an excellent therapeutic target in cancer [638] (Table 4) (Fig. 5).

Targeting neutrophils

Myeloid-derived cells such as neutrophils have also gained importance in recent years. Neutrophils also represent a potential therapeutic target in tumors since several studies have shown their tumor-promoting functions especially in metastasis [639–642]. The role of neutrophils is ambiguous due to their pro-cancerous and anti-cancerous effects depending on the context [643]. However, various clinical studies employing agents associated with the activation, recruitment, and functional response of neutrophils have shown encouraging results to improve the specific targeting of these specific myeloid cells [644–645].

Thus, current studies targeting MDSCs and neutrophils through strategies such as inhibiting their development, blocking their immunosuppressive functions, and depleting their population, have shown promise in preclinical models. However, the clinical success has been limited due to the heterogeneity and plasticity of MDSCs in different tumors. Future research should aim to better understand the mechanisms driving MDSC expansion and function, identify specific markers for targeting, and develop combination therapies to enhance the efficacy of MDSC-targeted treatments.

Targeting DCs

Highly heterogeneous APC DCs originate from human bone marrow $CD34^+$ precursors, and possess a strong competence in processing and presenting antigens compared to other APCs [646–299]. Importantly, the identification of each DC subtype, such as conventional/classic DCs (cDC) and plasmacytoid DCs (pDC) [647], and their trafficking or location during progression of tumor, have contributed to elucidate the actual role of DCs in cancer immunity, in order to establish valuable approaches for their manipulation [648]. The most critical DC-intrinsic characteristics to determine a potent and stable anticancer response, are: *1)* prime strong effector responses against cancer through tumor-associated antigen (TAA) cross-presentation to $CD8^+$ cytotoxic T-cells; *2)* high migratory capacity between lymphoid and non-lymphoid tissues; and *3)* proper release of cytokines and chemokines to modulate immune response and T-cell homing [649]. Increased DC density, especially DCs within the TME has been shown to improve prognosis in breast, lung, and ovarian cancers. Thus, DCs can be considered as valuable targets for cancer immunotherapy [50, 650]. Nevertheless, the TME can depress important functions of DCs, such as the generation of CC-motif chemokine ligand 4 (CCL4) and/or CCL5 chemoattractants, hindering DC recruitment to cancer sites, and survival signals [e.g. Fms-related tyrosine kinase 3 ligand (FLT3L)] necessary for differentiation and viability of DCs [651]. These caveats lead to incomplete activation of T-cells, and possibly endorsement of T-cell tolerance to TAAs [652] (Fig. 8).

Manipulation of DCs DCs have been manipulated using several approaches, such as: *1)* modulation of DC activity, proliferation, maturation, and survival through GM-CSF; *2)* administration of FLT3L to increase in vivo survival of circulating DCs and enhance subsequent DC trafficking to diverse tissues; and *3)* production of DC vaccines to boost anti-cancer immunity involving manipulation of patient-derived ex vivo DCs to augment various features [50, 653].

- GM-CSF

The inflammatory cytokine GM-CSF, released by diverse cell types, including T-cells, B-cells, macrophages, and mast cells, is responsible for activation and expansion of DCs, macrophages, and granulocytes [654, 253]. In line with this, experiments using several cancer models have indicated that GM-CSF-induced effects on DCs can determine durable anti-cancer immunity [655]. Current early phase clinical trials are testing new potential GM-CSF-based approaches including: *1)* intratumoral monotherapy by direct injection into metastatic lesions; *2)* exposure as an adjuvant therapy via systemic administration; *3)* co-treatment with ICI or chemotherapy; *4)* as GM-CSF-secreting vaccines; and *5)* GM-CSF-secreting oncolytic viruses [50]. The administration of GM-CSF as monotherapy augments mature DCs and significantly improves the OS, in melanoma patients [656, 657]. Regarding GM-CSF-secreting vaccines, cancer cells have been successfully harvested from metastatic or stage II-III breast cancer patients to develop autologous GM-CSF-secreting vaccines that determine coordinated immune responses with moderate toxicity (NCT00317603 and NCT00880464) [658]. Besides, in a phase 2 clinical study (NCT01349569), allogeneic MM GM-CSF-secreting vaccine with lenalidomide induced long-term immunity and lasting clinical responses in MM patients [659]. Nonetheless, other clinical trials have not led to positive outcomes mainly due to the negative effects of GM-CSF when administered in high concentrations [660]. The limited potency of GM-CSF is also caused by its enhancement of immune-suppressive populations, such as MDSCs and Tregs, that suppress the

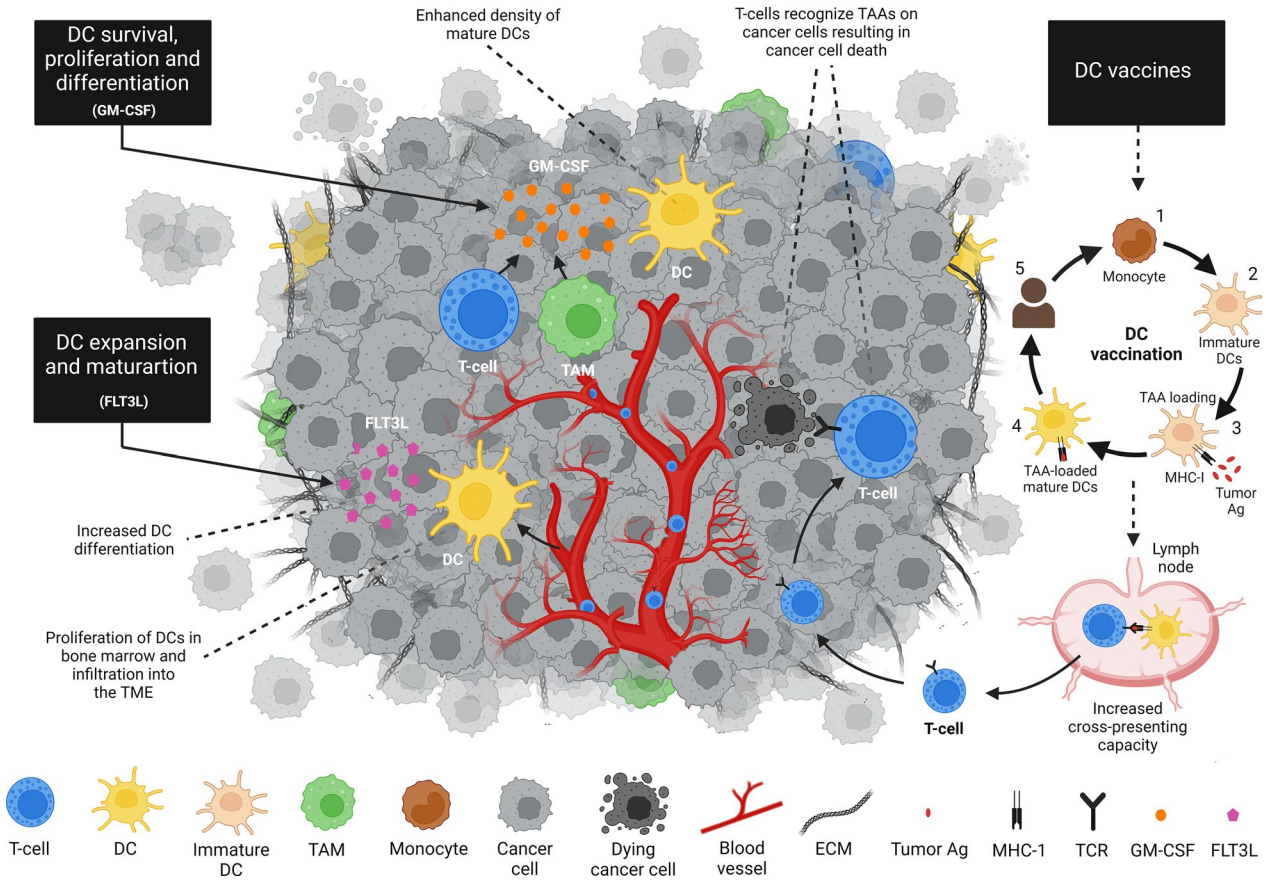

Fig. 8 Therapeutic targeting of DCs to increase anti-cancer activity. Different targeting strategies have been established to enhance DC-promoted T-cell priming, such as *1*) GM-CSF administration-mediated endorsement of DC survival, proliferation, and differentiation; *2*) FLT3L administration-induced DC expansion and maturation; and *3*) ex vivo DC manipulation and administration in the form of a DC vaccine. The DC vaccines manipulate DCs ex vivo to augment their presentation capacity for specific TAAs in vivo, and are created through the following steps: *1*) $CD14^+$ monocytes or $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) are isolated from the blood of a cancer patient; *2*) $CD14^+$ monocytes or $CD34^+$ HSPCs are differentiated into immature monocyte-derived dendritic cells (moDCs); *3*) immature moDCs are subjected to TAA loading normally attained from tumor lysates; *4*) DCs are genetically engineered to increment their cell-intrinsic features, e.g. cross-presentation, cytokine production, and lymph node migration, thereby augmenting their anticancer functions, and complete maturation of DC is accomplished by diverse maturation cocktails; finally, *5*) TAA-loaded matured DCs are then injected back into the cancer patient, either subcutaneously or intradermally, resulting in the increase and improvement of cancer-specific immune responses. The types of DC vaccines can differ depending on cells used for ex vivo manipulation, strategy for TAA delivery, or activation status of DCs infused into the cancer patient. Ag: antigen. This figure has been created with BioRender.com

function of antigen-specific T-cells [661]. In melanoma patients, TVEC, a GM-CSF secreting oncolytic virus derived from herpes and administered by intra-tumor injection was granted FDA approval after demonstrating its superiority to GM-CSF subcutaneous injections [662] (Table 4) (Fig. 5).

- FLT3L

A crucial biochemical mechanism regulating DC development is the interaction between tyrosine kinase receptor fms like tyrosine kinase 3 (FLT3) and its ligand FLT3L [663, 664]. FLT3L exposure augments circulating DCs and their successive spread to distinct tissue in vivo. Thus, FLT3L increases the number of DC in the TME and also contributes to DC maturation, enhancing anti-cancer T-cell priming [665, 666]. A phase 1 clinical trial (NCT00003431) has shown the immunogenicity and safety of recombinant FLT3L CDX-301 in advanced solid tumor patients; however, possible effects on cancer remission as a monotherapy are still being investigated. In a phase 3 clinical trial (NCT00006223), CDX-301 has been delivered as a single agent in acute myeloid leukemia patients; but results have not been fully described. According to positive results obtained with murine models [667], in a phase 2 clinical trial (NCT02839265), the

combination of CDX-301 and stereotactic body radiotherapy, has exhibited activity as systemic therapy and favorable safety, in advanced NSCLC patients [668]. Additionally, in a phase 2 clinical trial (NCT04491084), the triple combination of CDX-301, costimulatory molecule CD40, and stereotactic body radiotherapy, is currently under investigation in lung cancer patients. Understanding the contribution of specific subsets of DCs in acquiring and presenting TAAs, which is informative and important to properly interpret results, remain to be resolved in these aforementioned clinical trials (Table 4) (Fig. 5).

- DC vaccines

Tumor-specific immune responses can be obtained by directly injecting DC vaccines into patients [669–[294]. DC vaccines are classified into distinct categories depending on the strategy adopted for molecular modifications and activation status of DCs or the delivery of TAAs, before being injected back into patients [673]. DC vaccines are produced through the manipulation of ex vivo patient-derived DCs to augment various properties, including: *1)* increasing migration to lymph nodes; *2)* enhancing DC presentation efficiency for specific TAAs; *3)* endorsing recruitment of specialized cells e.g. NK cells, lymphocytes, and supplementary DCs; and *4)* overcoming cancer immunosuppression [649]. The DC vaccination-based therapy augments antigen-specific T-cell activity, as well as antigen-specific B-cell activity, and increase augmentation of $CD8^+$ cytotoxic T-cells (CTLs) within the TME; and thus, represents a promising strategy. Indeed, clinical trials displaying SD, PR, and CR, with favorable safety profile, have been observed [50]. Nevertheless, at present there is still limited evidence of anti-cancer efficacy, plausibly due to the large usage of ex vivo-based manipulation of monocyte-derived dendritic cells (moDCs) [674], which is not an ideal fount of DCs [675]. In fact, moDCs do not determine sufficient antigen presentation, cytokine production, and migration capacity, which are crucial features to overcome the immunosuppressive TME, and thus, leading to a successful DC-based immunotherapy [676, 677]. Notably, recent studies have shown the existence of two diverse cDC subsets, cDC1 and cDC2 [678], with distinct metabolic/functional characteristics, which modulate recruitment/activation of immune effector cells through diverse mechanisms, both in mice and humans [679]. Thence, clarifying the contribution to cancer immunity and the capacity in presenting TAAs of individual DC subsets is important to improve targeted expansion of DCs and their anti-cancer functions to establish more efficient vaccines. Recently, the combination of DC vaccines with ICIs and chemotherapy, which may augment the anticancer response, are being investigated in several clinical trials [519, 680] (Fig. 8).

Together, current strategies to target DCs include enhancing their antigen presentation capabilities, blocking inhibitory signals, and using DC-based vaccines to stimulate anti-tumor immunity. While DC-based therapies have shown potential in preclinical and early clinical studies, challenges remain in effectively activating and sustaining DC function within the immunosuppressive TME. Future research should focus on optimizing DC-activating approaches, exploring different DC subtypes functionally, and conducting larger clinical trials to establish the efficacy of DC-targeted therapies in various cancers.

Targeting stromal cells

Targeting CAFs

The targeting of stromal cells constantly represents an important research topic related to the TME field [681–687]. CAFs are among the main cell types generating ECM molecules in the TME, supporting cancer growth through a variety of mechanisms [688]. Beside depositing ECM, CAFs also release matrix-remodeling enzymes, endorsing EMT, cancer invasion, metastasis, and therapy resistance [689]. Additionally, CAFs increase cancer progression through the secretion of multiple cytokines, exosomes, and growth factors [690, 691]. Notably, CAFs can also influence other components of the TME, including the immune cells and vasculature. In fact, cytokines TGF-β, IL-6, and chemokine (C-X-C motif) ligand 9 (CXCL9) can also regulate T-cell responses, and CAF-derived VEGF can contribute to angiogenesis [50]. Based on these results, targeting CAFs for anticancer therapy has become a priority for several studies. Currently, most anti-CAF therapies being developed target the fibroblast activation protein (FAP), which is overexpressed on the cell surface of CAFs in over 90% of cancers [692]. Besides, FAP-expressing CAFs have been related to immune-suppression in diverse animal models as well as human samples [693, 694]. Various agents targeting FAP, such as RO6874281 (FAP-IL2v) and sibrotuzumab, are being evaluated in early phase clinical trials in several ASTs (NCT02627274, NCT02198274, and NCT03386721). An oral DNA vaccine targeting FAP has also been developed and successfully enhances $CD8^+$ T cell-mediated killing of CAFs, leading to suppressed tumor growth and metastasis in mice [695]. FAP-specific CAR T-cells have also been shown to effectively eliminate FAP^+ CAFs and significantly reduce tumor growth in lung, mesothelioma and pancreatic cancer mouse models [696–698]. Monoclonal antibodies against FAP such as sibrotuzumab have also been tested in a phase 1 study in NSCLC patients

and in a phase 2 study in CRC patients, showing favorable safety profiles. However, these trials have been discontinued due to no significant responses [699, 700]. This low efficacy may be due to the lack of specificity of FAP for CAFs, given that FAP^+ cells reside in other normal tissues and play important roles such as regulating tissue homeostasis in skeletal muscle and bone marrow [111]. Since the CAF function is activated by signaling pathways such as fibroblast growth factor receptor (FGFR), hedgehog (Hh), ROCK, platelet-derived growth factor receptor (PDGFR), NF-κB, TGF-β, and C-X-C chemokine receptor type 4 (CXCR4), inhibitors which specifically target these axes are also under clinical investigation and evaluation [43–702]. Besides, several studies have confirmed that dysregulation of major CAF markers fibroblast-specific protein 1 (FSP1), integrin alpha 11 (*ITGA11*), and integrin β-1 (*ITGB1*), is clearly related to cancer development and progression of various solid tumors, implying that these molecules are prognostic biomarkers and therapeutic targets for cancer treatment that warrants further investigation [701–704]. Interestingly, clinical trials using drugs targeting FGFR and hedgehog have reported productive results. Alterations in the FGFR gene are frequent in urothelial carcinoma and are associated with lower sensitivity to immune interventions. Accordingly, the FGFR-targeting small molecule inhibitor erdafitinib monotherapy has demonstrated significant anti-cancer activity in metastatic or locally advanced FGFR-altered urothelial carcinoma patients, and therefore has been FDA-approved for use in this cohort [705]. There are some effective hedgehog-targeting small molecule inhibitors such as vismodegib and sonidegib that have shown significant clinical benefits in basal cell carcinoma (BBC) patients, and consequently have been FDA-approved for use in this cohort [706]. Nevertheless, in a phase 2 clinical trial (NCT01130142), the combination of saridegib, a potent and specific inhibitor of smoothened, a key signaling transmembrane protein in the hedgehog pathway, and gemcitabine, failed to improve the clinical outcome compared to gemcitabine monotherapy, in metastatic pancreatic cancer patients [43]. Several clinical trials have displayed encouraging results by targeting PDGFR [707]. In particular, in a phase 2 clinical trial (NCT00076011), PDGFR inhibitor axitinib exhibited anticancer activity in metastatic RCC patients. The majority of patients had grade 3–4 treatment-related adverse events, which were manageable by dose modification and/or supportive care [708]. Besides, in a phase 3 clinical study (NCT02684006), axitinib is also being tested in combination with avelumab, versus sunitinib, in advanced RCC. Interestingly, another approach is vitamin A- or vitamin D-mediated CAF reprogramming/normalization. Accordingly, treatment using vitamin D analogue contributes to reverting CAFs to stellate cells and improves anti-cancer potency in pancreatic cancer preclinical models [709–711]. The vitamin D analogue paricalcitol is currently being tested in early phase clinical studies in various ASTs. In particular, a phase 1 clinical study is testing the co-treatment of paricalcitol and chemotherapy in metastatic breast cancer patients (NCT00637897); and a phase 2 clinical study is investigating the triple co-administration of paricalcitol, gemcitabine and nab-paclitaxel in metastatic pancreatic cancer patients (NCT03520790). Other targeted molecules include TGF-β using the small-molecule inhibitor galunisertib in combination with protein kinase inhibitor sorafenib or chemoradiotherapy, which has shown favorable or acceptable safety profile and improved ORR, in advanced solid tumor patients (NCT01246986) (NCT02688712) [712, 713]; CXCR4 using the small-molecule inhibitor AMD3100 in co-treatment with granulocyte colony-stimulating factor (G-CSF), which has determined stronger autologous hematopoietic stem cell mobilization, compared to G-CSF singly, in MM and NHL patients [714, 715]; ROCK using the small-molecule inhibitor AT13148 (NCT01585701), which exhibited favorable safety profile [716]; and vitamin A using the vitamin A metabolite ATRA, which displayed encouraging results in combination with low-dose anti-angiogenic drug apatinib in recurrent/metastatic adenoid cystic carcinoma (ACC) (NCT04433169) [717], as well as in co-treatment with chemotherapy or arsenic trioxide (ATO) in acute promyelocytic leukemia (APL) (NCT00482833) [718]. Besides, in a phase 2 clinical trial (NCT05345002), ATRA is being investigated in combination with the PD-1 inhibitor retifanlimab in recurrent isocitrate dehydrogenase (IDH)-mutant glioma patients. Further research into CAFs as a therapeutic target is still needed, which might include strategies to revert CAFs to their normal quiescent state, and targeting CAF-derived factors, in addition to directly depleting CAFs in the TME. In spite of recent progress in targeting CAFs, there is considerable lack of understanding regarding the biology of this cell type. A recent study has identified a greater number of somatic copy-number alterations in CRC patient sample-isolated CAFs in comparison to normal adjacent tissue-isolated fibroblasts, which undoubtedly complicates the targeted therapy toward these genetically unstable CAFs [719]. Moreover, the specifical target of CD10 positive and GPR77 positive CAFs augments susceptibility to chemotherapy in breast cancer preclinical models [720]. Furthermore, variation in metabolism of lipids in aged fibroblasts can induce therapy resistance in melanoma cells via FATP2 [721]. Thus, understanding the biology of different CAF subtypes, and their specific changes during cancer progression is crucial for a

Table 5 Inhibitors/agonists, antibodies, and vitamin A metabolites targeting CAFs in the TME for cancer therapy used in clinical trials or approved by the FDA

Drugs targeting CAFs in the TME							
Targeting CAFs							
Targeted molecule	**Drug name**	**Type of agent/s**	**Mechanism of Action**	**Status**	**Cancer Type/s**	**NCTs**	**References**
FAP	RO6874281	Blocking antibodies, Antibody-cytokine fusion proteins	Interfere with CAF function and promotes T-cell responses	Phase 2	ASTs	NCT02627274	PMID: 38,630,781
	Sibrotuzumab			Phase 2	ASTs (CRC, NSCLC)	NCT02198274	PMID: 12,624,517
TGFβ	Galunisertib	Small-molecule inhibitor, blocking antibody	Precludes CAF activation and interferes with CAF signaling	Phase 2	ASTs	NCT02688712	PMID: 35,952,709
FGFR	Erdafitinib	Small-molecule inhibitor	Prevents CAF activation	Approved	ASTs (UC)	NCT03390504	PMID: 36,186,154
PDGFR	Axitinib	Small-molecule inhibitor	Interferes with CAF recruitment	Phase 2	ASTs (RCC)	NCT00076011	PMID: 17,959,415
CXCR4	AMD3100	Small-molecule inhibitor	Interferes with CAF signaling	Phase 3	MM, NHL	NCT00103662 (MM), NCT02221492 (NHL)	PMID: 19,363,221 (MM), PMID: 19,720,922 (NHL)
Hedgehog	Saridegib	Small-molecule inhibitors	Prevent and reduce CAF activation	Phase 2	ASTs (PC)	NCT01130142	PMID: 31,035,664
	Vismodegib			Approved	BCC	NCT01367665	PMID: 25,981,813
	sonidegib			Approved	ASTs (BCC)	NCT01327053	PMID: 31,545,507
ROCK	AT13148	Small-molecule inhibitor	Interferes with CAF function	Phase 1	ASTs	NCT01585701	PMID: 32,616,501
Vitamin A	ATRA	Vitamin A metabolite	Induces CAF normalization	Phase 2	ASTs (ACC), LEU (APL)	NCT04433169 (ACC), NCT00482833 (LEU)	PMID: 34,091,189 (ASTs), PMID: 27,400,939 (LEU)
Vitamin D	Paricalcitol	Small-molecule agonist	Induces CAF normalization	Phase 2	ASTs (PC)	NCT03520790	PMID: 36,299,300

Data has been collected from http://www.fda.gov, and http://www.clinicaltrials.gov accessed in November 2023

Targeted Molecules: CXCR4: C-X-C chemokine receptor type 4; *FAP*, Fibroblast activation protein; *FGFR*, Fibroblast growth factor receptor; *PDGFR*, Platelet-derived growth factor receptor; *ROCK*, Rho-associated protein kinase; *TGF-β*, Transforming growth factor-β. *Cancer Types*: *ACC*, Adenoid Cystic Carcinoma; APL: Acute Promyelocytic leukemia; ASTs: Advanced Solid Tumors; BCC: Basal Cell Carcinoma; CRC: Colorectal Cancer; LEU: Leukemia; MM: Multiple myeloma; NHL: Non-Hodgkin lymphomas; NSCLC: Non-Small Cell Lung Cancer; PC: Pancreatic Cancer; RCC: Renal Cell Carcinoma; UC: Urothelial Carcinoma. In case drugs targeting CAFs in the TME for cancer therapy are being used in several ASTs, only a representative clinical trial (NCT) and its related reference (PMID) have been selected for (a) specific AST(s)

successful design of subgroup-specifically targeted treatments (Table 5) (Fig. 4) (Fig. 5) (Fig. 9). Moreover, future research should focus on identifying specific markers for different CAF subtypes, developing selective targeting approaches, and exploring combination therapies to improve outcomes.

Targeting other TME components

Targeting extracellular vesicles (ECVs)

ECVs include exosomes, microvesicles and apoptotic bodies [722]. ECVs have been used as diagnostic markers, as well as therapeutic applications [723–728].

Use of ECVs as diagnostic markers Due to their high accessibility cancer-derived ECVs hold the potential to be used as diagnostic markers [729–734]. Indeed, ECVs have been shown to function in most steps of cancer progression and contain exclusive biomolecules such as nucleic acids, proteins, and lipids [735]. Given that the molecular content of ECVs is greatly dependent on the cell of origin they can provide important information regarding the pathological condition of ECV-generating cells. Cancer-derived ECV biomarkers have recently been investigated to establish novel strategies for cancer diagnosis and prognosis; albeit there is no complete understanding regarding the cancer-specific path-

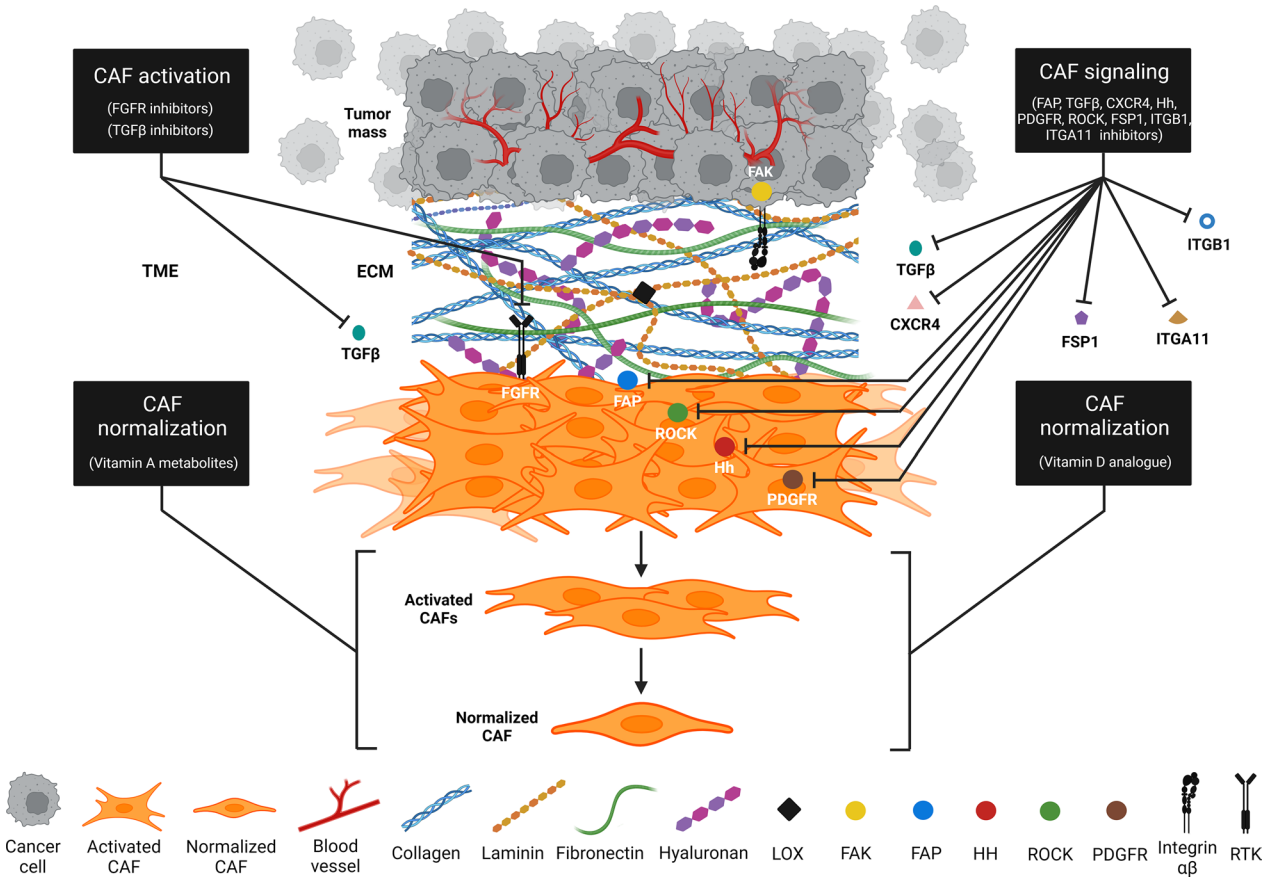

Fig. 9 Therapeutic targeting of CAFs to augment anti-cancer activity. CAFs can be targeted using various strategies, such as interfering with CAF activation using TGFβ and FBFR inhibitors, CAF signaling using TGFβ, CXCR4, FAP, ROCK signaling and Hedgehog signaling inhibitors, or CAF normalization using vitamin A metabolites and vitamin D analogues, which are either FDA-approved or currently being evaluated in clinical trials. CAFs: Cancer-associated fibroblasts; CXCR4: C-X-C chemokine receptor type 4; FAK: Focal adhesion kinase; FAP: Fibroblast activation protein; FGFR: Fibroblast growth factor receptor; FSP1: Fibroblast-specific protein 1 (FSP1); Hh: Hedgehog; ITGA11: Integrin alpha 11; ITGB1: Integrin β-1; LOX: Lysyl oxidases; PDGFR: Platelet-derived growth factor receptor; RTK: Receptor tyrosine kinase; TGF-β: Transforming growth factor-β. This figure has been created with BioRender.com

ways regulating ECV biogenesis in tumor cells, which would certainly contribute to generating novel classes of cancer therapies [736]. Since the levels of ECVs and exosomes are associated with cancer stage, as well as other clinically-related parameters, numerous clinical trials are investigating ECVs as biomarkers. In other studies, the collection of cancer patient tissue biopsies has been incorporated with the TME determined by collecting samples at baseline, after cycle 1 of neoadjuvant chemotherapy, and lastly during surgery [737]. Further investigation into the biogenesis, biomolecule content, and functions of cancer-derived ECV biomarkers is warranted. A comprehensive understanding of the ECV biology and heterogeneity will permit the establishment of better criteria for the use of optimal ECV sub-populations for diagnostics purposes.

Use of ECVs as therapeutic agents Targeting the exosomal secretion and uptake is another strategy against exosome mediated-EMT and metastasis [738–742]. Low pH has been shown to increase exosomal release and uptake by melanoma cells due to increased membrane fusion efficiency, and this can be successfully inhibited when treated with proton pump inhibitors [743]. The small GTPase Rab27a has also been reported to regulate exosomal secretion thereby contributing to the modification of the TME and promoting tumor progression [744]. In line with this, silencing Rab27a in mammary carcinoma cells in a mouse model decreased exosomal secretion and reduced lung metastasis [745]. In addition, silencing Rab27a determined a reduction of exosome production by melanoma cells, as well as decreased cancer growth and metastasis, implying that this strategy can be considered a potential

therapeutic target [746]. Lastly, several studies have demonstrated that heparin pre-treatment blocks exosomal uptake by oral squamous cell carcinoma [747] and urothelial cells [748], inhibiting exosome-induced increase in migration and invasion. This is possibly due to competitive inhibition with cell surface heparan sulfate proteoglycans (HSPGs), which are used for exosome internalization [749, 750]. These studies suggest that targeting different stages in the exosomal pathway is potentially a valuable strategy in preventing cancer spread and metastasis.

Though preclinical studies have shown promise in disrupting the communication pathways mediated by ECVs, clinical translation is still in its infancy due to challenges in effectively targeting and delivering therapies to ECVs. Future research should focus on developing specific inhibitors of ECV biogenesis and uptake, understanding the cargo and functional roles of ECVs in various cancers, and conducting clinical trials to assess the therapeutic potential of ECV-targeted interventions.

Targeting tumor vasculature

The tumor vasculature comprises leaky blood vessels, characterized by impaired vascular perfusion and increased vascular permeability [751–755]. This abnormal vessel structure results in inefficient oxygen and nutrient supply to tumor cells and metabolic waste removal [756, 757]. Coupled with the stagnant and variable blood flow, these factors contribute to hypoxia and acidosis in the TME, in addition to elevated interstitial fluid pressure [758–762]. Moreover, in certain solid tumors, hypoxia in the TME is worsened by the compressive forces exerted on tumor vessels by CAFs and their production of ECM components [763, 754]. The hypoxic and acidic TME reduces the cytotoxic activity of tumor-infiltrating effector T-cells, as well as facilitates the accumulation of immunosuppressive cells such as MDSCs, TAMs, and Tregs and secretion of immunosuppressive factors such as VEGF, TGF-β, and IL-10 to promote angiogenesis and aggravate vascular abnormalities [764–754]. In response to the hypoxic TME, signaling pathways such as HIF-1α are activated, subsequently inducing the EMT program [94]. Antiangiogenic therapies primarily target the endothelial cells of the tumor vasculature. These therapies induce vascular normalization, wherein immature tumor blood vessels are selectively regressed while the vascular integrity and function of mature vessels are enhanced [766–775] (Fig. 10).

Critical role of pericytes as a particular modulator of tumor angiogenesis and vascular integrity Recent research has emphasized the critical role of pericytes as a particular modulator of tumor angiogenesis and vascular integrity. Pericytes support blood vessels by regulating vascular stabilization, permeability, and blood flow. In tumors, the interaction between pericytes and endothelial cells often becomes defective, leading to chaotic and dysfunctional vasculature. This disruption is pivotal in creating a hypoxic and immunosuppressive tumor microenvironment, contributing to cancer progression and therapeutic resistance [776]. Pericyte-targeting strategies, combined with traditional anti-VEGF therapies, are being explored to enhance therapeutic efficacy. However, evidence suggests that the absence of pericytes does not significantly increase the sensitivity of tumor vasculature to vascular endothelial growth factor A (VEGFA) blockade, indicating a complex role of pericytes in tumor resistance mechanisms [777]. Innovative immunotherapeutic approaches are being developed to target antigens expressed by pericytes. One such strategy involves targeting the high molecular weight melanoma-associated antigen (HMW-MAA), which is found on pericytes as well as on various tumor cells. Immunotherapies that target HMW-MAA have shown promise in reducing pericyte populations within the tumor vasculature, thereby impairing tumor growth and enhancing immune cell infiltration [778]. This approach leverages the immune system to disrupt the supportive role of pericytes in the TME, contributing to a more effective antitumor response. Thus, targeting tumor vasculature and pericytes simultaneously offers a more effective approach to disrupt the supportive tumor microenvironment, potentially improving treatment responses and overcoming therapeutic resistance.

Current clinical approaches and challenges in antiangiogenic therapies for cancer treatment Currently, most of the ongoing clinical studies focus on assessing the efficacy of combining antiangiogenic therapies with chemotherapeutic drugs [779] or immunotherapeutic agents [780]. Bevacizumab, an anti-VEGFA inhibitor, is an antiangiogenic agent widely used as the standard-of-care treatment for advanced cancers [781]. Bevacizumab has demonstrated anticancer activity in various ASTs, either as monotherapy or in combination with other drugs, and has received FDA approval for use in these patient cohorts [781–783]. Notably, there are currently over 1,000 registered clinical studies investigating the use of bevacizumab, often in combination with different immunotherapies, highlighting the importance of targeting tumor vasculature in enhancing clinical response [50]. In a meta-analysis of clinical trials, the combination of bevacizumab and chemotherapy showed beneficial effects, leading to improved ORR, PFS, and OS, in metastatic CRC patients, compared to chemotherapy alone [784]. However, in a phase 3 clinical trial (NCT00528567), the combination of bevacizumab with chemotherapy agent anthracycline and/or taxane determined no difference in OS among

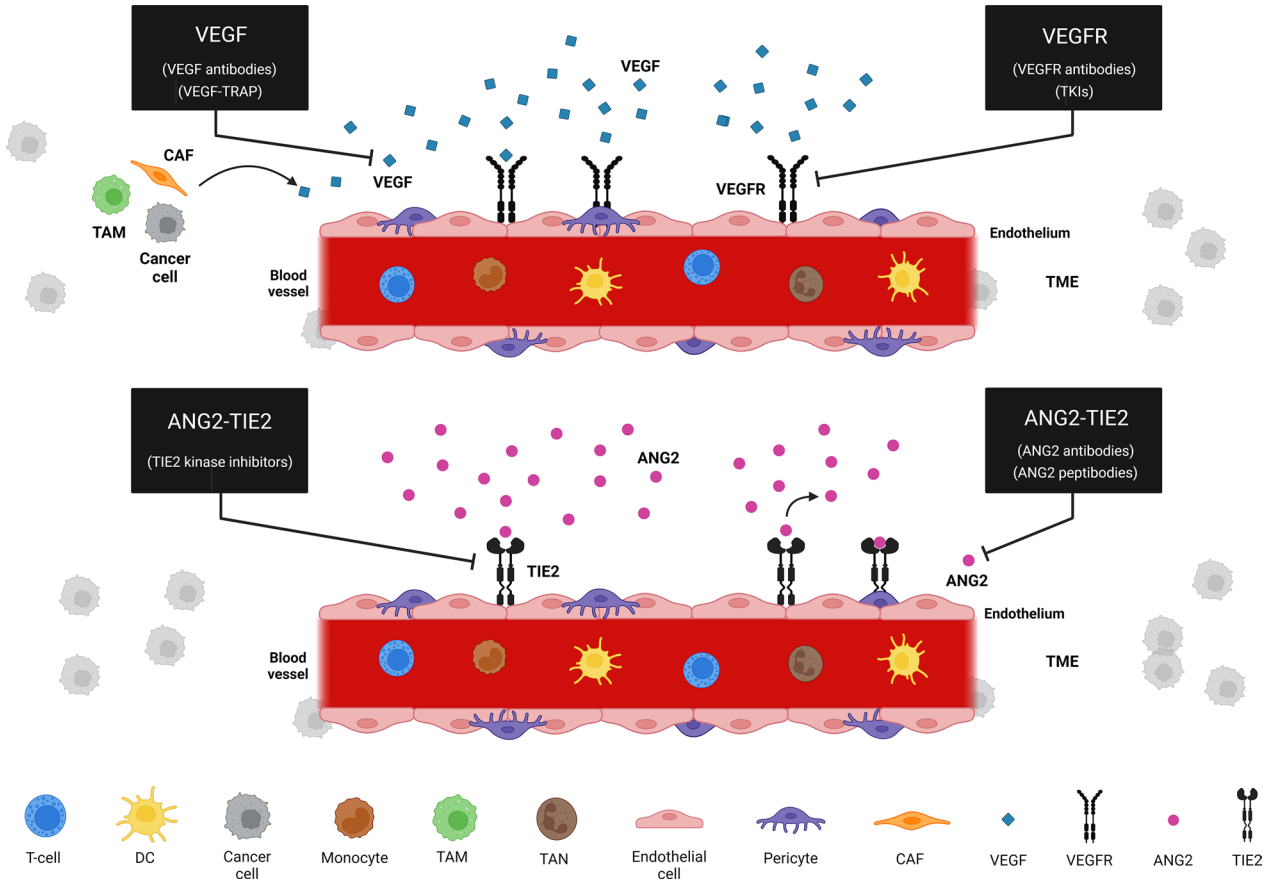

Fig. 10 Therapeutic targeting of tumor vasculature to enhance anticancer activity. Inhibition of VEGF and/or VEGFR is the most used antiangiogenic strategy accomplished with several FDA-approved agents, such as anti-VEGF and VEGF-TRAP (VEGF decoy receptors), and/or VEGFR-specific antibodies and tyrosine kinase inhibitors (RTK inhibitors), respectively. Alternatively, ANG2-TIE2 inhibitors currently being tested in the clinic can also be used to promote antiangiogenesis. The drugs targeting tumor vasculature, either FDA-approved or being-evaluated at different stages of clinical development drugs, are referenced in the text. VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor; ANG2: Angiopoietin-2; TIE2: TEK receptor tyrosine kinase; TKIs: Tyrosine kinase inhibitors. TME: Tumor microenvironment. This figure has been created with BioRender.com

TNBC patients [785]. Aflibercept, a fusion protein acting as a decoy receptor for placental growth factor, VEGFA, and vascular endothelial growth factor B (VEGFB) [786], has demonstrated significant clinical benefits when combined with folinic acid + fluorouracil + irinotecan hydrochloride (FOLFIRI) chemotherapy in metastatic CRC patients, leading to FDA approval for use in this patient cohort [787]. Ramucirumab, a recombinant IgG1 monoclonal antibody specifically binding to vascular endothelial growth factor receptor-2 (VEGFR-2) (anti-VEGFR-2) [788], has shown significant clinical benefits as monotherapy in gastric cancer, HCC, CRC, and NSCLC patients, and thus has gained FDA approval [789–792]. In addition, in a phase 3 clinical study (NCT00777153), cediranib displayed clinical activity, although it did not prolong PFS as monotherapy or in combination with lomustine, in recurrent glioblastoma patients [793]. Interestingly, other studies have shown the therapeutic potential of targeting the urokinase-type plasminogen activator (uPA)/urokinase-type plasminogen activator receptor (uPAR) system, which modulates VEGF-induced angiogenesis through co-internalization with integrin α5β1 [794, 795]. Indeed, in a phase 2, two-arm, double-blind, multicenter, randomized study, combined treatment of uPA inhibitor WX-671 with capecitabine (NCT00615940) has shown an increase ORR and PFS in human epidermal growth factor receptor 2 (HER2)-negative metastatic breast cancer patients [796, 797]. Other FDA-approved antiangiogenic agents include the receptor tyrosine kinase (RTK) small-molecule inhibitors, targeting a broader range of receptors such as fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and vascular endothelial growth factor receptors (VEGFRs). These inhibitors have shown promising results as monotherapies in clinical tri-

als compared to VEGFA inhibitors since they target both tumor vasculature and several dysregulated pathways in tumor cells [798]. Indeed, RTK-targeting inhibitors such as sorafenib monotherapy have exhibited significant clinical benefits in RCC, HCC, and thyroid cancer patients, and have subsequently received FDA approval for use in these cohorts [799–801]. Furthermore, sunitinib monotherapy has displayed significant clinical benefits in gastrointestinal stromal tumors (GISTs), RCC, and pancreatic neuroendocrine tumor patients, leading to FDA approval for use in these cohorts [802, 803]. Additionally, pazopanib monotherapy has demonstrated significant clinical benefits in RCC and STS patients, resulting in FDA approval [804]. Besides, in a phase clinical trial (NCT00678392), axitinib determined a longer PFS compared to sorafenib, in metastatic RCC patients, establishing this RTK-targeting inhibitor as a second-line treatment option in this specific cohort [805]. Alternative antiangiogenic approaches include angiopoietin-2 (ANGPT2)-TEK receptor tyrosine kinase (TIE2) inhibitors, such as MEDI3617, rebastinib, and trebananib, all of which are currently being investigated in clinical trials [806]. However, in a phase 3 clinical trial (NCT01493505), the co-treatment of trebananib, paclitaxel, carboplatin failed to demonstrate clinical benefit in ovarian cancer patients [807]. Additionally, various endothelial cell inhibitors, including endostatin, are currently undergoing clinical evaluation in combination with various drugs [808]. Prominent examples for TME-directed treatments combined with traditional chemotherapy are anti-angiogenic therapies. Their beneficial effects are primarily due to the restricted formation of new blood vessels within tumors [809], the normalization of existing malformed vessels, a decrease in interstitial fluid pressure, the alleviation of hypoxia, and the subsequent enhancement in drug delivery to the tumor site [810]. In fact, clinical trials using pan-VEGFR inhibitors have demonstrated vascular normalization [811] and increased tumor vascular perfusion [812] in glioblastoma patients. Moreover, a single infusion of bevacizumab reduces interstitial fluid pressure and microvascular density in human CRC [813]. Additionally, nanoparticles, liposomes, ultrasound-mediated techniques, and antibody–drug conjugates, represent other strategic approaches to improve drug delivery [814, 815]. Nevertheless, though antiangiogenic therapy has shown clinical benefits in some specific tumors, the overall results have not been as promising as initially expected. In fact, high doses of antiangiogenic agents can increase hypoxia and enhance invasiveness and metastasis of tumor cells. Therefore, judicious dosing of antiangiogenic therapy can support vascular normalization, leading to improved patient outcome [816]. Since continuous suppression of VEGFA can result in compensatory upregulation of other angiogenic factors, various approaches are being evaluated to achieve more durable vascular normalization. Notably, a murinized monoclonal antibody that blocks both ANGPT2 and VEGFA has determined improvement of vascular stability and promotion of antitumor immunity [817]. In the first-in-human phase 1 clinical trial (NCT01688206), vanucizumab also demonstrated promising clinical anticancer activity, accompanied with significant impact on tumor vascularity and an acceptable safety profile, in ASTs patients [818]. Other strategies of vasculature normalization are currently being evaluated in preclinical studies, including the suppression of regulator of G protein (RGS5) signaling, the re-expression of specific semaphorin family members, and the use of endogenous antiangiogenic molecules that are often downregulated in cancer [50, 810]. Other modes of tumor vascularization include the vascular co-option, where tumor cells can hijack pre-existing host vasculature for their growth, vascular mimicry, and the trans-differentiation of tumor cells into endothelial cells. A newly identified regulator implicated in vascular mimicry is the transmembrane glycoprotein receptor CD44 [819]. Consequently, a recent first-in-human phase 1 clinical study (NCT01358903) testing the anti-CD44 monoclonal antibody RG7356 showed moderate clinical anticancer activity, with a 21% SD rate lasting a median of 12 weeks and an acceptable safety profile, in AST patients [820]. The heterogeneity of tumor vascular components must also be taken in consideration [821–823]. In line with this, a recent study discovered previously unknown phenotypes of tumor endothelial cells at the single-cell level in mouse lung tumor models and patient samples [824]. Providing answers to these interrogative studies will be essential for designing new treatments as alternatives to existing antiangiogenic therapies. The tumor vascular networks are significantly related to the efficacy of tumor immunotherapy since modulating tumor vasculature can enhance immune cell infiltration into tumors using various approaches. Vasculature-mediated CTL infiltration can be impaired by various mechanisms: *1)* T-cell inhibition through the expression of suppressive molecules and receptors; *2)* deregulation of cell-adhesion molecules including vascular adhesion molecule 1 (VCAM1) and intercellular adhesion molecule 1 (ICAM1), and chemokines; and *3)* T-cell death activation by endothelial cell-mediated production of selective Fas ligand (FasL) [754, 825]. Thus, new strategies of combined antiangiogenic therapy and immunotherapy have been investigated in animal models [817, 826] and are currently being evaluated in ongoing clinical studies [827, 828], mostly with a primary focus on combinations of antiangiogenic therapies with ICI approaches and vaccines [829]. Indeed, in a phase 3 clinical trial (NCT02853331), the combination of

Table 6 Inhibitors and antibodies targeting tumor vasculature in the TME for cancer therapy used in clinical trials or approved by the FDA. Data has been collected from http://www.fda.gov, and http://www.clinicaltrials.gov accessed in November 2023

Drugs targeting tumor vasculature in the TME							
Targeting Tumor Vasculature							
Targeted Molecule	**Drug Name**	**Type of Agent/s**	**Mechanism of Action**	**Status**	**Cancer Type/s**	**NCTs**	**References**
VEGF/VEGFR	Cediranib	Neutralizing antibodies, fusion proteins (VEGF-TRAP)	Antiangiogenic therapy	Phase 2	ASTs (GLI)	NCT00777153	PMID: 23,940,216
	Bevacizumab			Approved	ASTs (BC, CC, CRC, FTC, GLI, HCC, NSCLC, OC, PPC, RCC)	NCT03829410	PMID: 38,231,047
	Aflibercept			Approved	ASTs (CRC)	NCT00561470	PMID: 24,368,879
	Ramucirumab			Approved	ASTs (CRC, GC, HCC, NSCLC)	NCT00917384	PMID: 24,094,768
uPAR	WX-671	Small-molecule inhibitor	Antiangiogenic therapy	Phase 2	BC	NCT00615940	PMID: 35,158,766
RTKs	Axitinib	Small-molecule inhibitors	Antiangiogenic therapy	Phase 3	ASTs (RCC)	NCT00678392	PMID: 23,598,172
	Sorafenib			Approved	ASTs (HCC, RCC, TC)	NCT00073307	PMID: 17,215,530
	Sunitinib			Approved	ASTs (GIST, PC, RCC)	NCT00428597	PMID: 27,836,885
	Pazopanib			Approved	ASTs (RCC, STS)	NCT00720941	PMID: 23,964,934
ANG2–TIE2	MEDI3617	Neutralizing antibodies/					
peptibodies, small-molecule							
inhibitors	Antiangiogenic therapy	Phase 1	ASTs	NCT01248949	PMID: 29,559,563		
	Rebastinib			Phase 2	ASTs	NCT03601897	PMID: 34,440,616
	Trebananib			Phase 3	ASTs	NCT01493505	PMID: 31,076,365

Targeted Molecules: ANG2-TIE2: Angiopoietin-2-TIE2; RTK: Receptor tyrosine kinase; uPAR: Urokinase-type plasminogen activator receptor; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor. *Cancer Types*: ASTs: Advanced Solid Tumors; BC: Breast Cancer; CC: Cervical Cancer; CRC: Colorectal Cancer; FTC: Fallopian Tube Cancer; GC: Gastric Cancer; GIST: Gastrointestinal stromal tumor; GLI: Glioblastoma; HCC: Hepatocellular Carcinoma; NSCLC: Non-Small Cell Lung Cancer; OC: Ovarian Cancer; PC: Pancreatic Cancer; PPC: Primary Peritoneal Cancer; RCC: Renal Cell Carcinoma; STS: Soft Tissue Sarcoma; TC: Thyroid Cancer. In case drugs targeting tumor vasculature in the TME for cancer therapy are being used in several ASTs, only a representative clinical trial (NCT) and its related reference (PMID) have been selected for (a) specific AST(s)

the RTK inhibitor axitinib and the anti-PD-1 pembrolizumab has shown enhanced ORR and OS, as well as prolonged PFS, compared to the treatment of RTK inhibitor sunitinib alone, in advanced RCC patients [830]. Furthermore, in a phase 2 clinical trial (NCT00678119), the co-treatment of sunitinib and autologous DC-based immunotherapy has exhibited significant anticancer activity in metastatic RCC patients [831] (Table 6) (Figure 4) (Figure 5). Despite the contradictory roles of tumor-associated lymphatic vessels and lymph node metastasis in cancer progression and distal metastasis, targeting the lymphatic vasculature, through inhibition of vascular endothelial growth factor C (VEGFC), vascular endothelial growth factor D (VEGFD), or their receptor vascular endothelial growth factor receptor-3 (VEGFR-3), has also been considered a potential therapeutic approach for tumor control [832, 833]. Specifically, while lymphatic vessels are known to facilitate metastasis and foster an immunosuppressive TME by regulating immune cell functions, they are also critical for promoting antitumor immunity and enhancing immunotherapies [834, 835]. Further research is necessary to elucidate the clinical relevance of lymphatic vessels in human cancer, the impact of lymph node metastasis, and the potential role of organ-specific lymphatic vessels as a supportive premetastatic niche.

Improving drug delivery via vessel normalization The integrity of the vasculature plays an important role in drug delivery and therapeutic efficacy [836–843]. Leaky blood vessels can impair effective delivery of anticancer agents to tumor sites. Poor vascular perfusion can increase IFP within the tumor, creating a physical barrier [844]. Moreover, the tumor ECM can collapse tumor microvessels, sequestering anticancer drugs and serving as can represent a principal physical obstacle [845]. The combination of antiangiogenic therapy and chemotherapy has been shown to yield beneficial effects by promoting vessel normalization and reducing interstitial fluid pressure (IFP), thus enhancing drug delivery [810]. In line with this, a phase 2 clinical trial (NCT00035656) demonstrated that cediranib, a potent inhibitor of all three VEGFRs [vascular endothelial growth factor receptor-1 (VEGFR-1), VEGFR-2, and VEGFR-3], induced vessel normalization and improved tumor blood perfusion in glioblastoma patients [812]. Additionally, anti-VEGFA agent bevacizumab has been shown to improve tumor blood perfusion accompanied with reduced microvascular density and IFP in human CRC [813].

Vascular-targeted therapies in combination with immunotherapies Since neovascularization is closely related to cancer survival, progression, and metastasis [846, 847], approaches targeting the tumor vasculature, often in combination with immunotherapies, can offer therapeutic advantages augmenting the efficacy of conventional antitumor treatments [848–851]. Glioblastoma blood vessels are known to selectively express p21-activated kinase 4 (PAK4) enzyme [852], a selective regulator of genetic reprogramming and abnormal vascularization. Interestingly, Ma et al. (2020) have shown that targeting PAK4 enzyme can reprogram the vascular microenvironment and enhance CAR-T immunotherapy in glioblastoma, a solid tumor notoriously featured by abnormal vascularity [853, 854] that generates an immune-inimical microenvironment and confers resistance to immunotherapy [855–857]. As a result, PAK4 inhibition combined with CAR T-cell engineered to target the EGFR variant III (EGFRvIII) mutation in glioma cells has led to reprogrammed vasculature, endorsing adhesion of immune cells and ability of engineered T-cells to successively penetrate the brain, thereupon determining a potent anticancer response in preclinical models [858]. Hence, targeting PAK4-induced endothelial cells plasticity can possibly represent an important strategy to reprogram the vascular microenvironment and improve cancer immunotherapy.

Taken together, current anti-angiogenic therapies, such as VEGF inhibitors, have shown effectiveness in various cancers by inhibiting new blood vessel formation and modulating the TME. However, resistance to these therapies often develops, and some patients do not respond. Indeed, resistance to antiangiogenic therapies remain a challenge since tumor cells may adopt alternative modes of tumor vascularization, one of which is known as vasculogenic mimicry (VM) [781]. This process, unlike angiogenesis which involves the formation of blood vessels lined by endothelial cells, is characterized by vessels lined with tumor cells [859]. The absence of endothelial cells, therefore, renders antiangiogenic therapeutic agents ineffective. The transmembrane glycoprotein receptor CD44 is a newly identified regulator implicated in VM [819]. Notably, a recent first-in-human phase 1 clinical study (NCT01358903) testing the anti-CD44 monoclonal antibody RG7356 has shown moderate clinical anticancer activity, with a 21% SD rate lasting a median of 12 weeks and an acceptable safety profile, in AST patients [820]. In relation to EMT, several studies have reported the upregulation of various EMT-TFs in VM-forming tumor cells [860, 861]. Consequently, targeting the TME and its downstream EMT activation pathways to inhibit VM formation emerges as a rational therapeutic strategy. For instance, the expression of *ZEB1* was found to be elevated in VM-positive CRC samples compared with VM-negative ones, with lower E-cadherin and higher vimentin expression, which are indicative of EMT [860]. Moreover, *TWIST1* nuclear expression was significantly associated with VM formation in HCC samples, and reduced VM formation was observed in TWIST1-knockdown HCC cells [861]. Furthermore, Ling et al. (2011) reported that the expression of the cytokine TGF-β, which regulates EMT-TFs, was significantly higher in VM-positive glioma cells than VM-negative ones, and accordingly, the depletion of TGF-β significantly impaired VM formation [862]. In addition, anti-angiogenic therapies can cause significant side effects due to their impact on normal vasculature. Future research should focus on understanding the mechanisms of resistance, developing more specific drug delivery methods to avoid side effects, and identifying biomarkers to predict which patients will benefit most from anti-angiogenic treatments.

Targeting ECM

The ECM exerts key roles in regulating TME and cancer [863–873]. Indeed, enhancing stiffness in surrounding tissue endorses EMT in cancer cells, leading to cancer invasiveness, stemness, and metastasis [874]. Additionally, the expression of specific ECM-related genes (e.g. *SPARCL1* and *TWIST*) is related to unfavorable prognosis and increased therapy resistance in numerous cancers [875–877]. Moreover, anomalous augmentation in the ECM can induce integrin and FAK signaling, which results in decreased apoptosis, augmented pro-survival

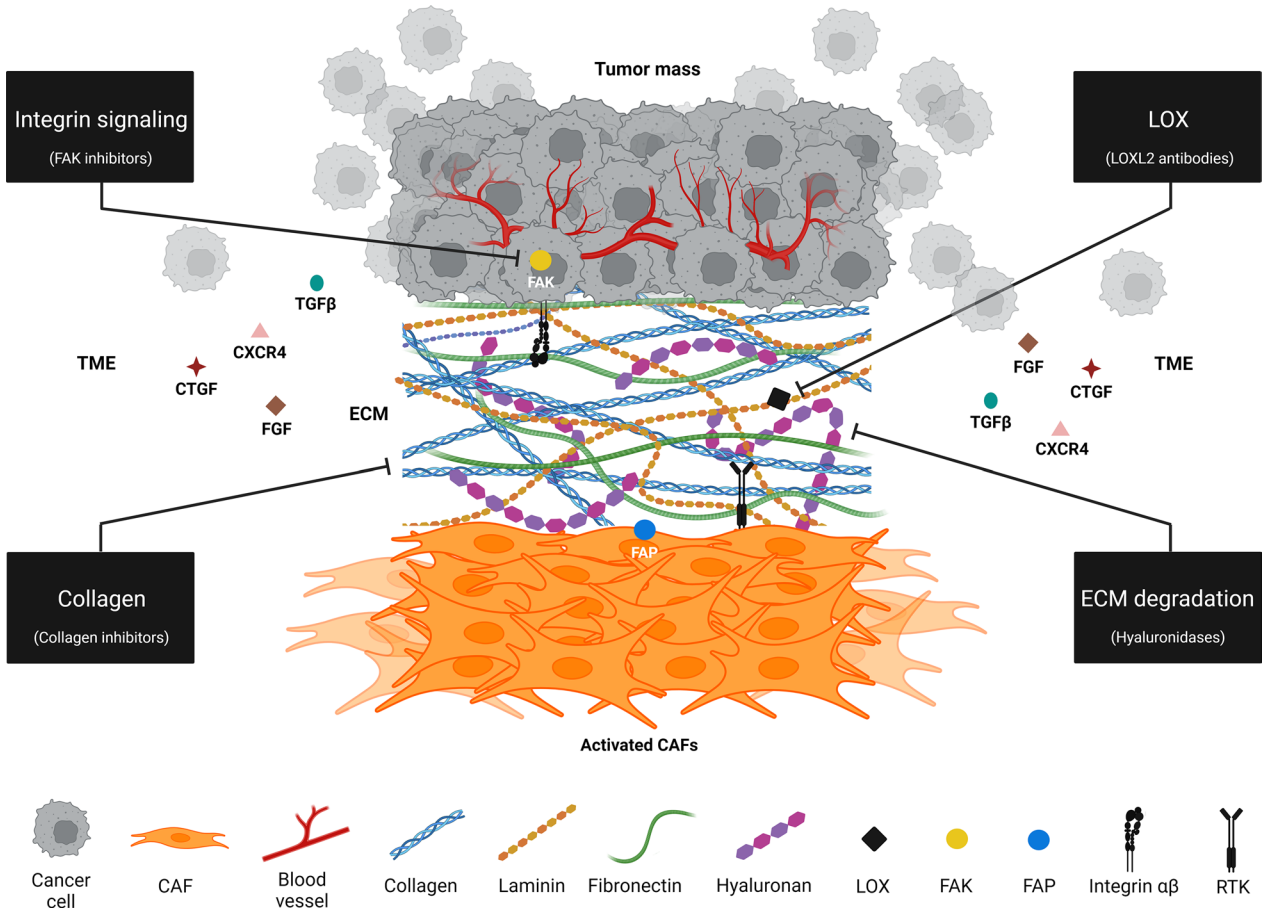

Fig. 11 Therapeutic targeting of ECM to increase anti-cancer activity. The secreted ECM can be targeted with different strategies, such as interfering with integrin signaling using FAK inhibitors, destabilizing collagen network through inhibition of LOX enzymes using LOXL2 antibodies, degrading hyaluronan using hyaluronidases, and enhancing antifibrotic properties by reducing collagen synthesis and production using collagen inhibitors. CAFs: Cancer-associated fibroblasts; CXCR4: C-X-C chemokine receptor type 4; CTGF: Connective tissue growth factor; FAK: Focal adhesion kinase; FAP: Fibroblast activation protein; FGF: Fibroblast growth factor; LOX: Lysyl oxidases; TGF-β: transforming growth factor-β. This figure has been created with BioRender.com.

signaling, and enhanced chemoresistance [878–870]. Furthermore, abnormal increase of the ECM negatively influences treatment potency by determining a sort of physical blockade to various drugs [882]. Targeting of secreted ECM is an active area of development of the TME field. ECM has been mainly targeted through four main different approaches: *1)* degrading the different components of the ECM; *2)* directly inhibiting de novo synthesis of the ECM components; *3)* repurposing of drugs with antifibrotic properties; and *4)* targeting integrins or the downstream effector FAK (Fig. 11).

Degrading the diverse components of the ECM The degradation of the diverse components of the ECM has been achieved by using hyaluronidases or collagenases allowing augmented distribution of therapeutic agents [883]. There are inconsistent results on the use of PEGylated human hyaluronidase (PEGPH20), often in combination with other therapeutic drugs, in clinical studies in advanced solid tumor patients. In a phase 2 clinical trial (NCT01839487), the co-administration of PEGPH20 with gemcitabine and nab-paclitaxel-based chemotherapy showed significant improvement of PFS in pancreatic cancer patients. The hyaluronan-high cancer patients have displayed a higher ORR (45% *versus* 31%) and OS (11.5 months *versus* 8.5 months) compared to the non-hyaluronan-high cancer patients. The most frequent grade 3/grade 4 undesirable effects after this co-treatment include muscle spasms, neutropenia, and myalgia [884]. Nevertheless, a successive phase 3 clinical study (NCT02715804) using the same combination did not exhibit positive results in hyaluronic-high stage IV pancreatic cancer [885]; and in another phase 1b/phase 2 clinical trial (NCT01959139), the combination of PEGPH20 and FOLFIRINOX chemo-

Table 7 Inhibitors, antibodies, and PEGylated enzymes targeting ECM in the TME for cancer therapy used in clinical trials. Data has been collected from http://www.fda.gov, and http://www.clinicaltrials.gov accessed in November 2023

Drugs targeting ECM in the TME							
Targeting ECM							
Targeted Molecule	**Drug Name**	**Type of Agent/s**	**Mechanism of Action**	**Status**	**Cancer Type/s**	**NCTs**	**References**
HA	PEGPH20	PEGylated enzyme	Degradation of HA	Phase 3	ASTs (PC)	NCT02715804	PMID: 29,235,360
LOXL2	Simtuzumab	Blocking antibody	Destabilization of collagen				
networks	Phase 2	ASTs (CRC, PC)	NCT01472198	PMID: 28,246,206			
Collagen	Pirfenidone	Small-molecule inhibitors	Reduce collagen and HA	N/A	ASTs	NCT00020631	PMID: 38,561,001
	Losartan			Phase 2	ASTs (PC)	NCT01821729	PMID: 31,145,418
	Metformin			Phase 3	ASTs (BC)	NCT01101438	PMID: 35,608,580
FAK	Defactinib	Small-molecule inhibitors	Prevent integrin signaling	Phase 2	ASTs	NCT01870609	PMID: 30,785,827
	GSK-2256098			Phase 2	ASTs (PC)	NCT02428270	PMID: 36,636,049
CTGF	Pamrevlumab	Blocking antibody	Prevents integrin signaling	Phase 2	ASTs (PC)	NCT02210559	PMID: 32,817,130

Targeted Molecules: CTGF: Connective tissue growth factor; FAK: Focal adhesion kinase; HA: Hyaluronan; LOXL2: Lysyl oxidase like-2. *Cancer Types*: ASTs: Advanced Solid Tumors; BC: Breast Cancer; CRC: Colorectal Cancer; PC: Pancreatic Cancer. In case drugs targeting ECM in the TME for cancer therapy are being used in several ASTs, only a representative clinical trial (NCT) and its related reference (PMID) have been selected for (a) specific AST(s)

therapy displayed unfavorable effects and enhanced toxicity in metastatic pancreatic adenocarcinoma patients [886] (Table 7) (Fig. 5).

Inhibiting de novo synthesis of ECM components The ECM component de novo synthesis can be directly suppressed by inhibiting crucial ECM-producing signaling axes including hypoxia-inducible factor 1-α (HIF-1α) or TGF-β; or alternatively, by suppressing the modifying enzymes needed for secreting and producing the various ECM components. This can be achieved by targeting LOX enzymes, whose role is crucial for the stabilization of collagen networks. However, the combination of antibody targeting lysyl oxidase homolog 2 (LOXL2) simtuzumab with FOLFIRI or gemcitabine has not shown significant improvement of clinical outcome in pancreatic cancer (NCT01472198) [887] and CRC (NCT01479465) [888] patients, respectively (Table 7) (Fig. 5).

Repurposing of drugs with antifibrotic properties In cancer therapy, using agents already FDA-approved for other indications, known as drug repurposing, is an attractive challenging strategy that can potentially overcome numerous issues related to de novo drug discovery, including dose-finding and safety profiles, thereby facilitating their clinical endorsement [889–892]. Hence, drug repurposing is inexpensive, time-efficient, and riskless in future clinical trials compared to de novo drug development [893]. Repurposing of drugs with antifibrotic properties (e.g. losartan, pirfenidone, and metformin) to treat advanced solid tumor patients is another strategy being used. Interestingly, in a phase 2 clinical trial (NCT01821729), the combination of losartan, FOLFIRINOX, and chemoradiotherapy (fluorouracil or capecitabine) showed clinical benefits in pancreatic cancer patients [894]. However, in a phase 3 clinical trial (NCT01101438), adding metformin to standard breast cancer treatment did not determine significant improvement of invasive disease-free survival in high-risk operable breast cancer patients [895]. There are other drug-repurposing drugs that can potentially be used as a co-treatment for pancreatic cancer, some of which are being investigated in clinical trials. Nonetheless, more studies are required to elucidate the efficacy and safety of several drug-repurposing agents [893] (Table 7) (Fig. 5).

Targeting integrins or the downstream effector FAK Another approach for targeting secreted ECM is through integrins or the downstream effector FAK, since ECM components trigger integrin-induce signaling to activate cellular responses [896–881]. Multiple preclinical studies have demonstrated the use of antibodies and synthetic blocking peptides against αvβ3, αvβ5 or β1 integrins which reduced tumor growth, angiogenesis and metastasis. However, due to their lack of efficacy in clinical trials these therapeutics are not present in the market yet. Possible reasons for this failure include the variable integrin expression in tumors, redundancy of integrins, where blocking one integrin may be compensated for by another

integrin, as well as some integrins having opposing roles during different stages of the disease, leading to variations in patient response to these drugs [902]. Other integrin-targeting approaches have also been explored, such as the anti-αvβ3 protein, ProAgio, which binds outside the classical ligand-binding site of integrin αvβ3, inducing apoptosis by recruiting and activating caspase 8 [904]. ProAgio is currently being tested in a phase 1 clinical trial (NCT05085548) recruiting for participants with advanced solid tumor malignancies including pancreatic cancer [902]. Another ongoing phase 1 study (NCT04389632) is assessing the antibody–drug conjugate SGN-B6A, an integrin β6-targeting antibody conjugated with antimitotic agent monomethyl auristatin E, in advanced solid tumor patients [905]. Additionally, screening of > 10,000 anti-MM antibody clones identified the MM-specific antibody MMG49, which targeted the active conformer of integrin β7 expressed on MM cells [906]. MMG49-CAR T-cells (OPC-415) have since been developed and are currently being tested in a phase 2 trial (NCT04649073). Besides direct targeting of integrins, modulating integrin signaling by targeting downstream kinases have also been studied. For example, FAK inhibitor compounds such as defactinib [907] are currently being investigated in the clinic [908]. In a phase 1 clinical study (NCT02546531), a combination of defactinib, monoclonal antibody pembrolizumab, and gemcitabine, showed promising preliminary efficacy and favorable safety profile, in AST patients [909]. On the other hand, in the COMMAND-A phase 2 clinical trial (NCT01870609), co-treatment of defactinib and a first-line chemotherapy did not determine clinical anti-cancer activity in malignant pleural mesothelioma patients [910]. Additionally, in a phase 2 clinical trial (NCT02428270), the FAK inhibitor GSK2256098 in combination with MEK inhibitor trametinib did not display significant anti-tumor activity in advanced pancreatic cancer patients [911] (Table 7) (Fig. 5). Thus, these studies demonstrate the challenges involved in targeting the ECM. Indeed, the ECM regulates multiple signaling pathways and is formed by various components, making it difficult to be targeted without off-target effects and toxicities often detected in many clinical trials [912].

Targeting physicochemical characteristics in the TME

Even though not being a TME component per se, the physicochemical aspects including oxygenation status and pH are promising targets within the TME [913]. Hypoxic microenvironment plays a key role in EMT induction through its activation of HIF-1α [94, 914], which is a potential therapeutic target [915]. In a phase 1 clinical trial (NCT00522652), the HIF-1α inhibitor PX-478 determined prolonged SD and favorable safety profile in advanced solid tumor patients, supporting the continued investigation of HIF-1α inhibition as a therapeutic target [916]. The antisense oligonucleotide EZN-2968 can downregulate HIF-1α expression and thus is currently undergoing phase 1 clinical trials [917]. Moreover, the molecular chaperone HSP-90 and histone deacetylases (HDAC) inhibitors can also promote HIF-1α degradation [918]. Furthermore, the chemotherapeutic agents doxorubicin and daunorubicin effectively inhibit HIF-1α transcriptional activity by blocking its binding to HREs in target genes [919]. While these studies have mostly examined the effect of these compounds on tumor growth and angiogenesis, it would be of interest to investigate if they are also effective in inhibiting EMT and preventing metastasis. Acidity (i.e., decreased pH of the ECM) is a hallmark of cancer, and is a useful biomarker for targeting metabolically active cells in the TME, including tumor cells and activated macrophages [920]. The pH-(low)-insertion peptide (pHLIP), a pH-sensing peptide, has been used to deliver cytotoxic payloads such as amanitin, and the immune-stimulating cytokine IL-2, for targeted therapy of acidic tumors [921] (Table 8) (Fig. 5).

Collectively, approaches targeting ECM include enzymatic degradation of ECM components, inhibition of ECM synthesis, and blocking interactions between

Table 8 Inhibitors targeting hypoxia in the TME for cancer therapy used in clinical trials. Data has been collected from http://www.fda.gov, and http://www.clinicaltrials.gov accessed in November 2023

Drugs targeting hypoxia in the TME							
Targeting Hypoxia							
Targeted Molecule	**Drug Name**	**Type of Agent/s**	**Mechanism of Action**	**Status**	**Cancer Type/s**	**NCTs**	**References**
HIF-1α	PX-478	Small-molecule inhibitor	Inhibits HIF-1α leading to G2/M phase cell cycle arrest and increased apoptosis	Phase 1	ASTs	NCT00522652	PMID: 18,729,192

Targeted Molecule: HIF1α: Hypoxia-inducible factor 1α. *Cancer Types*: ASTs: Advanced Solid Tumors. In case drugs targeting HIF-1α in the TME for cancer therapy are being used in several ASTs, only a representative clinical trial (NCT) and its related reference (PMID) have been selected for (a) specific AST(s)

ECM and tumor cells. Preclinical studies have shown potential in reducing tumor growth and enhancing the delivery and efficacy of other therapies. However, the clinical translation is challenging due to the complexity and diversity of ECM components in the TME. Future research should focus on identifying novel ECM targets, developing specific inhibitors, and conducting large population-based clinical trials to evaluate the safety and efficacy of ECM-targeted therapies.

Targeting the gut microbiome

Several recent studies have demonstrated the importance of gastrointestinal microbiota in tumor development and regulation of therapeutic response. The gut microbiota, an intricate community of microorganisms residing in the gastrointestinal tract, significantly influences the host's immune system and metabolism, thereby impacting cancer progression and treatment outcomes [922–925]. Specific microbial compositions have been associated with various types of cancer, indicating that the microbiome could serve both as biomarker and therapeutic target [926–929]. Emerging evidence suggests that the gut microbiome significantly influences EMT by modulating various signaling pathways and immune responses within the TME [928]. For example, beneficial bacterial species such as *Akkermansia muciniphila* and *Bifidobacterium* have been related to improved responses to ICIs including anti-PD-1 and anti-CTLA-4 therapies. These bacteria are believed to enhance anti-tumor immunity by promoting the maturation of DCs and increasing the production of pro-inflammatory cytokines, which in turn activate cytotoxic T-cells to target tumor cells [930]. Accordingly, in a recent phase 1 clinical study, the combination of fecal microbiota transplantation and re-induction of anti-PD-1 therapy exhibited positive responses in immunotherapy-refractory melanoma patients [931, 932]. This approach created an immune environment within the TME that is less conducive to EMT, thereby reducing the likelihood of metastasis. Conversely, an imbalance in the gut microbiome, known as dysbiosis, can foster a pro-tumorigenic TME that promotes EMT and cancer progression. Dysbiosis is often characterized by the overgrowth of pathogenic bacteria such as *Fusobacterium nucleatum*, which has been associated with increased production of pro-inflammatory cytokines like IL-6 and TNF-α. These cytokines activate key signaling pathways such as STAT3 and NF-κB, which are known to induce EMT by downregulating epithelial markers (e.g. E-cadherin) and upregulating mesenchymal markers (e.g. N-cadherin and vimentin) [933]. This shift towards a mesenchymal phenotype enhances the invasive and metastatic potential of cancer cells, underscoring the critical role of the gut microbiome in modulating EMT within the TME. The gut microbiome influences EMT through several interconnected mechanisms that involve immune modulation, metabolic regulation, and direct interaction with cancer cells. The interaction between gut microbiota and the immune system is particularly crucial since it modulates inflammation and immune responses, both of which are integral to cancer progression and therapeutic outcomes. Certain bacterial species can influence the production of pro-inflammatory or anti-inflammatory cytokines, altering the immune landscape within the TME. For example, butyrate-producing bacteria like *Faecalibacterium prausnitzii* have been shown to exert anti-inflammatory effects by inducing Tregs and suppressing pro-inflammatory cytokines. This anti-inflammatory environment can help maintain epithelial integrity and prevent EMT [934]. Contrarily, pathogenic bacteria can induce chronic inflammation, a well-established driver of EMT. Chronic inflammation in the TME is often associated with the recruitment of MDSCs and TAMs, both of which secrete factors that promote EMT and tumor progression. For instance, TAMs can secrete TGF-β, a potent inducer of EMT that promotes the transition of cancer cells to a more invasive and metastatic phenotype [935, 936]. Modulating the gut microbiome could therefore reduce inflammatory signals within the TME and inhibit EMT, potentially impeding cancer progression. The gut microbiome is a significant regulator of host metabolism, influencing tumor cells and the host's metabolic state through the production of metabolites and direct interactions with anticancer therapies. The most well-studied microbial metabolites are the short-chain fatty acids (SCFAs), such as butyrate, propionate, and acetate, which have known effects on cancer progression and EMT. In particular, butyrate has been extensively studied for its anticancer properties, especially in CRC cells [937]. Indeed, butyrate inhibits the proliferation of CRC cells through several mechanisms, including promoting autophagy-mediated degradation of β-catenin [938], inducing epigenetic changes [939], enhancing the expression of Toll-like receptor 4 (TLR4), and activating the MAPK and the NF-κB signaling pathways [940]. Additionally, butyrate triggers ferroptosis in CRC cells via the CD44/Solute Carrier Family 7 Member 11 (SLC7A11) pathway and significantly reduces the invasion ability of lung cancer cells, with considerable attenuation of the EMT, characterized by a decrease in the expression of mesenchymal marker and an increase of epithelial marker [941]. In contrast, other microbial metabolites, such as secondary bile acids and polyamines, have been implicated in promoting EMT and cancer progression. Secondary bile acids, produced by certain gut bacteria, can activate nuclear receptors like the farnesoid X receptor (FXR) and the pregnane X receptor (PXR), both of

which are involved in the progression of liver and gastrointestinal cancers. Activation of these receptors by bile acids can induce EMT by downregulating epithelial markers and upregulating mesenchymal markers, thereby enhancing the metastatic potential of cancer cells [942]. These metabolites not only directly impact the growth and proliferation of cancer cells but also modulate the immune environment to support anti-tumor activity. Moreover, the gut microbiome can influence the effectiveness and toxicity of anticancer therapies. For instance, certain bacterial species can metabolize chemotherapeutic drugs into toxic compounds, exacerbating side effects, while others can convert these drugs into less harmful substances, thereby reducing toxicity [943]. Studies have shown that microbiota can modulate the efficacy and toxicity of treatments like cyclophosphamide, an alkylating agent used in cancer therapy. Furthermore, gut bacteria can influence the pharmacokinetics of drugs, altering their absorption, distribution, metabolism, and excretion, which impacts their overall effectiveness and safety profile [944]. The gut microbiota, also regulate toxicity of numerous first-line/new therapies, such as chemotherapy, immunotherapy, and stem cell transplants [945, 946]. In line with this, various studies have shown that specific gut microbiota signatures are related to greater immune cell infiltration into cancer, augmented systemic immunity, and better response to ICI [947–950]. For instance, a microbiota enriched in *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* has been associated with increased infiltration of cytotoxic $CD8^+$ T-cells into the TME, promoting a robust anti-tumor immune response that enhances the efficacy of ICIs. This observation is supported by clinical data, where patients with these beneficial microbial profiles exhibit prolonged PFS and OS compared to those with dysbiotic microbiota [951]. Beyond immune modulation and metabolic regulation, certain gut bacteria can directly interact with cancer cells and influence their behavior. For instance, *Fusobacterium nucleatum* has been shown to adhere to CRC cells and promote their proliferation and invasion by activating the β-catenin signaling pathway. This direct interaction facilitates EMT and enhances the metastatic potential of these cancer cells. Such findings highlight the potential for targeting specific bacterial species to disrupt these pathogenic interactions and inhibit EMT [952]. Given the critical role of the gut microbiome in modulating the TME and EMT, therapeutic strategies aimed at manipulating the microbiome are gaining traction as potential adjuncts to conventional cancer therapies. Probiotics, prebiotics, and fecal microbiota transplantation (FMT), the process of transferring fecal bacteria from a healthy donor to a patient, are being investigated in both preclinical and clinical studies for their potential to modify the gut microbiome, and influence TME and EMT processes, all with the aim of improving the effectiveness of cancer treatments. Probiotics, which are live beneficial bacteria, confer health benefits to the host when administered in adequate amounts. In the context of cancer therapy, probiotics are being explored for their ability to restore a healthy gut microbiome composition, thereby counteracting dysbiosis-induced EMT. For example, the administration of *Lactobacillus rhamnosus* has been shown to reduce gut inflammation and inhibit EMT in CRC models by enhancing the expression of tight junction proteins and reducing the expression of mesenchymal markers [953]. Similarly, prebiotics, non-digestible fibers that promote the growth of beneficial gut bacteria, can enhance the production of SCFAs and suppress EMT. By modulating the gut microbiome, both probiotics and prebiotics offer promising approaches to mitigating EMT and reducing the risk of metastasis [954]. The use of antibiotics in cancer therapy presents a double-edged sword. While antibiotics can disrupt the gut microbiome and lead to dysbiosis, they can also be strategically employed to target specific pathogenic bacteria that promote EMT. For instance, antibiotics targeting *Fusobacterium nucleatum* have been shown to reduce tumor progression and metastasis in CRC models by decreasing the pro-inflammatory signaling that drives EMT [955]. However, the broad-spectrum effects of antibiotics on the gut microbiome necessitate careful consideration and targeted application to avoid unintended consequences. FMT has emerged as a promising approach to modulating the gut microbiome in cancer therapy. In a recent phase 1 clinical study, the combination of FMT and re-induction of anti-PD-1 therapy demonstrated positive responses in immunotherapy-refractory melanoma patients, highlighting the potential of microbiome-targeting therapies [931]. These results suggest that modifying the gut microbiota can overcome resistance to ICIs and improve patient outcomes. FMT may work by restoring a healthy microbiome composition supporting a more effective immune response against tumors. For instance, FMT can increase the abundance of *Akkermansia muciniphila* and *Bifidobacterium brave*, which have been related to heightened infiltration of cytotoxic T-cells into the tumor, thereby potentiating the effectiveness of ICIs [948, 956]. These results emphasize on the great potential of microbiome-targeting therapy that will be clinically investigated in the next decade. By leveraging the complex interactions between the gut microbiota and the host immune system, it may be possible to develop novel therapies that improve outcomes for cancer patients across a range of treatment modalities.

Cell metabolism in the TME

Cell metabolism in the TME is a complex and dynamic process that plays a crucial role in cancer progression [957–963]. Tumors can be viewed as dynamic pseudo-organs, where various components such as cancer cells, stromal cells, immune cells, endothelial cells, and the ECM interact with each other and collaboratively create a unique metabolic landscape that supports tumor growth and survival [964]. Within this intricate network, cancer cells adapt their metabolic properties to the local environment, for example through symbiotic nutrient sharing, nutrient competition, and the utilization of metabolites as signaling molecules [965]. While similar processes operate in normal physiology and development, in cancers, these metabolic interactions are hijacked to support the survival and growth of cancer cells. A comprehensive understanding of the TME can enhance our understanding of the tumor-immune cell interactions, thereby enabling prediction of clinical responses to immunotherapies by identifying predictive biomarkers. It is important to note that the cell metabolism within the TME is different between a solid tumor and a hematological malignancy. The primary differences lie in the structural and systemic characteristics of both environments: the former has a complex and often hostile tissue architecture with hypoxia, dense ECM and irregular vascularization while the latter relies less on physical structures and more on the interactions between the bloodstream and bone marrow niche.

Cell metabolism in the TME of solid tumors

The tumor ecosystem is a complex and heterogeneous patchwork of cancer cells and interconnected various host cells, including stromal cells, the endothelium, and the surrounding immune cells, all contributing to tumor proliferation and spread [647]. Tumor cells often maintain high mitotic and metabolic rates to support their growth, through molecular and physical interactions with a vascular network that they may have promoted themselves. Indeed, tumor cells can produce proangiogenic factors such as VEGF, TGF-β, FGF, and PDGF to induce rapid angiogenesis, albeit resulting in the formation of leaky, aberrant, and/or tortuous blood vessels]966] (Fig. 12). For example, the activation of CAFs leads to the recruitment of immune cells through cytokine secretion, initiating ECM remodeling and transforming the organ architecture into a rigid fibrotic matrix with heightened interstitial pressure [967, 763]. This impedes the vascular function due to deregulated proliferation and growth factor release. Consequently, this results in inefficient nutrient delivery and waste removal caused by leaky vessels, as well as poor T-cell infiltration due to the tortuous shape of the new vessels and their reduced pericyte population [965] (Fig. 12). Additionally, these endothelial cells often express low levels of leukocyte adhesion molecules or recruiting chemokines, thus impeding their ability to recruit immune cells to exert their anti-tumor function [968). This abnormal tumor vasculature limits gas exchange and leads to hypoxia. Consequently, there is an increase in glycolytic activity, resulting in lactate accumulation, and the manifestation of the "Warburg phenotype". Studies such as those by Brand et al. (2016) have demonstrated that this lactate buildup acidifies the TME and impairs the immune system's ability to respond to tumor in melanoma mouse models [969]. At pathophysiological concentrations, lactic acid induces apoptosis in T-cells and NK cells and interferes with regulation of nuclear factor of activated T-cells (NFAT), a transcription factor involved in the transcriptional control of IFN-γ, thereby reducing IFN-γ production. Interestingly, myeloid cells resist the lactate-induced cell death, resulting in elevated numbers of MDSCs which further promote tumor growth [970]. Moreover, the scarcity of nutrients in the TME creates a competitive environment where stromal cells, cancer cells, and immune cells vie for resources to sustain their rigorous anabolic demands and energy production needs. Immune cells are particularly disadvantaged in this competitive setting and the lack of nutrients almost invariably hinders their anti-tumor activity [971].

Cell metabolism in the TME of hematological malignancies

Though our understanding of the TME's role in the progression and treatment of hematological malignancies is not yet as advanced as it is for solid tumors, it is evident that the tumor niche is actively sustained and shaped by dynamic crosstalk between liquid cancer cells (lymphoma and leukemia) and the TME. The genesis of lymphoma is not merely the result of autonomous tumor processes but rather a combination of immune-escape mechanisms and promotion of tumor growth and proliferating factors, much like what it is observed in solid tumors [972]. Despite considerable progress in utilizing the genetic anomalies of blood malignancies for therapeutic purposes, the clinical outcome is often tumor reduction and remission rather than tumor eradication and cure. Minimal residual disease and immunosurveillance are dependent on molecular processes and interactions within the TME, underscoring the need for therapeutic targets within the TME aimed at enhancing antitumor immunity through the recruitment of immune cells and inhibition of tumor-promoting signals. B-cell lymphomas, including CLL, MCL, follicular lymphoma (FL), and Hodgkin's lymphoma (HL) are classic examples of sustained interactions between hematopoietic tumor cells and the supporting stroma [973]. The latter

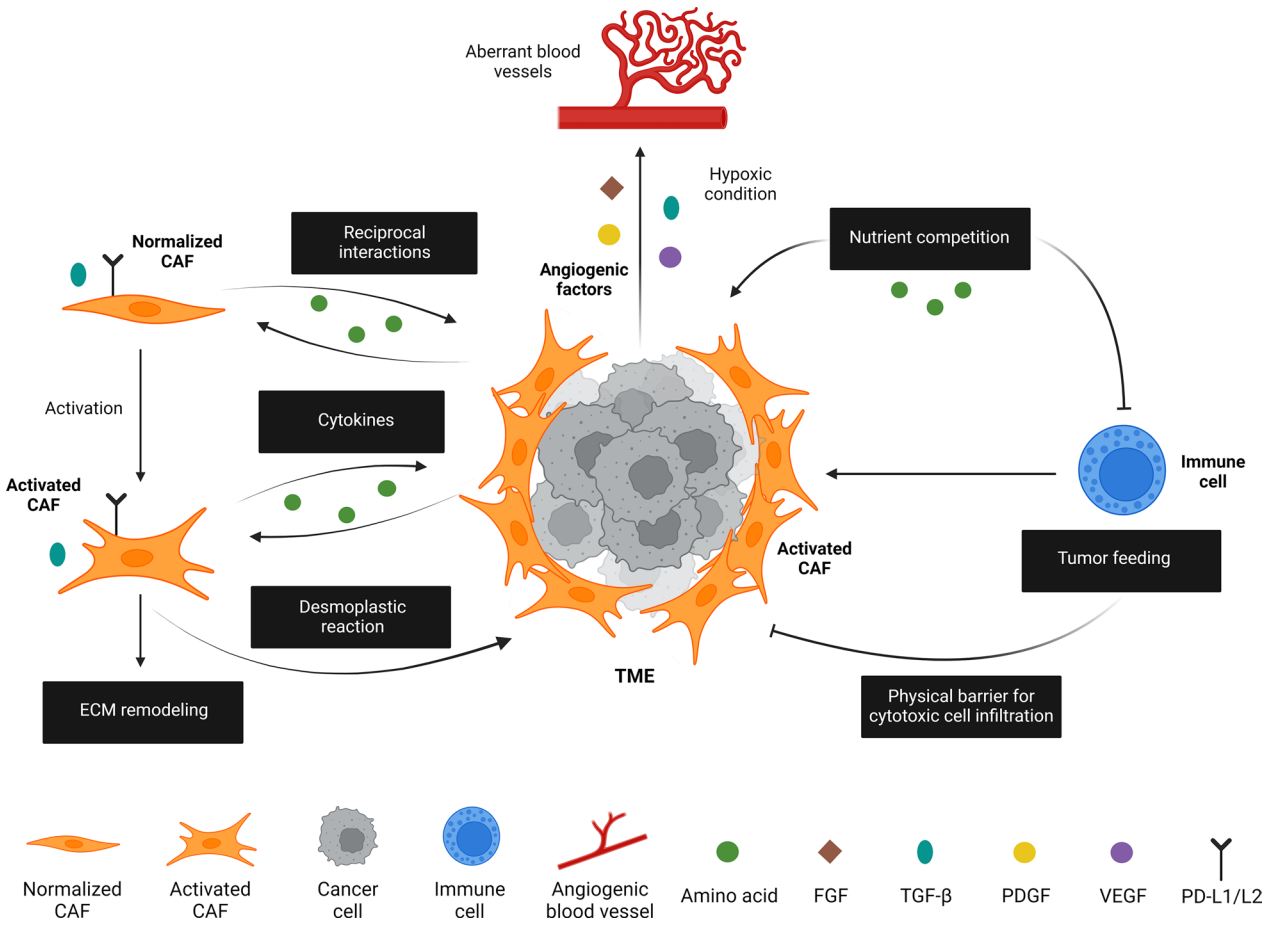

Fig. 12 Metabolic interactions in the TME. The TME is represented in the center by a group of cancer cells coated with activated fibroblasts and surrounded by CAFs and immune cells. *At the top*: In hypoxic condition, TME promotes the production of angiogenic factors (VEGF, TGF-β, FGF and PDGF) to induce rapid angiogenesis, resulting in the formation of aberrant blood vessels with reduced pericyte coverage, low levels of leukocyte adhesion molecules, and low levels of T-cell recruiting cytokines, therefore impeding the recruitment of anti-tumor immune cells. *On the left*: under certain stimuli, CAFs are activated and acquire a pro-inflammatory signature with the expression of immunomodulatory molecules (TGF-β and PDL-1) and lead to ECM remodeling into a rigid fibrotic matrix. They also form a stromal matrix surrounding the tumor core through the desmoplastic reaction. *On the right*: Cancer cells drain energy from the surrounding immune cells by competing for nutrients and amino acids, stealing their mitochondria through nanotubes, and hiding from them using protective stromal matrix formed by CAFs which limits cytotoxic cell infiltration. CAF: CAFs: Cancer-associated fibroblasts; VEGF: Vascular endothelial growth factor; TGF-β: Transforming growth factor-β; FGF: Fibroblast growth factor; PDGF: Platelet-derived growth factor; PDL-1: Programmed death-ligand 1. This figure has been created with BioRender.com

exerts basic nurturing functions, including neoangiogenesis, remodeling of the ECM, and production of growth factors and cytokines. The supporting stroma also plays a major role in regulating immune escape mechanisms. Indeed, stromal cells create a state of immune cell polarization within the TME by promoting tumor-associated immune cells such as TIMs, TAMs, TANs, MDSCs, and tumor associated dendritic cells (TADCs); all of which act to suppress the endogenous innate and adaptive anti-tumor immune responses, making them prime targets for targeted therapies, especially TIMs and TAMs. In mouse experiments, antibodies against myeloid surface markers or murine models with myeloid cell ablation have shown that the depletion of immunosuppressive myeloid cells can impede tumor growth [974]. Comparable results were observed in CLL xenografts, where macrophage depletion through targeting of the CSF1 receptor led to leukemic cell death via the extrinsic apoptotic pathway and switched the TME to a more antitumor phenotype, thereby reducing tumor burden in the bone marrow [975]. Tregs play a significant role in suppressing the immune response against lymphomas. They achieve this primarily through the secretion of immunosuppressive factors and upregulation of the PD-L1 ligand (inhibitory

ligands) on tumor cells, which further hinders the potential of adoptive T-cell therapies [976]. Traditional sequencing methods, while informative, often mask the intricate cellular diversity in hematological malignancies and their dynamic interactions with TME. Single-cell sequencing (SCS) technologies have emerged as transformative tools, offering unprecedented resolution to dissect these complexities at the single-cell level, and providing insights into the distinct cellular populations and their functional states within the TME. By dissecting the molecular profiles of various cell types, including cancer cells, stromal cells, immune cells, and endothelial cells, researchers can identify specific subpopulations and their unique roles in tumor progression and immune evasion [977]. There is a plethora of different modalities, with the main ones being single-cell DNA sequencing (scDNA-seq), single-cell RNA sequencing (scRNA-seq), and the single-cell assay for transposase-accessible chromatin with sequencing (scATAC-seq). Among SCS techniques, scRNA-seq has gained prominence for its ability to profile gene expression in individual cells. This granularity is crucial for understanding the diverse cellular subpopulations, lineage trajectories, and functional/metabolic states within hematological malignancies [365]. The TME plays a pivotal role in these cancers, and scRNA-seq can deconstruct its complexity by revealing cellular composition, intercellular communication, and underlying molecular mechanisms that drive various cancer and immune phenotypes [978, 979]. Moreover, scRNA-seq can uncover the complex signaling pathways that different cell types use to survive, grow, and communicate. By understanding these pathways, researchers can delve into how signaling circuitries drive the overall behavior of the tumor and its interactions with the TME. Furthermore, scRNA-seq can provide a detailed map of the molecular features of stromal cells, which are key components of the TME. With this information, scientists can create models to study how these cells develop and identify potential targets for new treatments. While scRNA-seq offers a snapshot of gene expression, scATAC-seq grants insights into the regulatory landscape by mapping chromatin accessibility at the single-cell level [980]. By integrating scRNA-seq and scATAC-seq, researchers can uncover the regulatory mechanisms that control gene expression in different cell types within the TME [365]. This combined analysis can identify key transcription factors and epigenetic modifications that influence the behavior of malignant and immune cells, suggesting potential therapeutic targets. Single-cell proteomics techniques, like mass cytometry (CyTOF) and single-cell western blotting, complement transcriptomic and epigenomic data by quantifying protein levels and modifications in individual cells. These methods validate and expand findings from scRNA-seq and scATAC-seq, providing a more comprehensive understanding of cellular functions and signaling pathways. Single-cell approaches have been used in CLL to investigate the role of the TME in progression and resistance. Purroy et al. (2022) applied scRNA-seq to characterize the circulating immune cells that coexist with CLL cells and found pronounced differences in immune cell composition between CLL samples and healthy donors, as well as a high number of differentially expressed genes at the time of progression[981]. Similarly, Wang et al. (2020) used this technology to investigate the effects of *SF3B1* mutations, usually associated with clinically aggressive disease, and found that cells carrying this mutation had significant changes in their cellular functions including apoptosis mechanisms, telomere maintenance and NOTCH signaling (downregulation of *DTX1* and altered splicing of *DVL2*) [979, 982]. In addition to abovementioned single cell approaches, advanced in vitro models like organoids and 3D tissue printers can be used to recreate the TME in the laboratory. These models can mimic the dynamic environment of the lymph nodes and bone marrow, which are critical sites in blood cancers [983]. By using these models, researchers can better understand how the TME interacts and metabolically shapes tumor growth and resistance to various therapies. This combined approach of using single cell sequencing and in vitro models holds great promise for developing more effective and personalized treatments for hematological malignancies.

Cell metabolism in the TME and immunotherapy

Over the past decade, immunotherapy has been described as a "game-changer" in the treatment of cancer. Immunotherapeutic agents work by "taking the brakes off" the immune system and manipulating and/or enhancing it to recognize and destroy tumor cells in both early- and advanced-stage patients [984]. As already described in chapter 4, the two most common types of immunotherapy strategies are ICIs and ACT. The ICIs block the receptor interactions between molecules intrinsically involved in T-cell regulation and function such as medications targeting the PD-1 and CTLA-4 antigens. On the other hand, ACTs utilize the host immune cells such as through via CAR-T or CAR-NK cells. While they have been successful in a subset of patients by showing long-term durable remissions [985], we still lack reliable biomarkers to better characterize the TME and potentially predict patient outcomes and response to these therapies [986]. Given the growing use of ICIs in clinical practice, the US FDA has approved the IHC assays to measure PD-L1 protein expression as a potential predictive marker in the context of NSCLC [987, 988]. An initial study examined 39 NSCLC patients and compared the

percentage of PD-L1 staining across four different assays. Though the assays demonstrated analytical and clinical comparability, disparities, and recurrent mis-classification of PD-L1 status were still an issue. Furthermore, the use of PD-L1 as a biomarker has been rigorously debated due to conflicting data on its relevance. While many studies show an increase in clinical response with increased PD-L1 expression [989, 990], several others reported that patients with no PD-L1 also respond to ICIs [417, 991]. Tumors with the same histological stage, according to TNM Classification of Malignant Tumor staging, often have drastically different clinical outcomes [992]. This highlights the need for a better classification system that considers cellular and molecular components of the TME. For instance, a study on colorectal malignancies revealed that T-cell infiltration provided a much better prognostic value than other traditional invasion criteria such as grade, staging, or presence of metastasis [993]. This renewed interest in the type, density, and location of immune cells within the TME has led to the development of a scoring system based on the quantification of $CD3^+$ and $CD8^+$ T-cells known as "immunoscore" [994, 995]. The immunoscores range from I0 to I4, with I0 referring to a low density of both cell types in the tumor center and invasive margin ("cold tumor"), and I4 indicating a high density in both locations ("hot tumor"). Since the immunoscore is dependent on T-cell infiltration, any mechanism that affects this process, including PD-L1 expression and pre-existing antitumor immunity, will have a direct or indirect impact on this score. Finally, gene-expression profiling of 130 frozen Hodgkin lymphoma samples found that a gene signature of TAMs was associated with primary treatment failure [996]. A subsequent analysis on an independent cohort of patients revealed a correlation between $CD68^+$ macrophages and shortened PFS, as well as increased likelihood of relapse after autologous haematopoietic stem cell transplantation (HSCT), thereby showing the potential influence of the TME on the prognosis of hematological malignancies.

Ultimately, a comprehensive understanding of the cell metabolism within the TME, whether in solid tumors or hematological malignancies, provides a valuable opportunity to target specific processes identified through novel techniques such as sequencing. This, in turn, can be utilized pharmacologically through immunotherapy to better hinder the ability of cancer cells to survive and proliferate.

TME/EMT-mediated therapeutic resistance

Despite significant advancements in cancer therapeutics, therapeutic resistance remains a major contributor to cancer relapse and poor patient outcomes. Therapeutic resistance can be categorized into two main types: intrinsic resistance and acquired/adaptive resistance. These two groups differ based on the origin of the resistance. Intrinsic resistance is present within the cancer cells prior to treatment, i.e. the cancer cells are inherently resistant to the initial therapy. In contrast, acquired/adaptive resistance develops in response to treatment. This section explores the roles of the TME and EMT in mediating resistance to chemotherapy, immunotherapy, radiotherapy, and targeted therapy. Collectively, these findings underscore the clinical potential of targeting the TME and the EMT program to improve patient outcomes across these therapeutic modalities.

Intrinsic resistance

Chemotherapy

Emerging evidence suggest a correlation between EMT and chemoresistance of cancer cells. For instance, Snail was found to regulate the expression of *ERCC1*, a gene known to contribute to cisplatin resistance, in HNSCC cells. Accordingly, the overexpression of Snail in HNSCC cells promoted resistance to cisplatin, and the depletion of Snail attenuated cisplatin resistance [997]. Meanwhile, Snail-knockdown sensitized lung adenocarcinoma cells to cisplatin, possibly by activating the JNK/mitochondrial pathway, and in turn, enhancing cisplatin-induced apoptosis [998]. Snail has also been reported to confer significant protection to pancreatic cancer cells against the chemotherapeutics 5-fluorouracil and gemcitabine [999]. Meanwhile, the overexpression of TWIST induced EMT of CRC cells and attenuated sensitivity to the chemotherapeutic oxaliplatin [1000]. In addition, the downregulation of ZEB1 promoted the sensitivity of MCL cells to the cytotoxic effect of doxorubicin, cytarabine and gemcitabine. Interestingly, the study reported a positive correlation between ZEB1 and the efflux transporters MRP1 and MXR, which mediate increased doxorubicin resistance; meanwhile, a negative correlation was observed between ZEB1 and the influx transporter CNT1, which is responsible for the cellular uptake of cytarabine and gemcitabine [1001]. A critical feature of the TME is hypoxia, a condition of low oxygen tension prevalent in many solid tumors. Hypoxia has been shown to promote chemoresistance in various acute lymphoblastic leukemia (ALL) derived cell lines [1002]. This observed chemoresistance is believed to be driven by HIF-1α, an important regulator of the cellular response to hypoxia, since its inhibition promotes chemosensitivity in ALL cells [1003]. Similarly, silencing HIF-1α promotes sensitivity to the chemotherapeutic melphalan in myeloma cells [1004]. TAMs and myofibroblasts were reported to support chemoresistance of pancreatic cancer cells to gemcitabine by secreting IGFs 1 and 2; in line with this, the blockade of insulin-like growth factor (IGF) sensitized pancreatic

tumors to gemcitabine [1005]. In addition, exosomes secreted by CAFs attenuate chemosensitivity, as reviewed by Li et al. (2021) [1006]. In ovarian cancer, CAF-secreted exosomal miR-98-5p promotes resistance to the chemotherapeutic cisplatin [1007] and CAF-secreted exosomal miR-22 promotes tamoxifen resistance in breast cancer cells [1008]. Moreover, esophageal squamous cell carcinoma CAFs secrete cytokines, such as IL-6, in turn activating the STAT3/NF-κB pathway to promote resistance to cisplatin [1009]. Senthebane et al. (2018) discovered a significant upregulation of ECM proteins, namely collagens, fibronectin and laminins, in esophageal squamous cell carcinomas tumor samples compared to the corresponding normal tissue. Decellularized ECMs containing collagens, laminins and fibronectin were found to promote resistance to cisplatin, 5-fluorouracil, and epirubicin in esophageal cancer cells by reducing drug-induced cell cycle arrest and apoptosis. In line with this, esophageal cancer cells cultured on ECMs deficient in collagen and fibronectin exhibited higher levels of cisplatin-induced apoptosis compared to cells cultured on normal decellularized ECMs [1010].

Immunotherapy

As reviewed by Pophali et al. (2024), among patients with hematological malignancies, ICI therapy has been approved only for those with classic HL and primary mediastinal B-cell lymphoma. Many clinical trials are currently underway to assess ICI therapy in other hematological malignancies [1011]. Meanwhile, six CAR T-cell products have been approved by the FDA and European Medicines Agency for the treatment of various hematological malignancies, as reviewed by Blüm et al. (2024) [1012]. The tumor vasculature, as outlined earlier, attenuates the recruitment of immune effector cells into the TME while inducing the accumulation of immunosuppressive cells and factors. Several studies have shown that the accumulation of immunosuppressive immune cells in the TME, along with immunosuppressive cytokines, confers resistance to both ICIs and CAR T-cells. However, by pairing ICIs with agents that diminish the immunosuppressive elements of the TME, such as Tregs or MDSCs, these therapies can effectively revive the immune system against cancer cells [1013]. Recent clinical trials have explored such combinations, revealing synergistic effects that amplify the antitumor immune response and offer new hope to patients with cancers previously considered resistant to immunotherapy. For instance, anti-PD-1 therapy alone did not have any effect on the immune composition of the TME in tumor-bearing mice; however, when combined with the depletion of TAMs, resulted in significantly higher percentages of $CD8^+$ T-cells, $CD4^+$ T-cells and NK cells [1014]. Interestingly, Arlauckas et al. (2017) utilized in vivo imaging to demonstrate the removal of anti-PD-1 antibodies from the surface of PD-1 + tumor-infiltrating $CD8^+$ T-cells by PD-1- TAMs within minutes of effective PD-1 blockade on T-cells [1015]. Clavijo et al. (2017) reported that the depletion of granulocytic MDSC sensitized mice bearing T-cell inflamed mouse oral cancer 1 tumors to anti-CTLA-4 induced tumor rejection and resulted in significantly prolonged survival compared to anti-CTLA-4 therapy alone [1016]. Meanwhile, in an orthotopic HCC mice model, anti-PD-1 therapy alone had no significant effect on survival time; however, when combined with the depletion of CAFs, resulted in significantly longer survival time [1017]. Furthermore, Taylor et al. (2017) reported that PD-1 and CTLA-4 blockade alone exhibited no benefit on tumor growth or survival in claudin-low tumor–bearing mice, but when combined with the depletion of Tregs, reduced tumor growth and significantly improved survival [1018]. In addition, the TME of refractory B-cell NHL patients who achieved complete remission to CAR T-cell therapy displayed lower baseline levels of chemokines that negatively regulate the recruitment of TAMs, Tregs, MDSCs, as well as lower levels of tumor-associated DCs and fibroblasts, and immunosuppressive cytokines (IL-10 and TGF-β1), than those in the partial remission group [1019]. The expression of inhibitory receptors on TILs may also be implicated in resistance to ICI therapy. Thommen et al. (2015) demonstrated that increased expression of inhibitory receptors, including PD-1, TIM3, CTLA-4, LAG-3, and B- and T-lymphocyte attenuator (BTLA), on intratumoral $CD8^+$ T-cells derived from NSCLC patients correlated with disease progression [1020]. The study found that patients expressing higher numbers of PD-1hi T-cells responded poorly to PD-1 blockade alone, and these cells displayed significantly higher expression of TIM3, CTLA-4, LAG3, and BTLA compared with compared with PD-1int subsets. Interestingly, in addition to its role in angiogenesis, VEGF expression in the TME has been associated with resistance to ICIs. Voron et al. (2015) demonstrated that PD-1 blockade induced a significant anti-tumor effect only in VEGF-knockout MEF tumor-bearing mice, but not in wild type MEF tumor-bearing mice. In line with this, anti-PD-1, in combination with anti-VEGFA, induced a strong anti-tumor effect in a mouse model of CRC as compared with anti-PD-1 alone. Notably, VEGFA was shown to increase the expression of PD-1 and other inhibitory checkpoints involved in $CD8^+$ T-cell exhaustion (TIM3, CTLA-4, and LAG-3), which

as discussed earlier, may be implicated in resistance to ICI therapy [1021].

Radiotherapy

Intrinsic resistance typically arises from oncogenic mutations; however, high levels of apoptotic inhibitor proteins such as cIAP1/2, XIAP, and survivin, lead to radioresistance in NSCLC [1022–1025]. CSCs in the TME express high levels of CD133 to repair DNA damage efficiently [1026]. CD133-positive glioma cells have higher levels of DNA damage checkpoint proteins, including ATM, Rad17, Chk1 and Chk2. Other similar proteins include RAD51 and Exo1 which promote cell-specific radioresistance. In NSCLC, CD44 overexpression promotes proliferation and upregulates PD-L1 expression to promote tumorigenesis, immunosuppression, and resistance [1027, 1028]. Intrinsic CSC radioresistance eventually leads to acquired radioresistance when CSCs are not killed by ionizing radiation (IR) [1029, 1030]. In a study on breast cancer, stromal cells, including CAFs and bone marrow cells, induced radioresistance in tumor cells through an IFN-related DNA damage resistance signature [1031] In another study on pancreatic cancer, CAFs enhanced tumor cell proliferation, migration, invasion, and colony formation resulting in radioresistance through factors such as interleukin-1β (IL-1β), and TGF-β [1032]. Effective DNA damage response and cell recovery are promoted by the production of IGF-1, insulin-like growth factor 2 (IGF-2), chemokine (C-X-C motif) ligand 12 (CXCL12), and β-hydroxybutyrate, which increase ROS levels and induce autophagy in cancer cells [1033]. CAFs also produce the signaling molecule chemokine (C-X-C motif) ligand 1 (CXCL1) and their interaction via an autocrine/paracrine signaling loop pushes the TME towards a radioresistant phenotype [1034]. Additionally, hypoxic tumor cells are primarily radioresistant and intrinsic resistance is therefore also closely tied to pre-existing hypoxic conditions within tumors, where oxygen deprivation hinders the formation of DNA-damaging ROS necessary for effective radiotherapy [1035]. Hypoxia exerts selection pressure and promotes the growth of cells with malignant potential, inducing EMT [1036]. The most prominent mechanism of hypoxia in radioresistance is the expression of HIF-1 which enhances glycolysis, serine synthesis pathway, and pentose phosphate pathways and increases antioxidant production to buffer radiation-induced ROS, conferring radioresistance [1037–1039]. ROS is also elevated by the process of hypoxia itself which triggers a feedback loop in favor of antioxidant generation and activates autophagy to accelerate the clearance of cellular ROS products, making cells radioresistant [1039–1043]. Hypoxia also keeps stem cells in a "quiescent" state which preserves proliferation and differentiation potential, decreasing radiosensitivity [1044].

Targetedtherapy

Hypoxia creates a selective pressure that drives tumor cells to adapt in ways that foster resistance to therapy. Under hypoxic conditions, tumor cells stabilize HIF-1α, which activates genes that promote EMT, including *TWIST* and *SNAIL* [1045]. In NSCLC, hypoxia-induced HIF-1α leads to downregulation of E-cadherin [1046, 1047]. This switch from an epithelial to a mesenchymal phenotype facilitates cell detachment and invasion, thereby contributing to resistance against EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib [1048, 1049]. Besides EMT, several cell populations within the TME are also key players in tumor progression and intrinsic drug resistance. For instance, CAFs facilitate drug resistance by secreting cytokines that suppress immune function and interacting with tumor cells [1045]. CAF-derived HGF and IGF-1 mediate primary resistance to TKIs [1050]. In osimertinib-resistant NSCLC, CAFs release IL-6, IL-8 and HGF, promoting EMT [1045] CAFs also secrete cardiotrophin-like cytokine factor 1 (CLCF1), inducing TGF-β which enhance cell stemness and chemokine (C-X-C motif) ligand 6 (CXCL6) which polarize TANs into the N2-like phenotype, creating a tumor microenvironment that promotes cancer cell stemness and immunosuppression [1051]. In breast cancer, CAFs produce NRG1β, stabilizing HER2-human epidermal growth factor receptor 3 (HER3) dimers and causing lapatinib resistance[1052]. Furthermore, CAFs promote angiogenesis via VEGFR-independent pathways, with pericytes potentially increasing VEGF production, further contributing to resistance to VEGFR TKIs [1053, 1054]. MDSCs impair the function of CTLs by generating nitric oxide and peroxynitrite, which reduces T-cell responsiveness [1055]. Additionally, MDSCs secrete pro-angiogenic factors like IL-8, matrix metalloproteinase 8 (MMP-8), and MMP-9, promoting tumor angiogenesis and progression [1056–1058]. In RCC, the anti-angiogenic drug sunitinib targets these pro-angiogenic pathways, but high levels of MDSCs persist in resistant cases due to increased production of GM-CSF, which protects MDSCs from apoptosis [1056]. Despite sunitinib treatment, MDSCs maintain their proliferation through the action of cytokines like IL-6 and GM-CSF, thereby bypassing the inhibition and further enhancing angiogenesis [1059]. TAMs, prevalent in the TME, polarize to M2 and promote drug resistance [1045]. Tumor cells secrete VEGF and IL-6 to recruit macrophages, promoting M2 polarization; and these M2 macrophages secrete chemokines like CC-motif chemokine ligand 15 (CCL15), which activates the NF-κB pathway, causing

gefitinib resistance [1060]. In NSCLC patients, their TAMs secrete *EREG* gene-encoded epiregulin, forming EGFR/HER2 heterodimers on cancer cells, induces phosphorylation of AKT, and attenuates TKI-induced apoptosis, therefore reducing TKI effectiveness [1061]. M2 TAMs also release HGF, causing sorafenib resistance in HCC and attracting more TAMs [1062]. In addition, a recent study illustrated that TAMs promote anti-androgen resistance of bone metastatic prostate cancer through induction of the fibronectin-Itga5-Src signaling cascade [1063]. Moreover, Tregs drive immunosuppression and drug resistance by upregulating cytokines and pathways that reduce T-cell function. In HCC, CC-motif chemokine ligand 22 (CCL22) mediates Treg migration into the TME, increasing sorafenib resistance [1045]. Combining TKIs with ICIs offers a promising strategy to overcome resistance by modulating the immunosuppressive TME. Clinical trials demonstrated that this combination significantly improves survival outcomes in RCC and NSCLC [1064–1066]. Targeting the TME and its cellular components is crucial for addressing drug resistance and enhancing cancer treatment efficacy.

Adaptive resistance

Chemotherapy

Interestingly, chemotherapy-induced EMT has been associated with chemoresistance. Li et al. (2015) reported that EMT-induction, evidenced by increased expression of the EMT-TFs Snail and Slug, was associated with acquired resistance to doxorubicin in colon cancer cells [1067]. In line with this, the reversal of EMT sensitized colon cancer cells to doxorubicin. Similarly, the induction of EMT, evidenced by the downregulation of E-cadherin and upregulation of vimentin, N-cadherin, and fibronectin, was observed in acquired cisplatin-resistant tongue squamous cell carcinoma cells, and a reversal of EMT sensitized these cells to cisplatin [1068]. Doxorubicin has also been shown to induce the expression of the EMT-TF TWIST1 in breast cancer cells [1069]. Accordingly, doxorubicin-treated cells displayed reduced expression of E-cadherin, and upregulation of vimentin. Notably, only doxorubicin-treated cells undergoing EMT displayed multidrug resistance (MDR) to vincristine, pacilitaxel, and bleomycin. Compared to their parental cells, trastuzumab-resistant HER2-overexpressing breast cancer cells displayed increased expression of Slug and TWIST; and in line with this, reduced expression of E-cadherin and increased expression of N-cadherin [83]. Furthermore, Kurrey et al. (2009) discovered that resistance to paclitaxel in ovarian cancer cells is associated with paclitaxel-induced expression of Snail and Slug. These factors induce resistance to apoptosis and promote stem-like characteristics, which lead to therapy failure, tumor cell recovery, and disease recurrence [1070]. The recruitment of immunosuppressive cells into the TME in response to chemotherapy contributes to TME-mediated chemoresistance. DeNardo et al. (2011) reported that paclitaxel significantly increased the infiltration of mammary tumors by TAMs, and the combination of macrophage depletion and paclitaxel treatment improved survival [1071]. Similarly, paclitaxel was shown to induce an influx of TAMs in mammary tumors, which conferred protection against the chemotherapeutics paclitaxel, etoposide, and doxorubicin through cathepsin-dependent and/or cathepsin-independent mechanisms [1072]. Hughes et al. (2015) found that M2-skewed TAMs were abundant in mouse tumors following treatment with various chemotherapeutics, and this promoted their relapse [1073]. In cisplatin-treated neuroblastoma cells, the exosomal transfer of miR-155 from TAMs to neuroblastoma cells was shown to enhance telomerase activity and promote chemoresistance [1074]. Meanwhile, in a mouse model of human luminal type B breast cancer, doxorubicin treatment induced the recruitment of CCR2-expressing myeloid cells, and Ccr2 null host mice responded better to doxorubicin [1075]. Sun et al. (2012) demonstrated that in response to chemotherapy, prostate fibroblasts upregulated the expression of the Wnt family member wingless-type MMTV integration site family member 16B (*WNT16B*) to reduce the effect of chemotherapy [1076]. Furthermore, a significant increase in CAFs was observed following chemotherapy in CRC specimens from patients, and chemotherapy was shown to induce colorectal CAFs to secrete IL-17A, which promotes chemoresistance through the NF-κB pathway [1077].

Immunotherapy

The compensatory upregulation of alternative inhibitory immune checkpoints in response to immune checkpoint blockade can exert immunosuppressive effects, potentially leading to the failure of ICI therapy. For instance, an upregulation of TIM-3 in PD-1 antibody-bound T-cells was observed in resistant lung cancer mouse models and patients following anti-PD-1 treatment [1078]. In prostate tumors following anti-CTLA-4 therapy, the expression of both PD-L1 and VISTA significantly increased on $CD4^+$ T-cells, $CD8^+$ T-cells and $CD68^+$ macrophages. In addition, PD-L1 expression on tumor cells increased. At the time of the study, ipilimumab monotherapy had not yet demonstrated significant clinical benefit in patients with prostate cancer [1079]. Similarly, Kakavand et al. (2017) reported that in a cohort of metastatic melanoma patients treated with either anti-PD-1 inhibitor or a combination of anti-PD-1 and anti-CTLA4 inhibitors, most patients with progressive disease displayed significantly increased density of $VISTA^+$ lymphocytes following

treatment [1080]. Though tumoral PD-L1 increased in the majority of patients with progressive disease, this change did not reach statistical significance. Interestingly, the study demonstrated a significant increase in the density of FOXP3 + Tregs following immune checkpoint blockade, which strongly correlated with the expression of VISTA. These findings are consistent with that of Le Mercier et al. (2014) where VISTA blockade was shown to attenuate the emergence of tumor-specific $Foxp3^{+}CD4^{+}$ Tregs from naïve $CD4^{+}$ T-cells [1081]. In CAR T-cell therapy, a single chain antibody variable fragment (scFv), often of murine origin, typically accompanies the tumor binding function of the CAR. It has been suggested that the production of anti-murine scFv CAR antibodies and CTLs, in response to CAR T-cell infusion, may lead to CAR T-cell rejection. Nevertheless, findings thus far are conflicting. In a phase 1 clinical trial in patients with malignant pleural mesothelioma (NCT01355965), anti-mesothelin CAR T-cell therapy triggered anaphylaxis in one patient with malignant pleural mesothelioma, most likely by inducing the production of IgE antibodies specific for the murine-based antibody sequences present in the CAR-modified T-cell product [1082]. Meanwhile, in a phase 1/phase 2 clinical trial in patients with relapsed/refractory B-ALL (NCT01865617), cytotoxic $CD8^{+}$ T-cell responses to anti-CD19 CAR T-cells occurred in five CAR T-cell therapy-resistant patients following a second infusion of CAR T-cells, resulting in the loss of CAR T-cells. Epitope mapping in one of these patients discovered immunogenic epitopes within the murine FMC63-derived CD19-specific scFv used to design the CAR in the clinical trial [1083]. However, a pooled analysis of two multicenter trials of relapsed/refractory paediatric B-ALL patients treated with anti-CD19 CAR T-cell therapy (NCT02435849; NCT02228096) found that treatment-induced anti-murine CAR19 antibodies neither affected the efficacy of therapy nor impacted the day 28 clinical response [1084].

Radiotherapy

Radiation therapy (RT) can alter the immune TME and thus the patient's immune profile [1085]. A study across several cancer types found that RT induced a systemic reduction of $CD3^{+}$ and $CD4^{+}$ T-cells, driving the TME towards an immunosuppressive phenotype [1086]. The release and accumulation of immunosuppressive cells in the TME further aggravate the immunosuppressive TME [1085–1090]. Suppressive immune cells such as MDSCs, Tregs, TAMs, and N2 neutrophils repress T-cell activation, increase infiltration of MDSCs and Tregs, and activate CAFs, offering tumor cells protection against treatment-activated cell death [1089–1093]. Moreover, IR induces GM-CSF secretion, promoting Arg1-rich MDSCs migration and suppression of T-cell function and activation [1094]. MDSCs also promote the destruction of T-cell receptors and can trigger the PD-L1 pathway or induce IL-10 secretion [1089]. Additionally, RT mediates STING activation which can similarly cause monocytic MDSC recruitment as shown in MC38 colon tumors, followed by inhibition of $CD8^{+}$ T-cell and DC activity [1095–1097]. A study on head and neck squamous cell carcinoma found that RT may also upregulate CCR2 in tumor cells, leading to the accumulation of TNFα-producing monocytes and Tregs [1088]. In the case of ovarian cancer, tumor cells and microenvironmental macrophages produce CCL22 which mediates tumor Treg recruitment [1098]. RT causes the overexpression of enzyme 12-LOX in oesophagal cancer cells, which upregulates CCL5 and promotes polarization of THP-1-derived macrophages to the pro-tumor M2 subtype, enhancing cellular metastasis and inducing radioresistance [1098, 1099]. Radioresistance induced by M2 macrophages promotes tumor cell survival and fibrosis [1100]. Following RT, tumor cells that survive upregulate HIF-1α and induce VEGF expression [1101, 1102]. VEGFA can increase inhibitory receptors which exert a combined effect on T-cell exhaustion [1103, 1104]. VEGFA secretion can also induce FasL expression, enhancing the pro-tumor environment [1105–1107]. IR-induced tumor vasculature can also influence pleiotropic alterations in the TME and worsen pre-existing hypoxia by directly or indirectly upregulating HIF-1α, enabling more effective T-cell suppression [1101–1108]. This upregulation aggravates tumor hypoxia by inhibiting myeloid cell differentiation and inducing radioresistance, angiogenesis, and malignant progression [1101–1113]. The upregulation and production of the aforementioned factors can induce tumor cell EMT and the combined effect of altered TME and EMT leads to tumor progression with poor prognosis [1114–1116].

Targeted therapy

EMT is a reversible biological process where epithelial cells lose their cell–cell adhesion and polarity, gaining mesenchymal traits like motility and invasiveness [1046]. This transition is regulated by key transcription factors such as SNAIL, ZEB, and TWIST [1117, 1118]. During EMT, epithelial markers like E-cadherin are downregulated, while mesenchymal markers such as N-cadherin and vimentin are upregulated, altering cellular characteristics and contributing to therapy resistance [1045]. In NSCLC, this shift enhances cell invasiveness and resistance to EGFR TKIs as they can activate alternative survival pathways and evade drug effects. Similar resistance mechanisms are observed in anaplastic lymphonic kinase (ALK)-positive NSCLC [1046]. Prolonged exposure

to ALK inhibitors such as crizotinib can further drive EMT, leading to enhanced drug resistance [1119–1124]. This resistance is mediated by the activation of alternative pathways like AXL and IGF-1R, which bypass the effects of ALK TKIs [1125–1129]. The TME is pivotal in initiating and sustaining EMT, with factors such as hypoxia, growth factors, and inflammatory cytokines secreted by stromal cells acting as potent inducers [1045). For instance, TGF-β, abundant in the TME, is a potent EMT inducer and its level increases with increasing gefitinib concentrations in NSCLC, a feedback regulation that promotes EMT and EGFR-TKI resistance established [1130]. TGF-β activates EMT transcription factors via both SMAD and non-SMAD pathways such as PI3K/AKT and RAS/MAPK [17]. TGFβR/SMAD also downregulates phosphohydroxylase 3 (PHD3), a negative regulator of EMT, further promoting metastasis and reinforcing the EGFR pathway, thus enhancing tumor cell invasion and metastasis [1045]. In cancers such as NSCLC and RCC, EMT is associated with resistance to TKIs. For example, in RCC, sunitinib resistance is linked to EMT-induced changes including sarcomatous differentiation, as shown in tumor histology [1131, 1132]. Also, EMT can lead to the downregulation of pro-apoptotic proteins like BIM, reducing the efficacy of TKIs [1133]. There is high expression of ZEB1 in erlotinib-resistant NSCLC cells, and knockdown of ZEB1 may restore the erlotinib sensitivity, suggesting that targeting EMT pathways could potentially restore drug response [1134]. In addition to EMT, immune cells in the TME are crucial to adaptive resistance. VEGF-TKI treatment can induce γδ T-cells to secrete IL-17A, leading to N2 TAN polarization, immunosuppression, and VEGFR-TKI resistance. In HCC, sorafenib treatment increases TAN infiltration, promoting resistance through HIF-1α/NF-κB signaling pathways [1045]. Combating drug resistance requires a multifaceted approach addressing both intrinsic and adaptive mechanisms within tumors. Targeting the TME, including pathways involved in EMT, is essential for overcoming resistance [1045, 1055]. While direct EMT inhibitors are still lacking, drugs that target metabolism and combined therapies, such as EGFR inhibitors with TGF-β receptor inhibitors, show potential in reversing EMT-induced resistance [1135]. The role of AXL inhibitors and Aurora kinase family inhibitors in restoring TKI sensitivity highlights the importance of addressing EMT-related processes and resistance pathways [1136, 1137]. Epigenetic modulation using HDAC inhibitors or DNA methyltransferase inhibitors can reverse resistance mechanisms by altering gene expression patterns related to the DNA damage response and apoptosis, including EMT reversal [1138, 1139]. Advanced radiotherapy techniques like dose escalation or stereotactic radiotherapy, which deliver highly focused doses to resistant tumor regions, further improve local control. High-linear energy transfer (LET) radiation is particularly attractive for overcoming resistance as it causes dense ionization tracks and complex DNA damage that is more difficult for cancer cells to repair, making it a potent option against radioresistant tumors, and can be delivered via external beam irradiation (e.g., carbon ion therapy) or molecularly targeted approaches (e.g., targeted alpha therapy). Moreover, addressing the multifaceted interactions within the TME through combined therapeutic strategies offers a promising path forward for overcoming resistance and improving patient outcomes.

Challenges related to TME heterogeneity and plasticity in targeting TME for *cancer* treatment

In Sect. "Developments in TME-targeting strategies", we detailed several potential TME-targeting therapeutic strategies, primarily focusing on the immune cells (in both innate and adaptive immune systems) and stromal cells especially CAFs. One of the major challenges in developing and implementing novel approaches to target the immune-suppressive and cancer-supporting stromal cells in the TME is the inherent heterogeneity of various TME stromal cell populations, as well as their tempo-spatial interactions and evolution during tumor progression/upon therapeutic interventions. In this section we further elaborate on these challenges when designing TME-targeting strategies by focusing on the TME cell heterogeneity and treatment-induced plasticity in both stromal and cancer cells and during their reciprocal interactions.

Stromal cell heterogeneity and *cancer* cell-reprogrammed stromal cell plasticity promote *cancer* aggressiveness and therapy resistance

The stromal cell compartment in the TME of most cancers is highly heterogeneous, and comprises, in addition to various immune cells, many subpopulations of fibroblasts, smooth muscle cells, myofibroblasts (i.e., myCAFs), cancer-associated endothelial cells and pericytes, MSCs, and cancer-specific tumor-promoting ECM produced by a myriad of stromal cells. Recent advances in single-cell techniques such as scRNA-seq/scATAC-seq/single-cell nuclear sequencing (scNuclear-seq), single-cell proteomics, imaging mass cytometry (IMC), and spatial transcriptomics (e.g., GeoMx) have allowed us to better appreciate the stromal cell heterogeneity in the TME down to the single-cell resolution. For examples, PDAC, an aggressive epithelial cancer with a 5-year survival of < 15% at advanced stages, is notorious in having a dense stroma, called desmoplasia, that

prevents immune cell infiltration and drug delivery. By combining scRNA-seq and spatial transcriptomic analysis, a recent study [1140] showed that the $SPP1^{+}APOE^{+}$ TAMs and $CTHRC1^{+}GREM1^{+}$ myCAFs were spatially colocalized and functionally cooperated to generate an immune-suppressive TME in PDAC through active ECM deposition and EMT. As another example, scRNA-seq of 13,857 mesenchymal cells in 9 treatment-naïve lung adenocarcinoma (LUAD) displayed significantly enriched $FAP^{+}PDPN^{+}$ CAFs and $ACTA2^{+}MCAM^{+}$ pericytes in tumors. Interestingly, these two stromal subsets were both topographically adjacent to the perivascular niche with close interactions with ECs as assessed by IMC. NOTCH signaling drove these stromal-EC interactions in tumors and as a result, pharmacological or genetic approaches in inhibiting NOTCH pathway in mesenchymal cells reduced collagen production and suppressed lung cancer cell invasion [1141]. As a final example, osteosarcoma is a bone cancer with unique TME populated by several types of MSCs, including inflammatory MSCs (iMSCs). scRNA-seq studies revealed that osteosarcoma cells secrete extracellular vesicles (ECVs) to transcriptomically reprogram regular MSCs to iMSCs via ECV-associated TGF-β and RNA cargo. iMSCs then mediated osteosarcoma chemoresistance and interestingly, blocking ECV-associated pathways using ladarixin and tocilizumab overcame iMSC-mediated chemoresistance and inhibited osteosarcoma metastasis [1142].

Induction of mesenchymal plasticity in *cancer* cells by immune cells and metabolic and physicochemical factors in the TME also promotes therapy resistance

Vice versa, (neuro)epithelial cancer cells may also become reprogrammed by the cells, ECM components, and soluble factors in the TME to non-epithelial drug-resistant states. For instance, glioblastoma multiforme (GBM) is an aggressive brain tumor thought to be derived from neural stem/progenitor cells. A recent study, by performing scRNA-seq studies in 76 GBM samples from either untreated or nivolumab-treated patients in a clinical trial, demonstrated that nivolumab treatment caused a significant mesenchymal transformation of GBM cells and increases in TAMs and exhausted T-cells [1143]. These findings imply that PD-1 expressing immune cells in the GBM TME may normally signal to GBM cells and keep them in a neuroepithelial state. Immune cells and physicochemical factors in the TME may influence tumor cell behavior through epigenetic mechanisms such as DNA methylation and histone modification, and these changes can reprogram cancer cells into more aggressive and therapy-resistant phenotypes. Furthermore, metabolic (e.g., hypoxia) and ECM alterations (e.g., increased collagen production) in the TME also promote cancer cell adaptability and cancer stemness. In prostate cancer patients receiving androgen deprivation therapy (ADT) and antiandrogens such as enzalutamide, CAFs in the TME secreted an increased amount of glucosamine, an abundant ECM proteoglycan, which subsequently promoted O-GlcNAcylation as well as increased expression of transcription factor Elk1 in prostate cancer cells. Increased Elk1 in turn induced the transcription of an enzyme called 3βHSD1 (*HSD3B1*) leading to de novo intratumoral androgen synthesis to overcome castration effects. As a result, Elk1 inhibitors could dampen the CAF-originated, glucosamine-initiated intracrine androgen biosynthesis using extragonadal substrates and inhibit the development of castration-resistant prostate cancer or castration-resistant prostate cancer (CRPC) [1144]. A follow-up study from the same group revealed that 3βHSD1, the rate-limiting enzyme in catalyzing the intra-tumoral androgen synthesis from non-testicular substrates such as dehydroepiandrosterone (DHEA), became stabilized by hypoxia via repressing autophagy-related genes [1145].

Treatment-induced plasticity in both *cancer* and stromal cells further promotes therapy resistance

Another major challenge is related to treatment-induced plasticity in both cancer cells in the tumor parenchyma as well as in fibroblasts and other stromal cells in the TME, which subsequently drives anticancer therapy resistance. The abovementioned example of nivolumab-induced reprogramming of GBM cells to a mesenchymal and chemo-resistant state illustrates this challenge. CAFs in the prostate cancer TME may contribute to therapy resistance by enhancing cancer cell survival, proliferation, and metastasis via secreting growth factors, cytokines, and ECM components, as well as by promoting angiogenesis, immune evasion, and EMT. ADT and antiandrogen treatments may also reprogram stromal cells, fostering CRPC. For example, a recent interesting study demonstrated that ADT treatment of prostate cancer models reprogrammed iCAFs in the TME to $SPP1^{+}$ myCAFs, which in turn interacted with prostate cancer cells and induced EMT in prostate cancer cells via TGF-b/SWI-SNF signaling. Consequently, depletion of the $SPP1^{+}$ MyCAFs in genetic mouse models of prostate cancer inhibited CRPC development and prolonged the lifespan of the mice [1146].

New developments in understanding and tackling TME heterogeneity and plasticity

Understanding TME heterogeneity and lineage plasticity opens new theranostic avenues. A recent study employed scRNA-seq to compare urine-derived cells (UDCs) in bladder cancer patients with the immune cells

and tumor cells [i.e., circulating tumor cells (CTCs)] in patients' blood with respect to their transcriptomic features. Surprisingly, they observed that UDCs were transcriptionally more similar to tumor cells in the patient's bladder cancer than the immune and tumor cells in the blood. In addition, UDCs were heterogeneous encompassing cytotoxic and activated $CD4^+$ T-cells, exhausted and tissue-resident memory $CD8^+$ T-cells, macrophages, germinal-center-like B-cells, tissue-resident and adaptive NK cells, and regulatory DCs found in tumor [1147]. Thus, this study suggests that bladder cancer UDCs may serve as surrogates for the TME and potential response/resistance biomarkers for clinical treatment such as ICIs.

This section clarified and emphasized that mechanism-driven development of novel combination strategies to holistically target *1)* cancer cells proper, *2)* TME-induced cancer cell state shifting, and *3)* therapy-induced plasticity in both cancer and stromal cells, may achieve better therapeutic efficacy and long-enduring clinical benefits. For example, the study in PDAC [1140] identified the crosstalk between stromal (myCAFs) and myeloid cells (TAMs) as critical mediators of immune-suppressive TME in PDAC, suggesting that, in principle, the combination treatment of pancreatic cancer cells with chemodrugs (e.g., gemcitabine), TAMs with anti-SPP (osteopontin) antibodies, and myCAFs with anti-GREM1 antibodies, may deliver a much stronger punch at the PDAC. Targeting TME-modulated lineage plasticity in prostate cancer cells may also enhance treatment effectiveness and reduce resistance to ADT/antiandrogens. For example, stromal cells like CAFs promote therapy resistance and CRPC development by triggering prostate cancer cell EMT through secreting cytokines such as TGFβ, by reprogramming CAFs themselves from iCAFs to $SPP1^+$ myCAFs [1146], or by secreting the ECM proteoglycan glucosamine to enhance intracrine androgen production [1144]. Simultaneous treatment of prostate cancer with ADT/enzalutamide (to target prostate cancer cells), anti-SPP1 antibodies (to target $SPP1^+$ myCAFs) [1146], and Elk1 inhibitors (to target glucosamine-initiated intratumoral androgen production) [1144] may elicit a more powerful and longer lasting prostate cancer-inhibitory effects than ADT/antiandrogens alone.

Limitations and challenges in exploiting the full clinical potential for TME modulation

Recent clinical trials targeting the TME have yielded mixed results, with some exhibiting significant benefits in specific subsets of patients or tumor types, while others have failed to demonstrate efficacy [1148]. This is not surprising given the complexity and heterogeneity of the TME, which can lead to variable responses among patients, not only between different types of cancer but also within individual tumors complicating the prediction of outcomes and personalization of treatment strategies. This unfavorable situation highlights the importance of precise patient selection, the need for identification of reliable biomarkers to predict therapeutic response and for combination therapies that target multiple components of the TME. The current limitations to the research or clinical application related to the TME are essentially caused by an incomplete understanding of the highly heterogeneous nature and complex dynamics of the TME, its numerous components and intricate network of cellular and molecular interactions. Moreover, the composition of the TME varies significantly between different types of cancer, individual tumors of the same cancer type and can even among different regions of an individual tumor. In addition, it may change over time during tumor growth/cancer development and in response to therapeutic interventions, often leading to the development of resistance mechanisms [1149]. Understanding the factors affecting the therapeutic outcomes after targeting the TME is essential to improve survival and safety of tumor patients. The mechanisms underlying intrinsic and acquired resistance need to be studied in a "real TME". In fact, past and present studies have largely been based on resistant cells in vitro, ignoring the complex spatial and temporal composition of the TME that can be crucial for acquired resistance in vivo. Current preclinical models often fail to capture the full complexity of TME, which can limit their predictive value for clinical outcomes. Advancing these therapeutic strategies depends on the availability of more accurate and sophisticated preclinical models that accurately mimic the human TME. Thus, further empirical research must adopt the development of models that accurately resemble the TME for bench investigations. To fully elucidate the important role of the TME in acquiring resistance, these should include tumor-tissue explants (i.e., patient biopsy-isolated human complex organoids, which incorporate multiple cell types alongside acellular aspects of the TME [1150, 1151], advanced methods of tissue engineering (multicellular tumor-spheroids), and "tumor on a chip". Once successful, the newly gained knowledge must be further evaluated in preclinical models such as suitable humanized mouse cancer models. All of the above challenges underscore the need for a multidisciplinary approach that integrates insights from oncology, immunology, and bioengineering to devise strategies that can safely and effectively manipulate the TME for therapeutic benefit. Since the TME is of dynamic nature, e.g., in response to treatment, the integration of new biomarkers and novel technologies is required to capture and monitor these changes; the latter include molecular imaging techniques, refinement of biomarker assays, the

integration of radiomics, AI and machine learning, and the design and development of more sophisticated autologous experimental ex vivo models. The future direction of research in this area will, therefore, have to focus on further elucidating the complex interactions within the TME, identifying new therapeutic targets, catching the unique characteristics of each patient's tumor and developing more sophisticated models for predicting treatment response. In addition, there is a strong need for novel and better biomarkers, which are invaluable for selecting the most appropriate treatment regimen [1152], predicting the response to TME-targeted therapies and monitoring alterations of the TME in response to treatment and eventually the development of resistance. Principally, interventions targeting the TME may have undesired side effects on normal tissue homeostasis that may require discontinuation of therapy [1153, 1154]. Since many components of the TME, such as stromal cells and ECM proteins, are also present in healthy tissues, there is a risk that *1)* targeting these components could disrupt normal physiological processes leading to adverse effects; *2)* the dynamic nature of the TME and its ability to evolve in response to therapy can lead to resistance characterized by its ability to adapt and reprogram in response to therapeutic pressures (this would necessitate adaptive treatment strategies that can foresee, counteract, or even prevent, the emergence of resistance) [1155]; *3)* disrupting ECM, or tumor vasculature or architecture to improve drug delivery could inadvertently enhance tumor cell dissemination, potentially increasing the risk of metastasis. Similarly, strategies that modulate immune cells of the TME could trigger inflammatory responses that in turn might support tumor progression by providing cancer cells with growth-stimulatory signals [1156]. If these mentioned limitations in revealing the secretes of the TME can be overcome and finally exploited for clinical application, then more effective treatment regimens can be tailored. These are expected to revolutionize the landscape of cancer therapy, moving towards more effective, less toxic, and highly personalized treatment regimens.

Characterization of inter- and intra-tumor heterogeneity of the TME and tumor subtypes

The inter- and intra-tumor heterogeneity of solid tumors is largely based on different compositions of the TME, and have been characterized only for some tumor types. Also, quantitative inference on spatial heterogeneity in the TME is still limited. Applying a framework of spatially annotated transcriptomics data in the TME of samples, spatially correlated patterns in the abundance scores were observed for the tumor cells, while immune cell types showed dispersed patterns in the TME. Intra-tumor (non-genetic) spatial patterns/variations in cell type abundance and pathway signatures in the TME were caused by hypoxia, EMT, and inflammation signatures. These data suggest that because of intra-tumor spatial heterogeneity, single biopsies may underappreciate the extent of clinically relevant, functional variations in the TME within individual tumors [1157]. Hence, multiple biopsy sampling covering different regions from the same tumor may be required to avoid this problem. This is a relevant issue given that tumor biopsies are integral to the diagnosis and clinical management of cancer patients. The different compositions of the TME are reflected in the various subtypes that have been characterized for some cancers. PDAC [1158], and neuroblastoma [1159] present with three immune subtypes/clusters, of which cluster #2 in both cases exhibit an EMT-related signature with elevated levels of TGF-β [1158, 1159]. Moreover, in glioblastoma, a tumor with poor prognosis for immunotherapy due to the complex TME, a ConsensusClusterPlus analyses revealed two subtypes (C1, C2), which were characterized by different EMT-related gene expression patterns. Subtype C2 had more malignant clinical and pathological manifestations, higher immune infiltration and tumor-associated pathways scores, and poorer response to treatment [1160]. Finally, a novel EMT-related lncRNA signature (EMTrLS) had strong prognostic value and potential clinical significance in lower-grade glioma. EMTrLS-high patients presented with high expression of immune checkpoints explaining their immunosuppressive state and EMTrLS stratification was able to predict therapy response to PD1 blockade. Since EMT-related signatures in the TME are predictive of the antitumor therapy response [1161], the use of EMTrLS as a novel biomarker may enable assessment of the responsiveness of LGG to chemotherapeutic drug efficacy and PD1 blockade. As previously mentioned, co-treatment with inhibitors of EGFR or the TGF-β receptors may be able to reverse EMT-like phenotype and increase therapy success [1135].

Novel agents specifically targeting the TME

Another ongoing challenge resides in the identification of the most effective targets within the TME; and once identified in the design and development of novel therapeutic agents that can selectively and efficaciously neutralize these without adversely affecting normal tissue function. These include drugs that normalize tumor vasculature, inhibit ECM-associated enzymes, deplete immunosuppressive cells, enable infiltration or activation of cytotoxic cells within the TME [1162], or phenotypically convert immunosuppressive into antitumor reactive cells, e.g., TAMs and CAFs. Ultimately, these drugs are designed to disrupt the tumor-supportive network within the TME,

thereby hindering tumor growth and metastasis. Examples for novel drugs include those that target the neoangiogenesis process and the immune microenvironment. As outlined in this review, anti-angiogenesis drugs can normalize these tumor blood vessels, improve perfusion and oxygenation, reduce interstitial pressure, and facilitate the delivery of chemotherapeutic and immunomodulatory agents (e.g., lefitolimod and minnelide) to the tumor site. These novel TME-targeting agents are currently in various stages of development, and some are already being evaluated in clinical trials.

Emerging therapeutic strategies targeting the TME

Emerging therapeutic strategies that target the TME are at the forefront in cancer treatment and might partially or fully replace standard chemotherapies in the future. Unfortunately, they are mostly in the early stages of experimental and preclinical testing and aim to disrupt the supportive network that tumors exploit for growth, invasion, and resistance to conventional therapies by the following strategies: reprogramming of the stromal compartment, ECM targeting agents, modulation of the immune microenvironment, metabolic reprogramming of the TME, and personalized medicine approaches.

Reprogramming of the stromal compartment

Stromal reprogramming is a novel treatment strategy that focuses on modifying the supporting cells in the TME, namely CAFs and MDSCs, which play crucial roles in tumor growth and spread. The various strategies seek to either suppress or delete the cancer-promoting functions of these cells or convert them into cells that prevent tumor growth [1163]. One effective means is to use pharmacological inhibitors of TGF-β to block the activation of CAFs and dampen immunological responses in the TME [1164]. TGF-β inhibitors that specifically target signaling crosstalk between cancer and stromal cells have demonstrated potential in preclinical studies. These studies have demonstrated that blocking TGF-β signaling can reduce the capacity of CAFs to promote progression of tumors and simultaneously enhance their sensitivity to alternative treatment approaches [1165]. Unfortunately, these techniques for reprogramming stromal cells are still in the early phases and are mostly studied in preclinical models. A particular challenge is to specifically target the tumor-promoting activities of these stromal cells, while maintaining their physiological roles in healthy tissues. Very recently, senescent cells arising in the TME have quite paradoxically been shown to contribute to tumor progression, in part through increasing therapeutic resistance. Selectively depleting senescent cells from affected organs in vivo with senolytics ("senotherapies") can impede tumor progression by restoring therapeutic responses [1166].

ECM targeting agents

The ECM not only provides structural integrity to tissues, but also plays an active role in cancer cell behavior, facilitating EMT, migration and invasion and hence tumor progression and metastasis. Moreover, a dense and fibrous ECM poses a physical barrier to drug penetration, which is a characteristic feature of many solid tumors. Agents targeting the ECM aim to disrupt these processes through various mechanisms, either by degrading ECM components to reduce tumor stiffness, or by inhibiting the enzymes or growth factors involved in ECM remodeling, like MMPs or TGF-β, respectively [1167]. Of note, reducing matrix stiffness or inhibiting TGF-β or MMPs in the TME will likely also reduce the EMT in the cancer cells. While these approaches have shown potential in preclinical studies, clinical translation has been challenging due to specificity issues. For instance, the broad activity of MMP inhibitors has led to side effects that limit their therapeutic window (see 2.3.3.). One of the major challenges is to achieve sufficient specificity in targeting tumor-associated ECM components without affecting the normal ECM, which is mandatory for preserving the function of non-cancerous tissues. Hence, there are ongoing efforts to identify ECM components that are uniquely altered in tumors. Enzymes that enhance the permeability of the tumor mass by breaking down key ECM components promote a deeper and more uniform distribution of therapeutic agents [67]. The challenge posed by physical barriers also involve the need for developing devices designed to penetrate the ECM to ensure a more complete and targeted drug delivery, such as nanoparticles and other drug carrier systems. Overcoming these barriers is critical for improving the efficacy of cytostatic drugs and other therapeutic agents in treating solid tumors.

Modulation of the immune microenvironment

The immune microenvironment plays a crucial role in the TME, since it has the capacity to both inhibit and facilitate tumor development. Its modulation therefore is crucial for enhancing the efficacy of immunotherapies: immunogenic tumors, characterized by their T-cell-infiltrated TME, respond better compared to non-immunogenic tumors [1168]. Approaches to reverse the immunosuppressive TME include the use of checkpoint inhibitors, the implementation of vaccines to activate the immune system against tumor-specific substances [1169], and adoptive cell therapies such as CAR-T-cell therapy. Other approaches that are conceptionally similar to stromal reprogramming include the depletion, or

M1-directed repolarization, of TAMs, or blockage of the recruitment of monocytes into the TME [1170, 1171]. The TME can protect tumors from being detected and attacked by the immune system using a variety of strategies, i.e., attracting Tregs or MDSCs, or blocking the infiltration of cytotoxic T-cells, while immunosuppressive processes in the TME might hinder the efficacy of immune-modulating therapy [1172]. Changing this situation requires the combined action of drugs that not only stimulate the immune response, but also remove the protective barriers of the TME against immune cells. Therapeutic agents that can selectively deplete these immunosuppressive cell populations or inhibit their functions have the potential to reactivate the immune system's ability to fight cancer. For instance, the MDSC-depleting drug cabozantinib is currently being studied in several clinical trials in combination with ICIs for the treatment of prostate cancer [1173]. Treatment with the Toll-like receptor 9 (TLR9) agonist lefitolimod modulated the TME via infiltration of activated $CD8^+$ cells and a M1-directed phenotype shift in the macrophage population, resulting in a pronounced antitumor effect that correlated with the magnitude of infiltrated immune cells and tumor-specific T-cell responses. In murine tumor models, lefitolimod stimulated a persistent antitumor memory after tumor rechallenge, an increase of tumor-specific T-cell responses and cross-reactivity against different tumor cells, and enhanced the limited antitumor effect of anti-PD1 and anti-PD-L1 [1168]. Likewise, minnelide, a prodrug of triptolide, has shown antitumorigenic activity in multiple malignancies in part via targeting super-enhancers, which in turn are known to induce an immunosuppressive TME. When used in combination with cyclophosphamide, minnelide reduces tumor growth and eliminates metastasis by reprogramming the TME and enhancing cytotoxic T-cell infiltration in 4T1 tumor-bearing mice [1174]. In summary, though efforts to regulate the immune microenvironment have demonstrated considerable success in preclinical cancer models, the necessity of overcoming an immunosuppressive TME still persists.

Metabolic reprogramming of the TME

Recently, metabolic reprogramming of tumors has been found to have a role in modulating the TME to enhance immune cell responses. Recent evidence highlights the critical role of altered glucose, amino acid, and lipid metabolism in the TME on the metabolism, function and effectiveness of antitumor immune cells [1175]. Hence, therapeutic interventions targeting these metabolic alterations have a great potential to be used in combinatorial treatments for diverse cancer types.

Personalized medicine approaches

The heterogeneity of the TME across different tumors and patients has prompted interest in personalized medicine approaches, where specific molecular markers(s) uniquely expressed within the TME of an individual patients' tumor are first identified and subsequently targeted with tailored treatments. These biomarker-driven therapies involve the use of patient-derived tumor models, such as organoids and patient-derived xenografts (PDXs), to test and optimize therapeutic regimens. These approaches hold great promise for improving treatment efficacy and reducing toxicity by ensuring that patients receive therapies most likely to benefit them based on the unique composition of their TME, while at the same time spare them unwanted side effects. Personalized medicine approaches are gaining credit in oncology due to significant heterogeneity among the TMEs of different patients. For example, the presence of particular genetic mutations or overexpressed proteins can guide the selection of targeted therapies, which ensures that the treatment regimen is specifically and maximally effective against the tumor's unique profile [1176]. Moreover, the use of patient-derived models is revolutionizing the preclinical evaluation of cancer therapies. These models closely mimic the patient's own tumor and hence its TME, thereby providing a more accurate prediction of how the tumor might respond to various treatments [1177]. By testing therapeutic regimens on these patient-specific models ex vivo prior to their clinical application, the most promising treatment strategies can be identified. Personalized regimens thus hold the potential to significantly improve the precision, effectiveness, safety of cancer therapies as well as the costs of the health system [1178].

Future directions

The TME is increasingly recognized as a key player in cancer progression and therapeutic response, offering both significant challenges and unique opportunities for the development of personalized medicine. In vivo molecular imaging modalities such as positron emission tomography (PET), magnetic resonance imaging (MRI), and optical imaging have become essential tools in probing the TME, providing critical insights into its cellular and molecular composition. Laboratory techniques like IHC, flow/mass cytometry, multiplexed immunofluorescence and spatial transcriptomics further enhance our understanding by enabling the detailed mapping of immune cells and gene expression within the TME, revealing the complex interplay that drives tumor behavior. These tools are becoming integral to theranostic strategies, where diagnostic markers guide the selection

of targeted therapies, allowing also for personalized treatment plans that are tailored to the specific characteristics of a patient's tumor, thereby improving efficacy while minimizing unnecessary interventions [1179, 1180]. Likely the most clinically advanced applications of theranostics to date are "radiotheranostics"; i.e., a theranostic approach based on nuclear imaging [e.g., PET or single-photon emission computed tomography (SPECT)] of molecular markers, whereafter the pharmacological behavior of a targeted therapeutic analog directed against the same marker may be projected reliably given the spatially and temporally quantitative nature of the imaging. Recent advancements have seen the development of various radiolabeled agents being investigated in clinical studies or preclinically in preparation for translation. Moreover, these agents may be specifically designed to target key components of the TME, such as hypoxic regions (e.g., 18F-FMISO) [1181–1183], chemokines [1184], or CAFs (e.g., breakthrough FAP-inhibitor molecule UAMC-1110, an anti-fibrotic molecule which paved the way for the current FAP-radiotheranostics with more than 100 active clinical trials currently running for solid cancers) [1185–1187], and provide a dual role application in therapy and diagnostic imaging. The integration of radiolabeled agents in therapeutic strategies extends to companion diagnostics, facilitating personalized treatment plans. For instance, radiolabeled antibodies targeting specific tumor antigens enable the visualization of antigen expression patterns, guiding the selection and monitoring of targeted therapies [1188]. The future of TME analysis likely lies in an integrative approach that combines molecular imaging, biomarker assays, radiomics [1189], and artificial intelligence (AI) [1190, 1191]. This synergy would ultimately offer a more comprehensive view of the TME, enhancing our ability to diagnose, prognosticate, monitor, and treat cancer more effectively. Integrating data from various sources allows for a more nuanced understanding of the TME. AI algorithms are poised to play a crucial role here, processing and integrating diverse data sets to uncover patterns and correlations that would otherwise be missed. Several studies have demonstrated the effectiveness of such integrative approaches. For instance, combining clinical variables, radiomic features and blood-based biomarkers have improved the accuracy of predicting treatment response in certain cancers, such as high grade serous ovarian cancer [1192] and NSCLC [1193], and may pave the way for more personalized treatment strategies. While promising, several challenges exist in their integration and application. One significant challenge relies in the complexity and heterogeneity of the TME, which requires not only comprehensive but as well also multi-dimensional analyses. Another hurdle involves the standardization of methodologies across different platforms and institutions and patient populations to ensure reproducibility and comparability of results [1194]. Collaborative efforts and data sharing are essential to foster a more cohesive approach in leveraging these technologies. Furthermore, the TME is of dynamic nature necessitating real-time monitoring and adaptation of therapeutic strategies. Addressing these challenges is crucial for realizing the full potential of this approach. As we look into the future, advancements in molecular imaging technologies, refinement of biomarker assays, and the integration of radiomics and AI hold the promise to enhance understanding and manipulating the TME, and, thus, to pave the way for personalized medicine.

Conclusion

In this comprehensive review, we have explored the multifaceted role of the TME in cancer development, focusing on metastasis and the complexities of EMT. The intricate interactions between cancer cells and various TME components, including CAFs, the ECM, and immune cells, are crucial in understanding cancer progression and the challenges in therapy. The emergence of TME-targeted therapies has been a significant development, promising to revolutionize cancer treatment. Immunotherapies, treatments targeting CAFs, antiangiogenic drugs, and ECM-directed therapies have shown potential in combating the adaptive nature of cancer. These strategies, by focusing on the TME, offer a pathway to overcome the limitations of conventional treatments, particularly in overcoming drug resistance and therapy failure. Up to now, the most prominent immune-based therapeutic strategies targeting the immune components of the TME are ICIs and ACT. Indeed, targeting the TME with immunotherapy using ICIs has maximized efficacy and benefit in cancer patients, although there is still significant limitation to their efficacy due to the immunosuppressive TME, as well as their potential toxicity due to immune related adverse events. Besides, targeting the TME with ACT using TILs or gene-modified T-cells expressing novel CARs or TCRs is another valuable strategy to modify the immune system to recognize cancer cells and thus achieve an anti-cancer effector function. Hence, research on the TME is currently focusing on developing strategic approaches to alleviate the TME-mediated immune-suppressive mechanisms, induce anti-cancer immunity, and increment the potency and the specificity of immune-targeted drugs. Current strategies to counteract TME-mediated resistance increasingly involve the development of combination therapies that integrate conventional cancer treatments with agents targeting the TME. These therapies represent a cutting-edge approach in oncology to overcome

the inherent limitations of conventional monotherapies and underscore the importance of a multifaceted attack in cancer treatment that targets both malignant cells and their supportive TME. Breaking down the protective barriers of the TME compromise possible resistance mechanisms, thereby synergistically enhancing the efficacy of conventional therapies. Moreover, it is essential to establish reliable biomarkers to guide TME-targeted therapies to achieve significant clinical efficacy in cancer patients. In addition, an important limitation related to TME-targeted cancer immunotherapies include primary and acquired resistance, suggesting that a proper understanding of the TME components and their interaction would provide an important aid to overcome these limitations. By leveraging this information, the potential of targeting the TME for the benefit of the majority of cancer patients will be considered an attainable goal. The FDA approval of various drugs and cell-based therapies on T-cells, immune checkpoints, and blood vasculature, has encouraged the ongoing investigation of the TME for further targets to possibly be exploited in future studies. Different mechanisms and cell types mediate immune suppression and thus suitable approaches targeting key vulnerabilities will be crucial. Moreover, the integration of advanced imaging modalities, theranostics, blood-based biomarkers, radiomics, and AI is setting new frontiers in oncological research. These technologies not only enhance our understanding of the TME but also pave the way for more accurate, personalized therapeutic approaches. Sophisticated imaging techniques enable us to visualize the TME with unprecedented clarity, aiding in the identification of novel therapeutic targets. As we move forward, the synergy between TME-focused therapies and advanced technological tools holds immense promise. The future of oncology lies in leveraging these innovations to develop more effective, personalized cancer treatments. However, challenges remain, particularly in standardizing these approaches and ensuring their accessibility and efficacy across diverse patient populations. Examining the impact of systemic factors like diet, metabolism, and physical activity, as well as specific conditions such as aging and inflammation, on the TME and its influence on therapy response is a compelling avenue for investigation. Gaining a deeper understanding of these aspects will enhance the precision of TME targeting, and thus, further improving treatment benefits for cancer patients. In conclusion, our understanding of the TME and its role in cancer biology has grown immensely, yet there is still much to uncover. The ongoing research in TME-targeted therapies and advanced diagnostic tools is crucial for the next leaps in cancer treatment, aiming to transform patient outcomes and usher in a new era of precision oncology. Critical elements for future exploration encompass pinpointing and selectively addressing vulnerabilities within the TME to enhance the stratification of cancer patients, formulating innovative combinations of treatments, and achieving early cancer detection.

Abbreviations

ACC	Adenoid cystic carcinoma
ACT	Adoptive T-cell transfer/therapy
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADT	Androgen deprivation therapy
AI	Artificial intelligence
ALK	Anaplastic lymphonic kinase
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
APC	Antigen-presenting cells
apCAFs	Antigen-presenting CAFs
APL	Acute promyelocytic leukemia
AR	Androgen receptor
ASTs	Advanced solid tumors
BCC	Basal cell carcinoma
BCR	B-cell receptor
bHLH	Basic helix-loop-helix
BTK	Bruton's tyrosine kinase
BTLA	B- and T-lymphocyte attenuator
CAFs	Cancer-associated fibroblasts
CAR(s)	Chimeric antigen-receptor(s)
CCL2	CC-motif chemokine ligand 2
CCL4	CC-motif chemokine ligand 4
CCL5	CC-motif chemokine ligand 5
CCL15	CC-motif chemokine ligand 15
CCL22	CC-motif chemokine ligand 22
CCR2	CC-chemokine receptor 2
CD40L	CD40 ligand
cDC(s)	Conventional or classical DC(s)
cDC1	Conventional/classical cDC type 1
cDC2	Conventional/classical cDC type 2
CEACAM1	Carcinoembryonic antigen cell adhesion molecule 1
cEMT	Complete EMT
CLCF1	Cardiotrophin-like cytokine factor 1
CLL	Chronic lymphocytic leukemia
CR	Complete response
CRC	Colorectal cancer
CRPC	Castration-resistant prostate cancer
CRS	Cytokine release syndrome
cSCC	Cutaneous squamous cell carcinoma
CSCs	Cancer stem cells
CSF1	Colony-stimulating factor-1
CSF1R	Colony-stimulating factor-1 receptor
CTLA-4	Cytotoxic T lymphocyte-associated protein-4
CTLs	Cytotoxic T-lymphocytes (Cytotoxic T-cells, T-killer cells, $CD8^+$ T-cells, killer T-cell)
CXCL1	Chemokine (C-X-C motif) ligand 1
CXCL2	Chemokine (C-X-C motif) ligand 2
CXCL6	Chemokine (C-X-C motif) ligand 6
CXCL9	Chemokine (C-X-C motif) ligand 9
CXCL12	Chemokine (C-X-C motif) ligand 12
CXCR1	C-X-C motif chemokine receptor 1
CXCR2	C-X-C chemokine receptor type 2
CXCR4	C-X-C chemokine receptor type 4
CTCs	Circulating tumor cells
DAP10	DNAX activation protein of 10 kDa
DAP12	DNAX activation protein of 12 kDa
DCs	Dendritic cells
DHEA	Dehydroepiandrosterone
DLBCL	Diffuse large B-cell lymphoma
DOR	Duration of response
ECM	Extracellular matrix
ECVs	Extracellular vesicles

EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EMT	Epithelial-mesenchymal transition
EMTrLS	EMT-related lncRNA signature
EMT-TFs	EMT-inducing transcription factors
ER	Estrogen receptor
FAK	Focal adhesion kinase
FAP	Fibroblast activation protein
FasL	Fas ligand
FDA	Food and Drug Administration
FGF	Fibroblast growth factor
FGF-1	Fibroblast growth factor 1
FGF-2	Fibroblast growth factor 2
FGFR	Fibroblast growth factor receptor
FGL1	Fibrinogen-like protein 1
FL	Follicular lymphoma
FLT3	Fms like tyrosine kinase 3
FLT3L	Fms-related tyrosine kinase 3 ligand
FMT	Fecal microbiota transplantation
FOLFIRI	Folinic acid + fluorouracil + irinotecan hydrochloride
FOLFIRINOX	Folinic acid + fluorouracil + irinotecan hydrochloride + oxaliplatin
5-FU	5-Fluorouracile
FSP1	Fibroblast-specific protein 1
FXR	Farnesoid X receptor
GBM	Glioblastoma multiforme
G-CSF	Granulocyte colony-stimulating factor
GISTs	Gastrointestinal stromal tumors
GM-CSF	Granulocyte–macrophage colony-stimulating factor
HAS3	Hyaluronan synthase-3
HCC	Hepatocellular carcinoma
HDAC	Histone deacetylases
HER2	Human epidermal growth factor receptor 2
HER3	Human epidermal growth factor receptor 3
HGF	Hepatocyte growth factor
Hh	Hedgehog
HIF-1α	Hypoxia-inducible factor 1-alpha
HL	Hodgkin's lymphoma
HLA	Human leukocyte antigen
HMGB1	High-mobility group protein B1
HMW-MAA	High molecular weight melanoma-associated antigen
HNSCC	Head and neck squamous cell carcinoma
HREs	Hypoxia-responsive elements
HRNs	Hypoxia-responsive non-coding RNAs
HSCT	Haematopoietic stem cell transplantation
HSPGs	Heparan sulfate proteoglycans
iCAFs	Inflammatory CAFs
ICAM1	Intercellular adhesion molecule 1
ICIs	Immune checkpoint inhibitors
IDH	Isocitrate dehydrogenase
IDO	Indoleamine 2,3-dioxygenase
IFN-α	Interferon alpha
IFN-γ	Interferon gamma
IFP	Interstitial fluid pressure
IGF	Insulin-like growth factor
IGF-1	Insulin-like growth factor 1
IGF-2	Insulin-like growth factor 2
IHC	Immunohistochemistry
ILK	Integrin-linked kinase
ILs	Interleukins
IL-1α	Interleukin-1α
IL-1β	Interleukin-1β
IL-2	Interleukin-2
IL4R	Interleukin-4 receptor
IL-6	Interleukin 6
IL-6R	Interleukin 6 receptor
IL-8	Interleukin 8
IL-10	Interleukin 10
IL-11	Interleukin 11
IL-12	Interleukin 12
IL-15	Interleukin 15
IL-17a	Interleukin-17A
IMC	Imaging mass cytometry
iMSCs	Inflammatory MSCs
IMVs	Immune modulatory vaccines
iNOS	Inducible nitric oxide synthase
IR	Ionizing radiation
irAEs	Immune-related adverse events
LAG-3	Lymphocyte activation gene-3
LET	Linear energy transfer
LIF	Leukaemia inhibitory factor
LOX	Lysyl oxidases
LOXL2	Lysyl oxidase homolog 2
LRG1	Leucine rich alpha-2-glycoprotein 1
LRP	Laminin receptor precursor
LSECtin	Liver sinusoidal endothelial cell lectin
LUAD	Lung adenocarcinoma
MAPK	Mitogen-activated protein kinase
MCL	Mantle Cell Lymphoma
MCP-1	Monocyte chemotactic protein-1
MDM(s)	Monocyte-derived macrophage(s)
MDR	Multidrug resistance
MDS	Myelodysplastic syndrome
MDSC(s)	Myeloid-derived suppressor cell(s)
MET	Mesenchymal-epithelial transition
MHC	Major histocompatibility complex
MHC-I	Major histocompatibility complex class I
MHC-II	Major histocompatibility complex class II
mIF	Low-plex multiplexed immunofluorescence
miRNA	MicroRNA or miR
MM	Multiple myeloma
MMP(s)	Matrix metalloproteinase(s)
MMP-2	Matrix metalloproteinase 2
MMP-3	Matrix metalloproteinase 3
MMP-7	Matrix metalloproteinase 7
MMP-8	Matrix metalloproteinase 8
MMP-9	Matrix metalloproteinase 9
MMP-13	Matrix metalloproteinase 13
MMP-14	Matrix metalloproteinase 14
MMP-26	Matrix metalloproteinase 26
moDCs	Monocyte-derived dendritic cells
MRI	Magnetic resonance imaging
MSC	Mesenchymal stromal cells
myCAFs	Myofibroblast-like CAFs
NETs	Neutrophil extracellular traps
NFAT	Nuclear factor of activated T-cells
NHL	Non-Hodgkin's lymphoma
NK	Natural killer
NSCLC	Non-small cell lung cancer
ORR	Overall response rate
OS	Overall survival
PAK4	P21-activated kinase 4
PCSCs	Prostate cancer stem cells
PET	Positron emission tomography
PD-1	Programmed cell death protein 1
PDAC	Pancreatic ductal adenocarcinoma
pDC(s)	Plasmacytoid DC(s)
PDGF	Platelet-derived growth factor
PDGFR	Platelet-derived growth factor receptor
PD-L1	Programmed cell death-ligand protein 1
PD-L2	Programmed cell death-ligand protein 2
PDXs	Patient-derived xenografts
pEMT	Partial EMT
PFS	Progression-free survival
PHD3	Phosphohydroxylase 3
pHLIP	PH-(low)-insertion peptide
PI3K	Phosphatidylinositol 3-kinase
PR	Partial response
PtdSer	Phosphatidyl serine
PXR	Pregnane X receptor
RCC	Renal Cell Carcinoma
RGS5	Regulator of G protein

ROS	Reactive oxygen species
RT	Radiation therapy
RTK	Receptor tyrosine kinase
scATAC-seq	Single-cell assay for transposase-accessible chromatin sequencing
scDNA-seq	Single-cell DNA sequencing
SCFAs	Short-chain fatty acids
scNuclear-seq	Single-cell nuclear sequencing
scRNA-seq	Single-cell RNA sequencing
scFv	Single chain antibody variable fragment
SCLC	Small cell lung cancer
SCS	Single-cell sequencing
SD	Stable disease
SDF-1	Stromal cell-derived factor-1
SLL	Small lymphocytic leukaemia
SPECT	Single-photon emission computed tomography
STS	Soft tissue sarcoma
TAA(s)	Tumor-associated antigen(s)
TADCs	Tumor associated dendritic cells
TAMs	Tumor-associated macrophages
TANs	Tumor-associated neutrophils
TCR(s)	T-cell receptor(s)
TGCT	Tenosynovial giant cell tumor
TGF-β	Transforming growth factor-β
TIGIT	T-cell immunoglobulin and ITIM domain
TILs	Tumor-infiltrating lymphocytes
TIM-3	T-cell immunoglobulin and mucin-domain containing-3
TKIs	Tyrosine kinase inhibitors
TLR4	Toll-like receptor 4
TLR9	Toll-like receptor 9
TMAs	Tumor microenvironment antigens
TME	Tumor microenvironment
TNs	Tenascins
TNBC	Triple-negative breast cancer
TNF-α	Tumor necrosis factor alpha
Tregs	Regulatory T-cells
TREM2	Triggering receptor expressed on myeloid cells 2
TSAs	Tumor-specific antigens
TWIST1	Twist-related protein 1
TWIST2	Twist-related protein 2
UDCs	Urine-derived cells
uPA	Urokinase-type plasminogen activator
uPAR	Urokinase-type plasminogen activator receptor
VCAM1	Vascular adhesion molecule 1
VEGF	Vascular endothelial growth factor
VEGFA	Vascular endothelial growth factor A
VEGFB	Vascular endothelial growth factor B
VEGFC	Vascular endothelial growth factor C
VEGFD	Vascular endothelial growth factor D
VEGFR-1	Vascular endothelial growth factor receptor-1
VEGFR-2	Vascular endothelial growth factor receptor-2
VEGFR-3	Vascular endothelial growth factor receptor-3
VEGFR(s)	Vascular endothelial growth factor receptor(s)
VM	Vasculogenic mimicry
WM	Waldenström's macroglobulinemia
ZEB1/2	Zinc finger E-box binding homeobox factors 1 and 2

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Declarations

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Competing interests
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