Therapeutic Targeting of the Tumor Microenvironment

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INTRODUCTION

The importance of the tumor microenvironment (TME) in dynamically regulating cancer progression and influencing therapeutic outcome is now widely appreciated, and multiple therapies directed to various components of the TME have been developed in recent years. In this review, we will introduce the key cell types and features of the TME; discuss how these can be therapeutically targeted, with an emphasis on therapies that are under clinical evaluation or have been approved; highlight how the TME is altered by various interventions including standard-of-care therapies; and conclude with an overview of the opportunities and potential challenges to consider in the coming years.

The TME is defined as the complex and rich multicellular environment in which a tumor develops. The TME typically comprises immune cells, including T and B lymphocytes, tumor-associated macrophages (TAM), dendritic cells (DC), natural killer (NK) cells, neutrophils, and myeloid-derived suppressor cells (MDSC); stromal cells such as cancer-associated fibroblasts (CAF), pericytes, and mesenchymal stromal cells; the extracellular matrix (ECM) and other secreted molecules, such as growth factors, cytokines, chemokines, and extracellular vesicles (EV); and the blood and lymphatic vascular networks, which are collectively enmeshed and in communication with each other and with the heterogeneous cancer cells themselves (refs. 1, 2; Table 1; Fig. 1).

Since the early days of the TME research field, therapeutic targeting of cancer-promoting cells in the TME was viewed with considerable promise for several reasons (3). The co-opted normal cells were believed to be genetically stable and thus more straightforward to target than genomically unstable cancer cells. The development of acquired drug resistance, at least via conventional mechanisms observed for cancer cell–targeted therapies, was considered to be less likely for similar reasons. There was also the notion that therapies directed to cells in the TME, such as the blood vasculature, might represent a “one size fits all” approach that could be applied to any cancer, regardless of the organ in which it develops.

Over the past several years, however, the immense complexity of the TME has become apparent, and these early perspectives can now be viewed as perhaps overly optimistic. Depending on the stage of cancer progression, and the organ in which the tumor arises, cells in the TME can be either tumor-suppressive or tumor-supporting (2, 4). These opposing functions are influenced by the cancer type, the ontogeny of TME cells, and their “education” within the tumor mass and/or at the systemic level. Given this complexity, multiple strategies to therapeutically target the TME have been developed, including depletion of cancer-promoting microenvironmental cells or their “reeducation” toward immune-stimulating, tumor-suppressive phenotypes.

The TME is also recognized to play essential roles in regulating the response to therapeutic intervention, which can manifest as intrinsic resistance, existing prior to treatment, or as acquired/adaptive resistance (1, 5, 6). The TME-intrinsic
properties include elevated interstitial fluid pressure (IFP) and an inefficient vascular supply, both of which contribute to the impaired delivery and distribution of drugs within the tumor mass (7, 8). High numbers of immunosuppressive cells, including TAMs and regulatory T cells (Treg), and the presence of protective niches that shield a subset of tumor cells from therapeutic effects, additionally contribute to intrinsic resistance (5, 9, 10). Regarding acquired resistance, a number of studies have revealed the pleiotropic adaptive effects on TME composition and phenotypes following a range of different therapeutic interventions, including standard-of-care treatments as well as TME-directed therapies (5). These alterations include elevations in chemokine production and the subsequent accumulation of immune cells, which would naturally be expected as part of the body’s reaction to massive therapy-induced cell death. However, these increases often show quite some specificity in terms of the cell type(s) implicated and the phenotypic alterations resulting from therapeutic intervention, indicating a selectivity to the underlying mechanisms.

Indeed, both radiotherapy and chemotherapies can increase the presence of immunosuppressive TAMs in tumors, protecting the cancer cells from therapy-induced cell death, which may ultimately lead to tumor recurrence (11–16). Chemotherapy may also induce DNA damage in stromal cells, resulting in the activation of NFKB and upregulation of the WNT pathway, thereby contributing to therapeutic resistance (17). Radiotherapy causes pleiotropic alterations in the TME as it affects the tumor vasculature, resulting in hypoxia and the subsequent activation of hypoxia-inducible factor 1 (HIF1), which promotes cancer cell survival and radioresistance (18). CAFs in the TME can also be activated,

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**Table 1. Major components of the tumor microenvironment**

<table>
<thead>
<tr>
<th>Macrophages</th>
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<tr>
<td>Myeloid cells of the innate immune system are crucial for the maintenance of tissue homeostasis and protection against infectious agents through cell engulfment, phagocytosis, and clearing of cellular debris. In cancer, these functions are often suppressed, and the macrophage pool can be composed of tissue-resident macrophages, as well as monocyte-derived cells, which are recruited from the circulation to the TME (30–32, 297). In situ, monocytes differentiate into MDMs and, together with the resident macrophages, represent the TAMs. TAMs can exert an immunomodulatory effect by secreting different factors including cytokines and chemokines (26, 34, 35).</td>
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<tr>
<th>T lymphocytes</th>
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<tr>
<td>T cells recognize antigens that are loaded onto MHC molecules on the cell surface of antigen-presenting cells (APC) via the TCR. T cells can be classified into two major classes: CD4+ and CD8+ T cells. CD8+ T cells detect antigens presented by MHC-I molecules, via a cross-presentation mechanism, leading to cytotoxic reactions that cause tumor cell death (298), and are therefore often termed CTLs. CD4+ T cells detect antigens in the context of MHC-II molecules and coordinate adaptive immune reactions by producing cytokines (299). Within the general CD4+ population, Tregs (CD4+CD25+) are essential to dampen the immune response following challenge (300), and their increased presence in tumors is often associated with poor patient prognosis (301).</td>
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<table>
<thead>
<tr>
<th>DCs</th>
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<tr>
<td>DCs are professional APCs that present extracellular antigens on MHC-II molecules to CD4+ T cells, but they also efficiently mediate cross-presentation, i.e., the presentation of extracellular antigens on MHC-I molecules to CD8+ CTLs, which is critical for anticancer immunity.</td>
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<table>
<thead>
<tr>
<th>Blood vessels</th>
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<tr>
<td>Both primary and metastatic cancers rely on an abundant supply of nutrients and oxygen for their growth. Thus, tumors can develop different mechanisms for tumor vascularization, including angiogenesis (the formation of new blood vessels), vascular co-option (use of preexisting vessels), and vascular mimicry (transdifferentiation of cancer cells to endothelial cells (EC); ref. 302). However, the resulting tumor vasculature is often tortuous, heterogeneous, and dysfunctional. Consequently, IFP within the TME can increase (impairing drug delivery), differential expression of integrins and cell adhesion molecules can block the entry of specific immune cell types (213), and there may be hypoxic areas in certain parts, which is associated with increased tumor aggressiveness (212).</td>
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<thead>
<tr>
<th>Lymphatic vessels</th>
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<tr>
<td>The primary function of lymphatic vessels, lined by lymphatic endothelial cells, is the removal of interstitial fluid (ISF) and tissue immunosurveillance. Along with the ISF, peripheral lymphatic vessels also transport antigens and immune cells to draining lymph nodes, where immune response or tolerance can be initiated (241). In cancer, these functions are frequently impaired, and the lymphatic vasculature can additionally be co-opted as a route for tumor cell dissemination in the body (303).</td>
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<th>CAFs</th>
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<tr>
<td>CAFs are a highly heterogeneous population in both origin and functionality. Although most CAFs result from the activation and expansion of local tissue-resident fibroblasts, several studies have reported CAFs originating from adipocytes, pericytes, ECs, and bone marrow–derived mesenchymal stem cells (283).</td>
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<table>
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<tr>
<th>ECM</th>
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<tr>
<td>The ECM can be produced by different cells inside the TME including CAFs, tumor cells, and ECs, and is predominantly composed of collagens, proteoglycans, hyaluronic acid, and laminins. The ECM acts as an important structural support for the tumor, serving as a scaffold for tumor cell invasion and chemotaxis (259, 260), and additionally functions as a storage depot for growth factors, chemokines, etc., which are released in a tightly regulated manner by proteases in the TME (304).</td>
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</tbody>
</table>
leading to radiation-induced fibrosis (19), secretion of lysyl oxidase (LOX) enzymes that favor collagen deposition (20), and elevated ECM production, which promotes cancer cell survival via integrin signaling (21). Although these brief representative examples highlight TME-mediated resistance, it is equally important to consider that certain therapies induce changes in the TME that can have a synergistic effect, for example, by promoting immunogenic cell death and enhancing T cell–dependent antitumor immunity (22–25).

Given the critical roles of the TME in tumor progression and regulating the efficacy of cancer treatment, strategies to target the TME have expanded significantly in recent years. These approaches mainly focus on the targeting of TAMs, DCs, T cells, tumor vasculature, ECM, and CAFs (Fig. 1), and will be discussed in the following sections, with a focus on therapies that either are in clinical evaluation or have been approved.

**TME THERAPIES**

**Targeting the Tumor-Associated Immune Landscape**

In many tumors, immune cells represent the most abundant noncancerous cells in the TME, comprising different innate immune cells such as TAMs, neutrophils, antigen-presentation cells (APC) including DCs, and adaptive immune cells including T cells (Fig. 1). We will summarize key functions of the major immune populations in the TME, with an emphasis on how to therapeutically engage these cells in mounting an active immune response against cancer.

**TAMs**

Most tissues contain long-living resident macrophages that have essential functions in regulating immune defense and tissue homeostasis (26). During tumor formation, this
resident macrophage pool expands through in situ proliferation and is complemented by the recruitment of monocyte-derived macrophages (MDM) into the TME (26). This creates a mosaic of ontogenetically distinct TAMs, which are further educated or modified within the TME, resulting in pronounced phenotypic and functional heterogeneity among diverse tumor types, including brain, breast, pancreatic, and lung cancers (27–33). Within the TME, TAMs can respond rapidly to local stimuli, such as cytokines or therapeutic perturbations, being polarized to adopt diverse phenotypes ranging from proinflammatory to anti-inflammatory states (26, 34). This plasticity is affected by the disease stage, affected tissue, and host-microbiota, which together determine whether TAMs either block or promote tumorigenesis (35, 36). Despite the spectrum of distinct functions that TAMs can execute, increased TAM abundance is most frequently associated with poor patient prognosis and therapeutic resistance (26, 34, 35), highlighting their potential as prognostic biomarkers and therapeutic targets.

TAMs regulate cancer progression through multiple mechanisms, including the promotion of tumor initiation by inflammation, and enhancing subsequent tumor growth and metastasis by enabling immune evasion, angiogenesis, cancer cell invasion, and immunosuppression (34, 35). Furthermore, TAMs can influence cancer relapse following treatment with conventional therapies, for example, as a result of enhanced MDM migration to the residual tumor driven by the elevated production of colony-stimulating factor 1 (CSF1; refs. 11, 37). Consequently, different approaches have been developed to therapeutically target TAMs, including blocking the recruitment and infiltration of MDMs into the TME, interfering with TAM differentiation into tumor-promoting phenotypes, and inhibiting proinflammatory cytokines and other stimuli responsible for chronic inflammation within the TME (35).

Although TAMs are subject to tissue-specific imprinting (26, 27, 38), strategies aimed at broadly targeting these cells across different organ sites have nonetheless shown considerable promise in preclinical models. Indeed, macrophage-targeted therapies not only have the potential benefit of blocking the ability of TAMs to directly promote cancer cell survival, but can also increase cross-presentation to CD8+ T cells and thereby enhance their antitumoral potency (35). A substantial number of these agents have entered clinical evaluation for diverse tumor types (Table 2). These include (i) inhibitors of CSF1 receptor (CSF1R) to deplete TAMs and/or alter their functions within the TME; (ii) CC-motif chemokine ligand 2 (CCL2) or CC-chemokine receptor 2 (CCR2) inhibitors to prevent TAM recruitment into the TME; (iii) CD47/SIRPα complex antagonists to enhance TAM-mediated phagocytosis of cancer cells; (iv) administration of costimulatory molecules such as CD40 to enhance T-cell activation; and (v) inhibitors of PI3Kγ and the triggering receptor expressed on myeloid cells 2 (TREM2) protein to reprogram TAMs toward antitumoral phenotypes (Fig. 2).

**CSF1R Inhibitors.** CSF1R is a transmembrane tyrosine kinase class III receptor required for macrophage differentiation and survival (39). CSF1R signaling is engaged by the binding of its ligands, CSF1 and IL34, whose expression patterns are spatially and temporally distinct in specific tissues such as the brain, skin, and liver (40). Although increased levels of CSF1 in the serum are often correlated with poor survival of patients, including those with ovarian and endometrial cancers (41), the role of IL34 in cancer has been less explored, in part due to its relatively recent identification as an alternative ligand for CSF1R (42), and a more restricted expression pattern compared with CSF1. Nonetheless, IL34 production by chemoresistant lung cancer cells has been reported to enhance the immunosuppressive profile of TAMs and contribute to cancer cell survival (43).

Neutralizing antibodies and small-molecule inhibitors directed against CSF1R have been used to either deplete intratumoral TAMs or promote their reeducation into a pro-tumoricidal phenotype in a context-dependent manner (refs. 44, 45; Table 2; Fig. 2). This approach resulted in antitumor efficacy in preclinical models of multiple primary tumors, including pancreatic cancer, breast cancer, and glioblastoma (44, 46), and reduced breast-to-lung metastasis (11). Given the increase reported in TAM numbers after treatment with standard-of-care therapies (11, 16, 34), CSF1R inhibitors have also been evaluated in combination treatments in preclinical studies. In breast cancer models, the efficacy of paclitaxel (Taxol) was enhanced by CSF1R inhibitor–mediated TAM depletion (11, 15). CSF1R inhibition similarly increased the effectiveness of radiotherapy and tyrosine kinase inhibitors in preclinical glioblastoma models, albeit via a different mechanism, by mediating TAM reeducation (14, 47). Preventing the entry of MDMs into the brain TME resulted in a comparatively modest effect in glioma models (14), indicating that TAM reeducation versus depletion may represent a more effective strategy (45).

Multiple drugs blocking CSF1R signaling (such as emactuzumab, ARRY-382, pexidartinib, PLX7486, and BLZ945, among others; Table 2) have been tested in the clinic, including in combination with conventional therapies targeting cancer cells. Phase I and II trials assessed emactuzumab in patients with advanced-stage solid tumors (NCT02323191 and NCT02923739), and ARRY-382 in patients with advanced solid tumors and metastatic disease (NCT01316822), for safety, tolerability, pharmacokinetics, and pharmacodynamics. Several clinical studies have now been published and have reported quite different outcomes depending on the tumor type. For example, the evaluation of pexidartinib (PLX3397) monotherapy in patients with recurrent glioblastoma in a phase II study (NCT01349036) showed good drug tolerance and penetration of the blood–tumor barrier. However, although TAMs were found to be depleted in the analysis of available patient tumor biopsies, the treatment failed to improve progression-free survival (PFS), and no partial responses (PR) or complete responses (CR) were observed (48). In contrast, in a phase III study of patients with advanced tenosynovial giant cell tumor (TGCT), characterized by the increased expression of CSF1 and CSF1R (including on the cancer cells; ref. 49), pexidartinib showed substantial efficacy (50). Robust tumor regression and improvement of symptoms were observed for many patients, and pexidartinib has now been approved by the FDA as an oral medication for TGCT (50).

There are a number of potential explanations for these divergent clinical results to date. First, resistance to CSF1R inhibition...
Table 2. Therapeutic targets directed toward tumor-associated immune and stromal compartments in interventional clinical trials or approved by the FDA (data collected from http://clinicaltrials.gov, accessed in December 2020)

<table>
<thead>
<tr>
<th>Target</th>
<th>Drug name</th>
<th>Drug type</th>
<th>Mechanism</th>
<th>Current status</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAMs</td>
<td></td>
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</tr>
<tr>
<td>CSF1R</td>
<td>Various, including BLZ945, edicotinib, emactuzumab, and PLX3397</td>
<td>Neutralizing antibodies and small-molecule inhibitors</td>
<td>Reduces macrophage survival or leads to macrophage reeducation</td>
<td>Several phase I and II studies ongoing; some reporting lack of efficacy. PLX3397 approved for TGCT patients</td>
</tr>
<tr>
<td>CCL2</td>
<td>Carlumab</td>
<td>Neutralizing antibody</td>
<td>Limiting monocyte and macrophage recruitment to the TME</td>
<td>Phase I trials completed; drug has been discontinued</td>
</tr>
<tr>
<td>CCR2</td>
<td>MLN1202, PF-04136309 and TAK202</td>
<td>Neutralizing antibodies</td>
<td>Limiting monocyte recruitment and infiltration into the TME</td>
<td>Clinical trials in phase I and II; few trials terminated</td>
</tr>
<tr>
<td>CD40</td>
<td>Chi Lob 7/4, CP-870,893, and dacetuzumab</td>
<td>Agonistic antibodies</td>
<td>To activate host APCs to induce clinically meaningful antitumor T-cell responses in patients</td>
<td>Clinical trials in phases I and II</td>
</tr>
<tr>
<td>CD47</td>
<td>CC-90002, magrolimab, and ZL-1201</td>
<td>Neutralizing antibodies</td>
<td>Interfere with recognition of CD47 by the SIRPα receptor on macrophages</td>
<td>Clinical trials in phase I and few in phase II; studies are at an early stage</td>
</tr>
<tr>
<td>SIRPα</td>
<td>TTI-621 and TTI-622</td>
<td>Recombinant fragment fusion proteins</td>
<td>Acts by binding to CD47 and preventing it from delivering an inhibitory “do not eat” signal to macrophages</td>
<td>Clinical trials in phase I</td>
</tr>
<tr>
<td>PI3K</td>
<td>Eganelisib</td>
<td>Small-molecule inhibitor</td>
<td>Leads to macrophage reeducation into antitumoral phenotypes</td>
<td>Several clinical trials in phases I and II</td>
</tr>
<tr>
<td>TREM2</td>
<td>PY314</td>
<td>Neutralizing antibody</td>
<td>Leads to macrophage reeducation into antitumoral phenotypes</td>
<td>One clinical trial in phase I</td>
</tr>
<tr>
<td>DCs</td>
<td></td>
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<tr>
<td>FLT3L</td>
<td>CDX-301 (FLT3L)</td>
<td>Recombinant cytokine</td>
<td>Expansion of DCs and infiltration in the TME</td>
<td>Clinical trials ongoing in phases I and II</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>GM-CSF, GM-CSF vaccines, leucine, and sangramostin</td>
<td>Cytokine</td>
<td>Booster of antitumor immunity by promoting differentiation of DCs</td>
<td>Several clinical trials ongoing in phases I and II and few trials in phase III</td>
</tr>
<tr>
<td>CTLA4</td>
<td>Ipilimumab</td>
<td>Neutralizing antibody</td>
<td>Blocking of the inhibitory signal CTLA4, allowing CTLs to destroy tumor cells</td>
<td>Several clinical trials ongoing in phases I, II, and III. FDA-approved immunotherapy for patients with melanoma</td>
</tr>
<tr>
<td>PD-L1</td>
<td>Atezolizumab, avelumab, and durvalumab</td>
<td>Neutralizing antibodies</td>
<td>Binds to PD-L1 to stop the interaction between PD-1 and PD-L1 in order to restore antitumor T-cell functions</td>
<td>Several clinical trials ongoing in phases I and II. FDA-approved immunotherapy for several cancers, including urothelial carcinoma, advanced renal carcinoma, and non–SCLC</td>
</tr>
<tr>
<td>PD-1</td>
<td>Various, including nivolumab, PDR001, and pembrolizumab</td>
<td>Neutralizing antibodies</td>
<td>Binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway-mediated inhibition of antitumor responses</td>
<td>Several clinical trials ongoing in phases I and II. FDA-approved immunotherapy for a number of cancers, including squamous cell lung cancer, non–SCLC, head and neck cancer, renal cell cancer</td>
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(continued)
<table>
<thead>
<tr>
<th>Target</th>
<th>Drug name</th>
<th>Drug type</th>
<th>Mechanism</th>
<th>Current status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune checkpoint blockade</td>
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<tr>
<td>LAG3</td>
<td>Various, including FS118, GSK2831781, IMP321, IMP761, LAG525, and relatlimab</td>
<td>Blocking and antagonistic bispecific antibodies</td>
<td>Blocking MHC-II-LAG3 interaction</td>
<td>Clinical trials ongoing in phases I and II</td>
</tr>
<tr>
<td>TIM3</td>
<td>Various, including cobolimab, INCAGN2390, MBG453, and Sym023</td>
<td>Antagonistic antibodies</td>
<td>Binding to TIM3 expressed on specific T cells, including TILs, thereby preventing T-cell inhibition</td>
<td>Clinical trials ongoing in phases I and II</td>
</tr>
<tr>
<td>TIGIT</td>
<td>Various, including tiragolumab, AB154, or BMS-986207</td>
<td>Blocking antibodies</td>
<td>Binding to TIGIT to prevent interaction with its ligands</td>
<td>Clinical trials ongoing in phases I–III; tiragolumab granted FDA BTD in combination with atezolizumab</td>
</tr>
<tr>
<td>Tumor vasculature</td>
<td></td>
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</tr>
<tr>
<td>VEGF/VEGFR</td>
<td>Various, including aflibercept, bevacizumab, and ramucirumab</td>
<td>Neutralizing antibodies, fusion protein (VEGF-TRAP)</td>
<td>Antiangiogenic therapy</td>
<td>FDA-approved; clinical trials ongoing in phases I–III</td>
</tr>
<tr>
<td>VEGFR/other RTKs</td>
<td>Various, including pazopanib, sorafenib, and sunitinib</td>
<td>Small-molecule inhibitors</td>
<td>Antiangiogenic therapy</td>
<td>FDA-approved; clinical trials ongoing in phases I–III</td>
</tr>
<tr>
<td>ANG2–TIE2</td>
<td>Various, including MEDI3617, rebastinib, and trebananib</td>
<td>Neutralizing antibody/peptibody, small-molecule inhibitor</td>
<td>Antiangiogenic therapy</td>
<td>Clinical trials ongoing in phases I–II; clinical trials in phase III completed or terminated (negative outcome)</td>
</tr>
<tr>
<td>ECM and CAFs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>PEGPH20</td>
<td>PEGylated enzyme</td>
<td>Degradation of HA</td>
<td>Clinical trials ongoing in phases I and II; terminated phase III clinical trial (negative outcome)</td>
</tr>
<tr>
<td>LOXL2</td>
<td>Sintuzumab</td>
<td>Blocking antibody</td>
<td>Destabilization of collagen networks</td>
<td>Clinical trials in phase II completed or terminated (negative outcome)</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>Various, including losartan, metformin, and pirfenidone</td>
<td>Small-molecule inhibitors</td>
<td>Collagen and HA reduction</td>
<td>FDA approved; clinical trials ongoing in phases I–III</td>
</tr>
<tr>
<td>FAK</td>
<td>Various, including defactinib (VS-6063, PF-04554878) and GSK-2256098</td>
<td>Small-molecule inhibitors</td>
<td>Prevents integrin signaling</td>
<td>Clinical trials ongoing in phases I and II</td>
</tr>
<tr>
<td>CTGF</td>
<td>Pamrevlumab (FG-3019)</td>
<td>Blocking antibody</td>
<td>Prevents integrin signaling</td>
<td>Clinical trials ongoing in phases I–III</td>
</tr>
<tr>
<td>FAP-expressing cells</td>
<td>PT630, RO6874281, and sibrotuzumab</td>
<td>Blocking antibody, small-molecule inhibitors, fusion protein</td>
<td>Interferes with CAF function, promotes T cell responses</td>
<td>Clinical trials ongoing in phases I and II</td>
</tr>
<tr>
<td>TGFβ</td>
<td>Various, including galunisertib and blocking antibodies</td>
<td>Small-molecule inhibitors and blocking antibodies</td>
<td>Prevents CAF activation and interferes with CAF signaling</td>
<td>Clinical trials ongoing in phases I and II</td>
</tr>
<tr>
<td>FGFR</td>
<td>Erdafitinib (INJ-42756493)</td>
<td>Small-molecule inhibitor</td>
<td>Prevents CAF activation</td>
<td>Clinical trials ongoing in phases I–III</td>
</tr>
<tr>
<td>Hedgehog</td>
<td>Various, including saridegib and vismodegib</td>
<td>Small-molecule inhibitors</td>
<td>Prevents/reduces CAF activation</td>
<td>Clinical trials ongoing in phases I and II</td>
</tr>
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</table>
Therapeutic Targeting of the Tumor Microenvironment

extravasate into tumor sites and differentiate into TAMs (ref. 59). Strategies to reprogram TAMs (35, 45) have the additional benefit of abrogating the potential for long-term side effects resulting from pan-macrophage depletion. Thus, deciphering the profiles and phenotypes of the diverse TAM populations is of direct clinical relevance, as it should reveal new strategies to exclusively target the tumor-promoting functions of TAMs.

**CCL2/CCR2 Inhibitors.** The increase of TAMs in the TME is largely driven by the release of chemokines, which leads to monocyte recruitment and MDM accumulation within the tumor, as well as the expansion of the tissue-resident macrophage pool (60, 61). The release of CCL2 by cancer cells recruits both tissue-resident macrophages and CCR2-expressing Ly6Chi monocytes from the blood circulation that extravasate into tumor sites and differentiate into TAMs (ref. 62; Fig. 2). High CCL2 levels in the serum and the TME are often associated with poor prognosis across diverse tumor types, including breast and prostate cancers (63, 64). In murine models of these cancers, inhibition of CCL2 by neutralizing antibodies was observed to sequester Ly6Chi monocytes in the bone marrow, thereby hindering the accumulation of TAMs and potentiating the antitumor efficacy of CD8⁺ T cells in the TME, which resulted in reduced tumor growth and metastasis (64, 65). However, cessation of treatment was shown to trigger rapid monocyte recruitment to the TME, accelerating the formation of lung metastasis and decreasing survival in mice (66), raising concerns regarding the long-term efficacy of anti-CCL2 agents as monotherapy in metastatic disease. Carlumab/CNTO888 (a human recombinant mAb targeting CCL2) entered phase I and II trials for patients with solid tumors including metastatic castrate-resistant prostate cancer (NCT00992186 and NCT01204996), but despite being well tolerated, it failed to significantly affect tumor growth and the drug was discontinued.

A handful of anti-CCR2 mAbs (such as PF-04136309 and MLN1202; Table 2) have been tested in phase I and II trials for patients with bone metastasis (NCT01015560) and with advanced pancreatic adenocarcinoma (NCT01413022). Some encouraging results were observed with PF-04136309 together with chemotherapy (FOLFIRINOX), which resulted in a tumor response in 49% of the patients and local tumor control in 96% (67). This effect was not observed for PF-04136309 as monotherapy, highlighting the likely need for rational combinatorial approaches in the clinic. With certain other CCL2/CCR2-targeting trials being subjected to early termination, the broader efficacy of this therapeutic strategy to treat patients with cancer still remains to be fully determined. The unexpected side effects and lack of evident clinical efficacy to date may be explained by the body’s capacity to overcome inhibition of the CCL2/CCR2 axis by boosting systemic levels of CCL2 (68), through still-unidentified compensatory mechanisms. Moreover, angiogenesis and local proliferation of resident TAMs may also dampen the effect of CCL2/CCR2 immunotherapy (66). As such, continuing discoveries regarding monocyte profiles and the biological mechanisms for their recruitment and differentiation within the TME may provide new targets to more selectively abolish specific monocyte subsets or prevent their differentiation into protumoral MDMs.

### Table 2. Therapeutic targets directed toward tumor-associated immune and stromal compartments in interventional clinical trials or approved by the FDA (data collected from http://clinicaltrials.gov, accessed in December 2020) (Continued)

<table>
<thead>
<tr>
<th>ROCK</th>
<th>AT13148</th>
<th>Small-molecule inhibitor</th>
<th>Interferes with CAF function</th>
<th>Clinical trials in phase I completed</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCR4</td>
<td>AMD3100</td>
<td>Small-molecule inhibitor</td>
<td>Interferes with CAF signaling</td>
<td>Clinical trials ongoing in phases I–III</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Paricalitol</td>
<td>Small-molecule agonist</td>
<td>Induces CAF normalization</td>
<td>FDA approved; clinical trials ongoing in phases I and II</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>ATRA</td>
<td>Vitamin A metabolite</td>
<td>Induces CAF normalization</td>
<td>FDA approved; clinical trials ongoing</td>
</tr>
</tbody>
</table>

NOTE: Cell-based therapies are discussed in the text.
CD47 Antagonists. Although TAMs are generally associated with tumor-promoting effects, they may also suppress tumor growth by activating different immune responses and by phagocytosing cancer cells (34). However, multiple mechanisms can subvert the phagocytic functions of TAMs in the TME, with one prominent example being via CD47–SIRPα interactions (69). CD47 is a “don’t eat me” immune checkpoint signaling receptor, which is constitutively expressed by normal cells and overexpressed on cancer cells (70). CD47 binds to the signal regulatory protein α (SIRPα), mainly expressed by TAMs and DCs (71). In macrophages, binding of SIRPα to CD47 initiates a signaling cascade that inhibits their phagocytic capacity (71). Consequently, blocking CD47–SIRPα interactions removes this inhibitory checkpoint signal and augments the macrophage-mediated clearance of cancer cells (ref. 72; Fig. 2). In addition to enhancing phagocytosis, the targeting of CD47 also induces DC endocytosis and activation, thereby stimulating T cell–mediated tumor clearance (69, 73, 74).

The CD47–SIRPα axis represents a promising innate immune checkpoint bolstered by a substantial body of data in multiple preclinical models (reviewed in ref. 69). As an example, the anti-CD47 antibody magrolimab (Hu5F9-G4) showed encouraging results in mouse pediatric brain tumors, with efficacy against cancer cells (75). CD47 blocking agents have started to be evaluated in patients, including various anti-CD47 mAbs (Hu5F9-G4, CC-90002, and ZL-1201) and recombinant SIRPα-crystallizable fragment fusion proteins (TTI-621 and TTI-622; Table 2). There are several ongoing phase I studies for both solid tumors and hematologic and B-cell malignancies (NCT03558139, NCT03248479, and NCT04599634), and a phase II trial (NCT02953782) for the treatment of solid tumors and advanced colorectal cancer has recently been completed, but the results have not yet been published. Results from the ongoing trials should soon provide crucial information on the therapeutic potential in patients.

CD40 Agonists. As innate immune cells can share similar cell-surface receptor repertoires, and may respond to common environmental cues during disease, many of the agents used to target TAMs could have broader effects by also affecting other APC subsets. CD40, a TNF receptor superfamily member, is such an example; it is expressed on APCs
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inhibition was further shown to decrease levels of IL10, which ultimately led to recruitment of TAMs increased their MHC-II and IL12 expression and induced the cancer cells (86). In addition, PI3K family members are being evaluated in multiple trials for solid tumors (Table 2; Fig. 2). The PI3K signaling pathway is important for regulating reprogramming TAMs, are currently under early clinical evaluation. These include inhibition of PI3K and TREM2 (Fig. 2), which generated a proinflammatory TME that reinforced an effective T-cell response (81).

CD40 monoclonal agonistic antibodies (e.g., rhuCD40L, CP-870,893, and RO7009789) are being evaluated in several trials for solid tumors (Table 2, Fig. 2). An early trial of rhuCD40L in patients with advanced squamous cell cancer of the head and neck resulted in a broad spectrum of efficacy, with some patients showing only modest responses, several with PR, and one patient entering into long-term complete remission (82). However, a phase I trial of another agent, CP-870,893, in patients with advanced cancer showed no objective clinical responses (83). More recently, for example, in a phase Ib study that has just been published, the tolerability and efficacy of a CD40 antibody (APX005M) combined with chemotherapy (gemcitabine plus nab-paclitaxel), with or without nivolumab, was achieved for patients with metastatic pancreatic adenocarcinoma (84). Thus, although promising (78), the overall outcome of CD40 agonistic antibodies in cancer therapy remains inconclusive at present, as these agents have also demonstrated differential capabilities to boost antitumor activity (85). The reasons underlying these differences remain to be determined in patients, as does the potential synergistic effect of CD40 immunotherapy when combined with CSF1R inhibition or other immune checkpoint inhibition (ICI) approaches.

Emerging TAM Targets. Additional strategies, aiming at reprogramming TAMs, are currently under early clinical evaluation. These include inhibition of PI3K and TREM2 (Fig. 2). The PI3K signaling pathway is important for regulating cell growth, motility, cell survival, metabolism, and angiogenesis, and correspondingly has been investigated in multiple clinical trials from a primary perspective of directly targeting the cancer cells (86). In addition, PI3K family members have important effects on the immune system. For example, PI3Kγ is a key regulator of TAM-mediated immunosuppression (87). In mouse models, selective inactivation of PI3Kγ in TAMs increased their MHC-II and IL12 expression and decreased levels of IL10, which ultimately led to recruitment of immune cells with antitumoral activity and subsequent tumor regression (87). PI3Kγ inhibition was further shown to overcome resistance to ICI, reshaping the TME and promoting T cell–mediated tumor regression (88). Given the importance of PI3Kγ in regulating the switch between immune suppression and stimulation (Fig. 2), phase I and II clinical studies are now evaluating the PI3Kγ inhibitor eganelisib (IPI-549; Table 2) in diverse cancers, either as a monotherapy or in combination with ICI (NCT03719326, NCT02637531, NCT03795610, and NCT03980041). After the tolerability of the drug was demonstrated (NCT03980041), in 2020 the FDA granted fast-track designation to a phase II study investigating eganelisib combined with ICI and chemotherapy for first-line treatment of patients with inoperable locally advanced or metastatic triple-negative breast cancer (NCT03961998).

Another approach to change TAM phenotypes is via inhibition of the TREM2 receptor, which is a member of the Ig superfamily and a major signaling hub, interacting with the adaptor proteins DNAX activation protein of 10 kDa and 12 kDa (DAP10 and DAP12, respectively), as well as numerous extracellular ligands (89). In mice, deficiency of TREM2 skewed TAMs from an immunosuppressive to an antitumoral phenotype, resulting in slower tumor progression (Fig. 2; refs. 90, 91). Moreover, TREM2 deletion combined with PD-1 inhibition reduced tumor growth in several different animal models (90, 91). Analysis of clinical samples showed that TREM2 was expressed in TAMs in > 200 human cancer cases, and high levels correlated with poor outcome in colorectal and breast cancers (90). Given these findings, a humanized mAb (PY314; Table 2) has been designed to bind and deplete TREM2-expressing TAMs, and a phase I trial has recently been initiated to evaluate PY314 as a monotherapy or in combination with pembrolizumab (NCT04691375).

In sum, the agents highlighted here will likely require combination with standard-of-care therapies, including immunotherapies, if they are to advance clinically. We can take some cues from preclinical trials for the optimal combinations to evaluate. As one representative example, CSF1R inhibition was shown to enhance the efficacy of ICI in a model of pancreatic cancer (46), overcome resistance to chemotherapy in breast cancer models (11, 15), and significantly extend survival in combination with fractionated radiation in glioma models (14). We will need to carefully consider patient selection/stratification, and some clinical trials are incorporating evaluation of the unique tumor profile of each patient by genomic analysis into their studies (e.g., NCT03784014) as a means to determine optimal drug combinations. Immune-related toxicity profiles, as observed in some early-phase clinical studies, may represent a challenge with these therapies (92, 93). Although some of these adverse events may be manageable, they will require careful and rigorous assessment of the various combination options.

MDSCs and Neutrophils

Even though most of the studies discussed here have focused on targeting TAMs, other myeloid-derived cells, including MDSCs and neutrophils, have also gained importance in recent years. MDSCs, a heterogeneous population of immature myeloid cells, can promote tumor growth by suppressing T-cell and NK-cell activity, and they also contribute to resistance to immunotherapy. In fact, several clinical trials have revealed a correlation between MDSC abundance and poor response to ICI interventions (94). MDSCs can
be targeted by multiple mechanisms, as reviewed in ref. 54. Interestingly, some of the TAM-targeting agents that are now under evaluation in clinical trials, such as CSF1R inhibitors, can additionally interfere with MDSC recruitment into the tumor (54). Conversely, adaptive mechanisms of resistance to CSF1R inhibitors involving MDSCs have also been reported. For example, following CSF1R inhibition, CAFs can release granulocyte-recruiting chemokines, leading to an increase in tumor-promoting MDSCs and cancer outgrowth in several mouse models (95). These adaptive effects could be reduced by the addition of a CXCR2 inhibitor, and further enhanced in combination with anti-PD-1 treatment (95). Neutrophils are also under consideration as potential therapeutic targets in cancer, as many studies have revealed tumor-promoting functions for these cells, particularly in metastasis. However, the role of neutrophils is quite controversial, as there are studies supporting both protumoral and antitumoral effects of these cells in a highly context-dependent manner (96). Nevertheless, there are several preclinical and clinical studies involving drugs that affect different aspects of neutrophil biology (although not exclusively), including their recruitment, activation, or functional response (as reviewed in ref. 97). Ongoing studies will inevitably provide important answers about how to selectively target these specific myeloid cell types in the coming years.

**DCs**

DCs represent a group of highly heterogeneous professional APCs derived from CD34+ bone marrow precursors, which show an enhanced capacity to take up, process, and present antigens by comparison with other types of APCs (98). DCs present extracellular antigens on MHC-II molecules to CD4+ T cells and can also cross-present antigens on MHC-I molecules to CD8+ cytotoxic lymphocytes (CTL). DCs can be characterized into two distinct cell populations comprising conventional or classic DCs (cDC) and plasmacytoid DCs (pDC; ref. 99). cDCs are further classified into two subsets: (i) cDC1, which express CD141 in humans (CD8α or CD103 in mice), and (ii) cDC2, which express CD1c in humans (CD11b in mice; ref. 100). Each of these subsets has been linked to distinct functional characteristics, showing contrasting immunostimulatory and immunosuppressive roles in the TME (101). cDCs have a high potential for lymph node migration, with cDC1 inducing a strong antitumoral CTL response, whereas cDC2 induce Th17 cells. In mice, vaccination with cDC2 resulted in reduced tumor growth, in association with decreased MDSCs and reprogramming of protumoral TAMs in the TME (101). Monocyte-derived DCs (moDC) have generally been associated with a high capacity for tumor antigen processing but modest T cell–stimulatory capacity due to nitric oxide (NO)–mediated immunosuppression (101). On the other hand, pDCs are linked to the elevated production and release of cytokines such as type I IFNs (98). Thus, the identification of each DC subtype, and their location/ trafficking during cancer progression, will be essential to understand their precise role in tumor immunity in order to design highly targeted strategies to manipulate them.

Three major DC-intrinsic characteristics are critical to trigger a robust and durable antitumoral response, including an optimal migratory capacity between lymphoid and nonlymphoid tissues, cross-presentation of tumor-associated antigens (TAA) to CD8+ CTLs to prime potent effector responses against the tumor, and chemokine and cytokine release to modulate the overall immune response and T-cell homing (102). In this context, DCs can be viewed as promising targets for cancer immunotherapy. Indeed, increased DC density, mostly of cDCs, within the TME is associated with better prognosis in ovarian carcinoma, lung cancers, and breast cancers (103–105). However, the TME can elicit multiple mechanisms to perturb DC functions, including decreased production of chemoattractants (e.g., CCL4 and CCL5), which impair the recruitment of DCs to the tumor bed, and a reduction in survival signals [such as the growth factor FMS-like tyrosine kinase 3 ligand (FLT3L)] required for DC differentiation and viability (106). Together, this results in insufficient T-cell activation and, potentially, the induction of T-cell tolerance to TAAs (106). For this reason, different strategies have been investigated to manipulate DCs, including (i) administration of FLT3L to trigger expansion and survival of the DC pool in vivo, (ii) modulation of DC activity through GM-CSF, and (iii) creation of DC vaccines to boost antitumoral immunity (Fig. 3A and B, Table 2).

**FLT3L.** Signaling via engagement of the FLT3 tyrosine kinase receptor with its ligand, FLT3L, is a critical regulatory mechanism for DC commitment and development. Administration of FLT3L induces the expansion of circulating DCs in vivo and their subsequent trafficking to different tissues (ref. 107; Fig. 3A). Consequently, FLT3L not only augments DC numbers in the TME but also provides a maturation stimulus to DCs, thereby improving antitumor T-cell priming (ref. 108; Fig. 3A). Recombinant FLT3L (e.g., CDX-301) was shown to expand DCs and hematopoietic precursors in healthy human volunteers (109). In patients with acute myeloid leukemia, CDX-301 was delivered as a single agent in a phase III trial (NCT00062223), but the results have yet to be fully described. It is also under clinical evaluation in patients with solid tumors, and although the immunogenicity and safety of the drug have been demonstrated in phase I and II trials (NCT00003431), any effects on tumor remission as a monotherapy remain to be determined. In murine models of non–small cell lung cancer (NSCLC), FLT3L administration enhanced the impact of local radiotherapy (110), and a similar strategy is in phase II trials for patients with advanced NSCLC (NCT02839265). Moreover, a further combination of radiotherapy with the administration of both FLT3L and the costimulatory molecule CD40 is currently in phase II trials in patients with lung cancer (NCT04491084). Notably, the relative contributions of individual DC subsets in acquiring and presenting TAAs remain to be determined in these trials, which will be critical for the interpretation of the results.

**GM-CSF.** GM-CSF is an inflammatory cytokine produced by diverse cell types [including T cells, B cells, macrophages, mast cells, and endothelial cells (EC)] initially reported to be responsible for the expansion and activation of granulocytes and macrophages. Subsequent studies revealed its broader effect on additional cell types, including DCs, by promoting their proliferation, maturation, and survival (ref. 111; Fig. 3A). Preclinical experiments in several cancer models...
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**Figure 3.** Therapeutic strategies targeting DCs and T lymphocytes to enhance antitumor activity. A, Diverse targeting approaches have been developed to enhance overall DC-mediated T-cell priming, including (1) stimulation of DC expansion and maturation triggered by the administration of FLT3L; (2) promotion of DC survival, expansion, and differentiation mediated by administration of the cytokine GM-CSF; and (3) trafficking of DCs manipulated *ex vivo* and administered in the form of a DC vaccine (darker purple DC color used to indicate exogenous cell therapy). On the other side, T-cell antitumor activity can also be augmented through (4) the inhibition of various immune checkpoint molecules, or (5) via adoptive transfer of TILs, TCR T cells, or CAR T cells.

B, DC vaccines manipulate DCs *ex vivo* to enhance their presentation capacity for specific TAAs *in vivo*. CD14⁺ monocytes or CD34⁺ HSPCs are isolated from the cancer patient’s blood and differentiated into immature moDCs, which are subjected to TAA loading (typically obtained from tumor lysates). At this point, DCs can be genetically engineered to boost their cell-intrinsic characteristics (cross-presentation, lymph node migration, and cytokine/chemokine production), thus enhancing their antitumoral functions. Complete DC maturation is achieved by different maturation cocktails. Matured, TAA-loaded DCs are then injected back into the patient (intradermally or subcutaneously; ref. 119), leading to the enhancement of tumor-specific immune responses. The types of DC vaccines can vary depending on the cell type used for *ex vivo* manipulation, the approach for TAA delivery, or the activation status of the DCs infused into the patient. C, TCR T-cell and CAR T-cell therapies involve the collection of T cells from the blood of the patient with cancer and subsequent introduction of a TCR or CAR gene by a viral vector. The engineered T cells are then expanded *ex vivo* and infused back into the patient, where trafficking to the TME occurs.

In *vivo* targeting
- DC expansion and maturation
- FLT3L (CDX-301)

In *ex vivo* targeting
- DC survival, proliferation, and differentiation
- GM-CSF (GM-CSF, leucine, sangramostin)

Immune checkpoint blockade
- αCTLA4 (ipilimumab)
- αPD-1 (e.g., pembrolizumab, nivolumab)
- αPD-L1 (atezolizumab, avelumab, durvalumab)
- αLAG3 (e.g., relatlimab, LAG525, IMP321)
- αTIM3 (e.g., cobolimab, MBG453)
- αTIGIT (e.g., tiragolumab, AB154, BMS-986207)

**BC** suggested that long-lasting antitumor immunity could be driven by the effects of GM-CSF on DCs (112), resulting in the evaluation of recombinant GM-CSF in clinical studies (113). Several strategies are under investigation in phase I and phase II trials (Table 2), including administration of GM-CSF as an adjuvant therapy via systemic administration; as intratumoral monotherapy by direct injection in metastatic lesions; in combination with chemotherapy or with ICI; or as GM-CSF–secreting vaccines (NCT02703714, NCT01134614, NCT02977156, and NCT00317603). In line with the preclinical results, administration of GM-CSF as monotherapy in patients with melanoma increased mature DCs (113), and improved the overall survival (OS) by 60% in comparison with matched controls (114) in early clinical trials. Data from...
DC Vaccines. The generation of DC vaccines involves the manipulation of patient-derived DCs ex vivo to enhance several properties. This includes boosting the DC presentation capacity for specific TAAs, enhancing migration to lymph nodes, overcoming tumor immunosuppression and promoting recruitment of specialized cell populations such as lymphocytes, NK cells, and supplementary DCs (102). These vaccines are injected into the patient to elicit a tumor-specific immune response (ref. 119; Fig. 3B). DC vaccines can be classified into different categories depending on the approach used for TAA delivery, or the molecular modifications and activation status of the DCs, before they are injected back into the patient (as reviewed in ref. 102). Most of the vaccines used to date have resulted from the isolation of monocytes or hematopoietic stem and progenitor cells (HSPC) from the blood and their differentiation in cell culture with recombinant cytokines (102). Immature DCs are then matured and pulsed with TAAs, often derived from tumor lysates, and additionally stimulated with diverse DC maturation cocktails (120). DC vaccination therapy represents a promising approach, as increased antigen-specific T- and B-cell activity and accumulation of CD8+ CTLs into the TME has been reported (121). Disease stabilization has also been observed, and a few cases of PR or CR (122, 123), with little or no evidence of toxicity in clinical trials (124).

However, evidence of substantial antitumor effects has remained limited to date. One possible explanation for this is the broad usage of moDCs for ex vivo manipulation, which may not be the optimal source of DCs (vs. cDCs or pDCs). MoDCs elicit insufficient antigen presentation, limited migration capacity, and cytokine production, all representing essential features for their capacity to overcome the immunosuppressive TME and thus directly affecting the success of the DC-based immunotherapy (125, 126). Interestingly, a recent study has revealed two distinct cDC2 subsets in both mice and humans with different metabolic and functional profiles, which modulated the recruitment and activation of immune effector cells via different mechanisms (127). Thus, a deeper understanding of the individual DC subsets, their contribution to tumor immunity, and their relative capacity in presenting TAAs is critical to enhance the targeted expansion of specific DC subsets with improved antitumor functions and create more effective vaccines. Moreover, combination of DC vaccines with other immunotherapy strategies may enhance the extent of antitumoral responses. Such approaches are currently being assessed in clinical trials, including the combination of DC vaccines with ICIs and chemotherapy (NCT04567069, NCT04201873, and NCT03092453).

T Cells

Current cancer immunotherapies targeting T cells include those directed toward unleashing the antitumor efficacy of T cells by the inhibition of immune checkpoints, or strategies aiming to boost adaptive immunity via the adoptive transfer of genetically engineered T cells equipped with chimeric antigen receptors (CAR) or T-cell receptors (TCR; ref. 128; Fig. 3A and B).

ICI. Several negative regulators of T-cell activation can function as checkpoint molecules to tightly control the immune system and avoid immune hyperactivation. These include CTLA4 and the PD-1 receptor, which represent the most frequent targets of current ICI therapies (129, 130). Although CTLA4 regulates T-cell proliferation at early stages of the immune response, and mostly in the lymph nodes, PD-1 is considered to have a similar biological effect, but at later stages of the immune response within peripheral tissues (130). Besides CTLA4 and PD-1, other immune checkpoint molecules have been discovered, including TIM3, the LAG3 molecule, and the TIGIT protein, which represent attractive emerging targets for T-cell immunotherapy (Fig. 3A; Table 2).

CTLA4 Blockade. CTLA4 is expressed by T cells and binds to B7-1/B7-2 costimulatory molecules on APCs (ref. 131; Fig. 3A). The recognition of CTLA4 as a negative regulator of T-cell activation gave rise to the hypothesis that blocking this molecule could unleash a therapeutic response of T cells against tumor cells (128). Although several preclinical studies showed that CTLA4 blockade can induce a long-lasting immunologic memory in multiple tumor types (132–135), in larger tumors with a more robust anti-inflammatory TME or in less immunogenic tumors, a significant impact was not observed (135–138). CTLA4 blockade by the mAb ipilimumab was further evaluated in the late 2000s in clinical trials for several tumor types, with a very positive outcome particularly in patients with melanoma (139), which ultimately led to its approval by the FDA in 2011. However, the therapeutic outcomes in NSCLC, small-cell lung cancer (SCLC), and prostate cancer have not been as striking, with a smaller subset of patients responding in comparison with melanoma (140–142). To extend the repertoire of patients with cancer who can potentially benefit from this therapy, many clinical trials are currently ongoing for diverse types of solid tumors, frequently in combination with other drugs.

Targeting the PD-1/PD-L1 Axis. PD-1 is expressed on T cells following TCR stimulation and binds to PD-L1 (also known as B7-H1) and PD-L2 (also known as B7-DC), both expressed on APCs and upregulated by proinflammatory cytokines (ref. 143; Fig. 3A). PD-1 restricts immune responses mostly through inhibitory intracellular signaling in effector T cells and Tregs (143). In contrast to CTLA4, the PD-1/PD-L1 axis is essential for controlling the continued activation and proliferation of other interventional studies are, however, inconsistent, and concerns have been raised regarding the detrimental effect of this cytokine when administered in high concentrations (115). The poor efficacy and sometimes opposite effects of GM-CSF may be explained by its augmentation of immuno-suppressive populations including Tregs and MDSCs that inhibit the function of antigen-specific T cells (116, 117). Another important point of consideration is that GM-CSF has been shown to promote obesity-associated neutrophilia and breast-to-lung metastasis in preclinical studies (118), and therefore its administration specifically to obese patients warrants meticulous evaluation.
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Given the promising results from PD-1/PD-L1 inhibition in various preclinical studies (128), mAbs against PD-1/PD-L1 were developed and tested in clinical trials (Table 2). Pembrolizumab and nivolumab became the first FDA-approved PD-1–targeted immunotherapies for patients with melanoma in 2014 (145, 146). In comparison with ipilimumab, pembrolizumab showed a more substantial six-month PFS and greater OS benefit, with an additional advantage of less toxicity (147). Pembrolizumab was subsequently approved for the treatment of PD-L1–expressing NSCLC, urothelial carcinoma, and head and neck squamous cell cancer (https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&ApplNo=021083). Nivolumab resulted in similar beneficial outcomes, demonstrating an OS of 72.9% compared with 42.1% survival at one year in a group of patients with melanoma treated with dacarbazine chemotherapy alone (148). Importantly, increased levels of PD-L1 in the target tumor correlated with improved efficacy of PD-1 blockade (149).

The PD-1 ligand, PD-L1, also plays an essential role in regulating immune responses (150). PD-L1 is typically expressed on APCs and can control Treg differentiation and suppressive activity (150). However, tumor cells and other TME components, such as infiltrating myeloid cells and DCs, often exploit this mechanism by upregulating PD-1 ligands to induce T-cell exhaustion, creating an immunosuppressive TME that facilitates tumor growth and invasion (151–153). The targeting of PD-L1 via atezolizumab was first approved in 2016 for urothelial carcinoma (154) and later expanded to patients with NSCLC, triple-negative breast cancer, and SCLC, in part due to its lower toxicity in comparison with docetaxel (155). Additional anti–PD-L1 mAbs, such as avelumab and durvalumab, subsequently entered the market and are used for the treatment of diverse tumor types, such as urothelial carcinoma, advanced renal cell carcinoma, and NSCLC (156, 157). Therefore, similar to PD-1, blockade of PD-L1 has been effective in difficult-to-treat cancers, and there are numerous ongoing clinical trials to potentially improve therapeutic outcomes in a broader set of cancers (Table 2).

Even though CTLA4 and PD-1/PD-L1–targeting strategies have been shown to be effective and to induce durable responses, many of the patients treated still fail to respond to these individual therapies. As CTLA4 and PD-1 functionally complement each other in principle, the combination of these two strategies has also been evaluated as a means to augment the response rate (158). However, resultant toxicity has been a major concern (159). In this regard, a low-dose combination regimen is now being evaluated in a phase II trial for a large number of tumors (NCT02834013). Data from this and other studies exploring a more localized delivery of immunotherapeutic strategies (160) are expected to facilitate the administration of ICIs to minimize toxicity profiles while preserving clinical efficacy.

**LAG3 Inhibitors.** LAG3 signaling has been described as a negative regulator of T-cell activation, proliferation, and cytokine production (161). LAG3 is expressed by activated CD4+ and CD8+ T cells, Tregs, and subsets of NK cells, B cells, and pDCs (162). This protein shows structural similarities with the CD4 receptor and has MHC-II as one of its ligands (Fig. 3A), which binds to LAG3 with a higher affinity than CD4 (163, 164). In addition to MHC-II, several other cell-surface proteins including galectin-3, liver and lymph node sinusoidal endothelial cell C-type lectin (LSECtin/CLEC4G), α-sinuclein, and fibrinogen-like protein 1 (FGL1) interact with LAG3 (163).

The levels of LAG3 and the infiltration of LAG3+ cells into the TME are associated with disease progression and poor prognosis in several types of tumors, including breast cancer (165), NSCLC (166), and renal cell carcinoma (167). The functional relevance of LAG3 in cancer immunity was revealed through preclinical studies of metastatic ovarian cancer, in which treatment with an anti–PD-1 antibody resulted in increased levels of both LAG3 and CTLA4, whereas treatment with an anti-LAG3 antibody upregulated the levels of PD-1 (168). These experiments showed that single-agent checkpoint blockade may lead to a compensatory upregulation of other checkpoint molecules in the TME (168). Indeed, combined blockade of LAG3 and PD-1 synergistically enhanced CD8+ T-cell cytotoxicity and decreased Tregs in the TME, thereby suppressing tumor growth (169). These results indicate that LAG3 contributes to immune-escape mechanisms, similar to PD-1, and support its consideration as a promising target for cancer immunotherapy.

On the basis of preclinical observations, multiple compounds aimed at blocking LAG3 signaling have been developed (e.g., LAG525, relatlimab, IMP321, and IMP761, among others; Table 2), and their efficiency is currently being investigated in several phase I and phase II trials either as mono-therapy or in combination, predominantly with anti–PD-1 or anti–PD-L1–neutralizing antibodies (NCT03459222, NCT03642067, and NCT02460224). Although the precise mechanism of action of these antibodies is not fully understood, it is thought that they primarily block the interaction between LAG3 and MHC-II (170), while potential effects on the other LAG3 ligands require further investigation. In a phase I/II study of LAG525 used in combination with anti–PD-1 for advanced malignancies (NCT02460224), antitumor activity was observed, with a durable response in approximately 10% of the patients. Relatlimab has also been investigated in phase I and II trials in which an overall response rate (ORR) of 11.5% was observed for patients with melanoma, including six patients with PR and one patient who achieved a CR (NCT01968109; ref. 171). A phase II/III study is currently evaluating whether combination therapy with nivolumab and relatlimab is more effective than nivolumab alone in treating patients with unresectable primary melanoma or metastatic melanoma (NCT03470922).

**TIM3 Inhibitors.** The TIM3 receptor is expressed by multiple cell types including T and B lymphocytes, Tregs, NK cells, DCs, monocytes, macrophages, and tumor-associated endothelium (161). Four ligands have been reported to bind to TIM3, including galectin-9, high-mobility group protein B1 (HMGB1), carcinoembryonic antigen cell adhesion molecule 1 (CEACAM1), and phosphatidyl serine (PtdSer; ref. 162). Ligand binding to TIM3 results in negative regulation
of T-cell responses and T-cell apoptosis (172, 173). Increased expression of TIM3 is associated with poor prognosis in solid tumors (174), and its blockade resulted in therapeutic benefits by restricting tumor growth, particularly in combination with anti–PD-1 antibodies (reviewed in ref. 175). Interestingly, some preclinical studies show that TIM3 inhibition also regulates DC function and response to chemotherapy in breast cancer (176), suggesting a broader role of this promising target.

Several TIM3 antagonist antibodies have been developed in recent years, including TSR-022, cobolimab, MBG453, and Sym023 (Table 2; Fig. 3A). The safety and efficacy of these antibodies are still at an early stage of clinical investigation, with several trials in phase I and II. However, similar to the LAG3 inhibitors, initial data show that the treatment of patients with advanced solid tumors with TSR-022 has a synergistic effect when combined with anti–PD-1 immunotherapy (NCT02817633; ref. 177). TSR-022 was also well tolerated in patients with anti–PD–1–refractory NSCLC and melanoma, and combination therapy with anti–PD-1 resulted in an ORR of 15% and stable disease in 40% of patients (178). Various clinical trials have recently been initiated to determine the safety and efficacy of TIM3 inhibitors in additional cancers (NCT03652077, NCT03099109, NCT03446040, among others).

**TIGIT Inhibitors.** TIGIT is an inhibitory receptor expressed by NK cells and T cells (161). The main ligand of TIGIT is CD155, which is expressed by both APCs and tumor cells, and acts as a negative regulator of NK-cell and T-cell functions. TIGIT may also act in a cell-intrinsic manner by interfering with the costimulatory molecule DNAM1 or by directly initiating an inhibitory signaling cascade via its cytoplasmic tail (179).

In recent years, TIGIT has emerged as an attractive target in cancer immunotherapy, as it can block multiple antitumor immunity mechanisms (179–181). TIGIT upregulation has been observed in several tumor types, and its expression correlated with poor clinical outcomes in melanoma (182) and acute myeloid leukemia (183) among others. These findings, together with various preclinical studies indicating that TIGIT deficiency may protect against several types of cancer (184, 185), led to the development of various mAbs against human TIGIT that are now being tested in clinical trials. Several phase I/II trials are evaluating the safety and efficacy of various anti-TIGIT antibodies, including tiragolumab, AB154, and BMS-986207, either alone or in combination with other immunotherapies (NCT03628677, NCT02913313, and NCT04300647). Tiragolumab has advanced to phase III clinical trials in combination with atezolizumab (anti–PD-1/ PD-L1), and in some cases with carboplatin and etoposide in patients with lung cancer (NCT04294810 and NCT04256421) and esophageal squamous cell carcinoma (NCT04543617). While we await results from these ongoing clinical trials, tiragolumab has just been granted FDA Breakthrough Therapy Designation (BTD) in combination with atezolizumab for PD-L1–high NSCLC based on the positive results of the CITYSCAPE phase II clinical trial (NCT03563716), highlighting the exciting therapeutic potential of yet another ICI.

**Adaptive T-cell Therapy.** Adaptive T-cell therapy (ACT) is a form of transfusion therapy in which mature T-cell subsets are infused into the patient to eliminate tumor cells and ideally also prevent disease recurrence (ref. 186; Fig. 3C). The initial experimental strategy used in this field consisted of the isolation of tumor-infiltrating lymphocytes (TIL) from surgically excised samples, with subsequent expansion and reinfusion into patients. Early preclinical studies using metastatic cancer models showed that the isolation and expansion of TILs ex vivo, followed by their infusion in vivo with IL2, led to the generation of lytic T cells with broad capacity to recognize cancer cells, resulting in the successful treatment of lung, liver, and subcutaneous tumors (187). Stimulated by these initial observations, ACT of TILs together with IL2 was evaluated in patients with metastatic melanoma (188). Although tumor regression was reported in approximately one third of the patients, the duration of response lasted only four months on average, and few patients showed a CR (189). In the two decades following these initial trials, numerous clinical studies have evaluated ACT with TILs, with mixed success to date in solid tumors. However, there are encouraging recent developments, including a phase I trial that reported durable responses in NSCLC (190).

One of the major challenges of TIL therapy is its dependence on the presence of functional effector T cells with antitumor activity, which does not happen for most cancer types (191). In addition, the activation and expansion of these cells in culture can be technically difficult. Therefore, strategies to genetically modify T cells have been developed, including TCR and CAR T-cell therapies (Fig. 3C). Both approaches involve manipulating the patient’s own T cells ex vivo to express receptors that recognize tumor-specific antigens, and reintroducing them to boost T cell–mediated cancer cell killing, but they differ regarding the mechanism for antigen recognition. TCR T cells have a broader range of targets as they can recognize both extracellular and intracellular antigen fragments, but only if they are presented by MHC molecules, which is a limitation of engineered TCR T cells (192). Although these therapies have proved successful in preclinical models and various clinical trials (192), there is not yet a TCR-based T-cell therapy approved by the FDA.

While TIL and TCR T-cell therapies can target only cancer cells that present their antigens via MHC/HLA, CAR T cells can bypass this requirement by recognizing target molecules on the surface of tumor cells (128). This is an important distinction, given that MHC/HLA molecules are frequently downregulated in tumors. In the clinic, CAR T-cell therapy has proved extremely successful for hematologic malignancies, resulting in the FDA approval of three independent CAR T-cell therapies directed toward CD19 for the treatment of B-cell acute lymphoblastic leukemia (tisagenlecleucel), large B-cell lymphoma (axicabtagene ciloleucel), and more recently mantle cell lymphoma (bruxolucabtagene autoleuce). In addition to these approvals, several CAR T-cell therapies are now being evaluated in clinical trials for the treatment of other subtypes of lymphoma, leukemia, and multiple myeloma (192). On the other hand, CAR T-cell therapies have been only moderately effective to date for solid tumors, including neuroblastoma (193), mainly due to the considerable heterogeneous nature of these tumors and the presence of a far more complex TME. Several trials are ongoing in various tumor types (192), and novel strategies are being developed for diverse TMEs,
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including CAR T-cell therapies against the pan-cancer B7-H3 (CD276) molecule, which was shown to be successful in murine models of pediatric tumors (194). Another approach involves engineering CAR T cells to act on the immunosuppressive TME and revive exhausted T cells (195–197). This is the case for so-called armored CAR T cells, typically modified second-generation CAR T cells, which constitutively express cytokines or ligands that further improve efficacy (191). Indeed, armored CAR T cells engineered to produce IL12 were shown to overcome immunosuppression mediated by Tregs and myeloid cells in the TME, promote CD8 T-cell cytolytic activity (198), and enhance myeloid cell recruitment and antigen presentation (199).

In summary, the use of engineered T cells has been very successful in several types of cancer. However, there are several challenges to be addressed in the coming years, including toxicity issues, the selection of novel antigens, and overcoming an immunosuppressive TME.

**The Gut Microbiome: An Emerging Target to Boost Immunotherapy Responses.** Over the last decade, the number of studies demonstrating the important role of the gastrointestinal microbiota in cancer development and response to therapy has significantly increased. Gut microbiota have been shown to regulate not only therapeutic response, but also the toxicity of several first-line and novel therapies, including chemotherapy, stem cell transplants, and immunotherapy (reviewed in refs. 200, 201). For instance, several studies revealed that certain gut microbiota signatures are associated with better response to ICI, higher immune cell infiltration into tumors, and enhanced systemic immunity (202–207). On the basis of this evidence, there is a growing interest in targeting the microbiome and creating bacterial “consortia” to use in combination with other therapies. In fact, a phase I clinical trial in patients with immunotherapy-refractory melanoma recently reported favorable responses when combining fecal microbiota transplantation with reinduction of anti–PD-1 therapy (208, 209). These pioneering findings underline the potential of microbiome-targeting therapies that will be evaluated in numerous clinical trials in the coming years (201).

**Therapies Directed toward the Tumor-Associated Stroma and Vasculature.** Targeting of vascular networks, stromal cells, and the secreted ECM continues to represent an active area of development, dating back to the early days of the TME field. The antiangiogenic agent bevacizumab (Avastin) was the first TME-targeted therapy approved by the FDA, in 2004, for the treatment of metastatic colorectal cancer (210). There are currently >1,000 registered clinical trials using this compound (https://clinicaltrials.gov), including in combination with numerous immunotherapies, indicating the enduring relevance of vascular-targeted therapies. In this section, we summarize the most clinically advanced therapeutic strategies directed toward the stromal compartment of the TME, focusing on the tumor vasculature, ECM, and CAFs (Fig. 4; Table 2).

**Tumor Vasculature.** Compared with healthy tissues, the tumor vasculature is often tortuous and dysfunctional, and exhibits a heterogeneous vascular permeability. This is due to several morphologic and functional changes, including a high proliferation rate of ECs, reduced cellular tight junctions, abnormal pericyte coverage, and increased ECM deposition (211). This aberrant vasculature can result in inefficient delivery of oxygen, thereby contributing to a hypoxic environment within the tumor mass, which is associated with increased cancer aggressiveness (212). Moreover, the dysfunctional vessels can selectively block the infiltration of specific immune cell types, including CTLs, and significantly interfere with the delivery and distribution of therapeutic agents (213). Therefore, it is not surprising that the tumor vasculature has been one of the most studied targets in the TME field. Most of the efforts have focused on targeting the vasculature from two different perspectives that will be covered below: (i) vascular depletion using antiangiogenic therapies, and (ii) improving the delivery of drugs (and immune cells) via vessel normalization.

**Antiangiogenic Therapies.** Tumor angiogenesis is a complex process driven by the increased production and/or enhanced bioavailability of several soluble growth factors, including members of the VEGF, PDGF, EGF, FGFR2, and ANG families (211, 214). Among these, inhibition of VEGFA/VEGFR2 signaling represents the most widely used antiangiogenic therapy (Fig. 4A). Bevacizumab, a humanized anti-VEGFA mAb, was the first approved antiangiogenic agent for several cancers, including metastatic colorectal cancer, cervical cancer, NSCLC, glioblastoma, and metastatic renal cell cancer (www.accessdata.fda.gov). Most of the ongoing trials are currently focused on assessing the combination of anti-VEGF therapy with chemotherapeutic agents (215) or immunotherapies (216, 217). A meta-analysis of randomized control trials comparing the effect of different chemotherapeutic drugs alone, or in combination with bevacizumab, in metastatic colorectal cancer showed a beneficial effect of the combination in extending OS, PFS, and ORR (218). However, many other trials have reported no clinical benefit in adding the anti-VEGF antibody to their combinations. For instance, a phase III clinical trial in triple-negative breast cancer showed no difference in OS by combining bevacizumab with anthracycline- and/or taxane-based chemotherapy (NCT00528567; ref. 219).

A second line of FDA-approved compounds includes tyrosine kinase inhibitors (TKI) targeting VEGF, FGF, and PDGF receptors, among other receptor tyrosine kinases (RTK; Fig. 4A). In comparison with VEGFA inhibitors, several of these compounds (pazopanib, sorafenib, and sunitinib) have fared better in clinical trials as monotherapies, likely because they not only target the vasculature but also block multiple dysregulated pathways in cancer cells (220). Additional antiangiogenic strategies include the use of ANG2–TIE2 inhibitors, such as trebananib, MEDI3617, and rebastinib, which are now undergoing clinical evaluation (221). However, phase III trials testing the combination of trebananib with carboplatin and paclitaxel failed to show clinical benefit in ovarian cancer (NCT01493505; ref. 222).

Several direct inhibitors of ECs, such as endostatin, are also being evaluated in clinical trials in combination with other therapeutic agents (223, 224).
**Figure 4.** Therapies targeting the tumor-associated stroma and vasculature. Multiple strategies targeting the tumor vasculature, the ECM, and CAFs are in clinical use or at different stages of clinical development, as indicated here and referenced in the text. 

**A.** VEGF/VEGFR inhibition represents the most widely used antiangiogenic strategy [1, 2], which can be achieved using multiple FDA-approved drugs, such as anti-VEGF or VEGFR-specific antibodies, VEGF decoy receptors (VEGF-TRAP), or RTK inhibitors. An alternative antiangiogenic strategy is the use of (3) ANG2–TIE2 inhibitors, including trebananib, rebastinib, and MEDI3617, which are now under clinical evaluation.

**B.** CAFs and the secreted ECM can also be targeted using several approaches. For example, (1) different components of the ECM can be degraded by collagenases or hyaluronidases (PEGPH20), or (2) ECM synthesis can be blocked by inhibiting LOX enzymes. (3) Additionally, some drugs such as losartan are being repurposed for their antifibrotic properties. Several strategies interfering with (4) CAF activation, (5) CAF signaling, or (6) CAF normalization are also being evaluated in clinical trials, including TGFβ inhibitors, FAP targeting agents, or the vitamin D analogue paricalcitol, among many others. An alternative strategy consists of (7) targeting integrin signaling or the downstream effector kinase FAK.
Even though antiangiogenic therapies show clinical benefits for a subset of specific cancers, the overall outcome was not as promising as initially hoped for a TME therapy that was hypothesized to show broad efficacy independent of cancer type. Thus, many preclinical and clinical studies subsequently focused on understanding the possible resistance mechanisms to these therapies. High doses of antiangiogenic treatments were found to result in hypoxia and increased invasiveness and metastasis of cancer cells (225). As an alternative, both preclinical and clinical studies indicated that careful dosing of antiangiogenic therapies could favor vessel normalization, resulting in a better patient outcome (226, 227). However, as continuous inhibition of VEGFA can lead to the compensatory upregulation of other angiogenic factors, different strategies have been explored to induce a more stable vessel normalization. Interestingly, a dual inhibitor of VEGFA and ANG2 (A2V or vanezumab) improved vessel normalization in preclinical models (228), which is being evaluated in clinical trials (229). Other vessel normalization strategies that are being explored in preclinical studies include the reexpression of specific semaphorin family members, endogenous antiangiogenic molecules that can be frequently downregulated in tumors (230), and the inhibition of regulator of G protein (RGS) signaling (231), among many others (reviewed in ref. 232).

Although tumor angiogenesis has been studied for decades, a more in-depth and comprehensive understanding of the complex biology driving the different modes of tumor vascularization is still needed to design optimal vascular-targeted therapies. Cancer cells can engage various strategies for tumor vascularization, including vascular co-option and vascular mimicry (transdifferentiation of tumor cells to ECs). Interestingly, the transmembrane glycoprotein receptor CD44 was recently identified as a novel regulator involved in the process of vascular mimicry (233), and this finding motivated the initiation of a phase I clinical trial using an anti-CD44 mAb for solid tumors (NCT01358903). The considerable heterogeneity of tumor vascular components has also been underestimated to date. Indeed, a recent study in mouse lung tumor models and patient samples identified previously unrecognized phenotypes of tumor ECs at the single-cell level (234). Extending these types of interrogative studies to different tumors and other vascular components, such as pericytes, in a comprehensive pan-organ manner will be critical to design novel therapies that could be used as alternatives to the existing antiangiogenic therapies, for example, by enhancing the infiltration of beneficial immune cell types.

**Combining Vascular-Targeted Therapies with Immunotherapies.** The tumor vascular networks (blood and lymphatic) play critical roles in regulating the efficacy of cancer immunotherapy, as these vessels can actively and specifically either block or enable the infiltration of different immune cell populations into tumors through diverse means. The mechanisms preventing infiltration of CTLs via the blood vasculature include the deregulation of cell-adhesion molecules such as intercellular adhesion molecule 1 (ICAM1) and vascular adhesion molecule 1 (VCAM1), the suppression of T cells by expression of inhibitory receptors and molecules, and the induction of T-cell death by selective Fas ligand (FasL) production by ECs (213, 235). Intriguingly, there is an exquisite mechanistic specificity underlying this regulation, as other immune cell types including Tregs, monococytes, and neutrophils can avidly cross the tumor vasculature (reviewed in ref. 236). These findings have led to a new perspective of targeting the tumor vasculature in combination with immunotherapy, with promising results in several preclinical models (228, 237, 238) and a number of ongoing clinical trials. Most of these trials are focused on combining antiangiogenic therapies with vaccines, cell therapies, or ICI strategies (217). For instance, a phase III trial evaluating the combination of axitinib (VEGFR TKI) and pembrolizumab (anti-PA-1) compared with sunitinib (a different VEGFR TKI) treatment alone in patients with advanced renal cell carcinoma resulted in increased OS, ORR, and PFS (NCT02853331; ref. 239). In addition, the combination of sunitinib with autologous DC immunotherapy showed clinical benefit in patients with metastatic renal carcinoma in phase II trials (NCT00678119; ref. 240), and the combination of a pox virus–based vaccine expressing GM-CSF with sorafenib (VEGFR TKI) has just completed phase III trials in patients with hepatocellular carcinoma (NCT02562755).

Although the current clinical activity is focused on targeting the blood vasculature, tumor-associated lymphatic vessels are additionally emerging as complex multifaceted regulators of cancer immunity (241, 242). Lymphatic-mediated transport of tumor antigens by DCs is central to the generation of antitumor immunity (108, 243). In murine melanoma models, lymphangiogenesis induction promoted the intra-tumoral accumulation of CD8+ T cells (244). In colorectal cancer and melanoma patient samples, a high tumoral lymphatic vascular density correlated with increased infiltration of CD8+ T cells (245, 246). Recently, upon the “rediscovery” of a central nervous system lymphatic network (247, 248), the lymphatic transport of TAA-loaded DCs was observed to be key for the priming of CD8+ T cells in the lymph nodes and their subsequent trafficking to the brain TME, where tumor regression was observed (249, 250). On the other hand, tumor-associated lymphatic endothelial cells can display an immunosuppressive profile and express PD-L1, thereby limiting DC maturation and the cytotoxic function of T cells (242). Moreover, as the lymphatic vasculature can also be associated with metastatic cell dissemination, it will be critical to identify precise strategies that selectively boost this vessel network’s immune-modulatory functions to enhance efficient responses to immune-based therapies and potentiate antitumor immunity.

**Improving Drug Penetration.** The composition and integrity of the vasculature is also a major player in drug delivery and consequently therapeutic efficacy. Even though certain features of the tumor vasculature, including heterogeneous permeability, leakiness, and reduced tight junctions, could be expected to enhance drug penetration into the tumors, the reality is that this leaky vasculature is functionally abnormal—resulting in inefficient delivery of both oxygen and antitumor agents. When blood perfusion through the tumor vasculature is aberrant, this can create an increase in IFP within the tumor, which acts as a physical barrier (251). The tumor ECM
additionally contributes a major physical obstacle as it can collapse tumor microvessels and sequester antitumor drugs (252), as will be discussed below in the ECM-targeting section.

One of the strategies to improve drug delivery into the tumor bed consists of vascular normalization (232). In fact, the beneficial effects reported when antiangiogenic therapies are combined with chemotherapeutic agents are probably due to vessel normalization, a reduction in IFP, and a consequent increase in drug delivery. Indeed, phase II trials with cediranib, a pan-VEGFR TKI, showed clinical evidence of improved tumor blood perfusion and vessel normalization in patients with glioblastoma (253, 254). Similarly, it has been demonstrated that a single infusion with bevacizumab decreased IFP, tumor perfusion, and microvascular density in human colorectal cancer (255). Other strategies to improve drug delivery include the use of nanoparticles, liposomes, antibody–drug conjugates, and ultrasound-mediated techniques (reviewed in refs. 7, 256, 257).

**ECM**

The ECM is composed of diverse proteins and macromolecules, including collagens, glycoproteins, elastin, fibronectins, and proteoglycans, which are secreted by cells into the extracellular space (258, 259). ECM composition varies substantially from organ to organ, and by comparison with healthy tissues, the tumor ECM is further characterized by increased coverage, density, and stiffness. ECM molecules can be produced by multiple cell types in the TME, and the ECM not only provides structural support but also plays a vital role in regulating the behavior of cancer and TME cells. For example, increasing stiffness in the surrounding tissue can promote the epithelial-to-mesenchymal transition in cancer cells, associated with tumor invasiveness, stemness, and metastasis (259, 260). In addition, the aberrant accumulation of ECM affects therapeutic efficacy by acting as a physical barrier to therapeutic agents, and by contributing to the activation of integrin and focal adhesion kinase (FAK) signaling, which leads to reduced apoptosis, increased prosurvival signaling, and chemoresistance (261). Moreover, the expression profile of specific ECM-related genes has been correlated with poor patient prognosis and resistance to therapy in several tumor types (262–264). For example, the expression of certain ECM remodeling genes such as desmopakin or SPARCL1 contributes to chemotherapy resistance in pediatric osteosarcoma (262), whereas in breast cancer, a high expression of TWIST and other ECM-related genes is associated with poor prognosis (263, 264).

Considering the negative effects of aberrant ECM composition and enhanced deposition in tumors, there has been considerable interest in developing novel ECM-targeting therapies (Table 2; Fig. 4B). Many studies have focused on degrading the different components of the ECM, for example, by using collagenases or hyaluronidases, which allows for enhanced distribution of therapeutic drugs (265). In this regard, PEGylated human hyaluronidase (PEGPH20) underwent clinical trials in several solid tumors, including pancreatic and gastric cancers, mainly in combination with other therapeutic agents. As an example, phase II trials testing the combination of PEGPH20 with standard gemcitabine plus nab-paclitaxel chemotherapy showed clinical benefit in patients with pancreatic cancer (NCT01839487; ref. 266). However, a subsequent phase III trial using this combination was terminated due to a negative study outcome (NCT02715804; ref. 267). In addition, a phase I/II clinical trial in patients with metastatic pancreatic adenocarcinoma showed increased toxicity and detrimental effects when combining FOLFIRINOX with PEGPH20 (NCT01959139; ref. 268).

As the ECM is continuously being remodeled and modified by enzymes produced by various cells in the TME, an alternative therapeutic approach could be to directly inhibit the de novo synthesis of ECM components. This may be achieved by blocking the key signaling pathways that promote ECM production, such as TGFβ or HIF1α, or by inhibiting the modifying enzymes necessary for the production, secretion, and maturation of the different ECM components. One such approach involves the targeting of LOX enzymes, which are important for stabilizing collagen networks. Simtuzumab, an antibody targeting LOXL2, has been tested in the clinic; however, phase II trials of simtuzumab in combination with gemcitabine and FOLFIRI in patients with colorectal cancer and pancreatic cancer, respectively, showed that the addition of simtuzumab did not improve clinical outcome (NCT01472198 and NCT01479465; refs. 269, 270). Another approach that is being explored is the repurposing of drugs with antifibrotic properties, such as losartan, metformin, and pirfenidone. Indeed, losartan has shown clinical benefits in phase II trials in combination with FOLFIRINOX and chemoradiotherapy with fluorouracil or capecitabine in pancreatic cancer (NCT01821729; ref. 271). Finally, as ECM components activate integrin-mediated signaling to trigger cellular responses, an alternative strategy consists of targeting integrins or the downstream effector FAK (272). This can be achieved by several compounds including the FAK inhibitor defactinib, which is currently being tested in phase II clinical trials. However, the combination of defactinib with first-line chemotherapy failed to show clinical benefits in patients with malignant pleural mesothelioma (NCT01870609; ref. 273).

In summary, strategies to block the synthesis or enhance the turnover of ECM have not resulted in striking clinical results to date, and these types of broad approaches will likely continue to face challenges for several reasons. First, the ECM is highly complex, as it is formed by numerous components and regulated by multiple pathways, which makes it difficult to target without off-target effects. This is reflected in the toxicities and secondary adverse effects that have been reported in some of the clinical trials (274). Second, several preclinical studies showed that targeting ECM could promote cancer aggressiveness in pancreatic tumors (275). And third, as with many other strategies targeting the TME, the future of ECM-targeted therapies most likely relies on identifying rational combinations with other treatment modalities.

**CAFs**

One of the major cell types that produce ECM molecules in the TME are CAFs, which can support tumor growth through multiple mechanisms. CAFs not only deposit ECM but also produce matrix remodeling enzymes, thereby promoting tumor invasion, metastasis, and resistance to therapies. In addition, CAFs promote tumor growth and invasion by secreting several cytokines, exosomes, and growth factors, for example, leukemia...
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Therapeutic targeting of the TME has long been viewed as a promising strategy in the anticancer armamentarium. The clinical approval of drugs and cell-based therapies directed toward the blood vasculature, immune checkpoints, and T cells has driven the continued exploration of the TME for additional targets to exploit. In this review, we have highlighted some examples of the successes of TME therapies, as well as those that have not lived up to initial expectations. Even the success stories, such as ICIs, are still only beneficial for a subset of cancers and a minority of patients, and a major mechanism limiting their efficacy is an immunosuppressive TME. Thus, the TME field will inevitably continue to focus its efforts on developing strategies to relieve immunosuppressive mechanisms, to activate antitumor immunity and/or boost the efficacy of immune-targeted agents.

Given that immune suppression is mediated via diverse mechanisms and often interconnected cell types, strategies to identify and selectively target key vulnerabilities will be critical. Several areas hold considerable promise, including the modulation of the tumor vasculature in combination with immunotherapies. One recent example involved inhibition of the PAK4 enzyme (which is selectively expressed in glioblastoma blood vessels) in combination with delivery of CAR T cells engineered to target the EGFRvIII mutation in glioma cells (290). In preclinical models, this therapy resulted in reprogramming of the vasculature, which promoted immune cell adhesion and engineered T cells’ subsequent ability to enter the brain, thereby eliciting a robust antitumor response (290). Another intriguing study in mouse colorectal cancer models showed that adaptive resistance to chemotherapy (5-FU and cisplatin) is associated with a pronounced stromal response and T-cell exclusion (291). Combined targeting of the desmoplastic stroma along with the vasculature (VEGF-ΔK) and a CD40 agonist resulted in a conversion from fibrotic immune-excluded tumors to enable the unleashing of a CTL-mediated anticancer response (291). These brief illustrative examples demonstrate the potential for such complex multitargeting strategies to be evaluated in animal models as a means to identify and stratify combinations for potential translation to patients. This type of preclinical “prescreening” will be critical given the immense number of planned immunotherapy clinical trials in which there are simply not enough patients to enroll for all the foreseen combinations (292).

In this regard, rational stratification of patients in advance and accurate monitoring of the immune response and other parameters while on trial will be invaluable to gain as much dynamic information as possible for responders versus non-responders. Several recent advances highlight the power of such approaches (reviewed in ref. 293). As an example, non-invasive liquid biopsies are routinely used to measure circulating tumor DNA in the blood; this was recently combined with peripheral immune analyses to predict clinical benefit from ICIs in patients with NSCLC while on treatment (294). Another tractable approach is the use of EVs as diagnostic markers, given their high accessibility (they are present in nearly all body fluids), and the fact that their molecular content highly depends on the cell of origin, thereby providing relevant information about the pathologic state of the EV-producing cells (295). Given that both the level of EVs/exosomes and their content can be correlated with multiple clinical parameters (e.g., tumor stage, response to therapy; ref. 295), several trials are evaluating EVs as biomarkers. Other studies have incorporated the collection of patient tissue

CONCLUSIONS AND FUTURE DIRECTIONS

Therapeutic targeting of the TME has long been viewed as a promising strategy in the anticancer armamentarium. The clinical approval of drugs and cell-based therapies directed

inhibitory factor (LIF) and growth differentiation factor 15 (GDF15; refs. 276–278). Interestingly, the secretome of CAFs not only influences tumor cells but also other components of the TME, including the vasculature and immune cells. For instance, CAF-derived VEGF drives angiogenesis (279), whereas IL6, CXCL9, and TGFβ modulate T-cell responses (280).

On the basis of these data, multiple studies have focused on targeting CAFs for anticancer therapy (Table 2; Fig. 4B). One strategy is to inhibit fibroblast activation protein (FAP), as FAP-expressing CAFs have been associated with immunosuppression in several preclinical models and in human samples (280–282). Several FAP-targeting agents (e.g., PT630, RO6874281, and sibrotuzumab) are undergoing phase I and II trials in different solid tumors (NCT02198274, NCT02627274, and NCT03386721). In addition, as CAF activation and function are driven by signaling pathways including Hedgehog, NFκB, CXCR4, FGFR, or TGFβ, specific inhibitors of these pathways are also being studied in clinical trials (283). However, phase II trials combining a Hedgehog inhibitor with gemcitabine unfortunately showed a worse clinical outcome than that with gemcitabine alone (NCT01130142; ref. 283). This may be due to the observations that CAFs can have both protumorigenic and anti-tumorigenic roles. For instance, the depletion of smooth muscle actin (SMA+) CAFs in a mouse model of pancreatic cancer had detrimental effects on survival due to an immunosuppressive effect (284). Thus, alternative strategies to CAF depletion have been investigated, such as CAF reprogramming or CAF normalization by vitamin D or vitamin A. Indeed, treatment of pancreatic cancer preclinical models with a vitamin D analogue induced CAF reversion to stellate cells and improved antitumor efficacy (285). The vitamin D analogue paricalcitol is now undergoing phase I and II trials in several solid tumors (NCT03520790 and NCT00637897).

Despite the recent advances in profiling and targeting CAFs, there are still many unknowns behind the biology of this cell type that must be addressed. For instance, as previously mentioned, CAFs are characterized by their high heterogeneity and their capacity to switch between different states (282, 283, 286). A recent study showed that specifically targeting a population of CAFs positive for CD10 and GPR77 increased sensitivity to chemotherapy in breast cancer (282, 283, 286). In another study, reported the identification of somatic copy-number alterations in CAFs isolated from colorectal cancer patient samples, with a substantially higher prevalence compared with fibroblasts isolated from normal adjacent tissue (289), potentially further complicating the therapeutic targeting of CAFs if genetically unstable. Together, these findings emphasize that a deeper understanding of the many different CAF subtypes and how they change during tumor progression is essential for designing subgroup-specific therapies.
biopsies, such as in breast cancer, where the tumor immune microenvironment was assessed in samples collected before treatment, after the first cycle of neoadjuvant chemotherapy, and at the time of surgery (296). Interestingly, this longitudinal study revealed that the on-treatment immune response was more predictive of treatment outcome compared with the paired baseline samples (296), supporting the inclusion of these types of dynamic real-time analyses in clinical trials wherever possible.

Looking forward, there are several areas of active investigation that will likely reveal further exciting insights into the TME in the coming years. It will be critical to move beyond the current focus on targeting individual cell types of interest and rather adopt a more comprehensive systems-level approach in which we analyze and integrate all TME components to identify and disable the critical nodes. We now recognize that the TME can differ quite profoundly from one organ to another, and thus we cannot simply extrapolate findings between different tumor types. We must additionally examine the patient as a whole, and not only focus on the tumor in isolation. For instance, it will be essential to investigate how systemic influences, for example the gastrointestinal microbiome, metabolism, diet, or exercise, or underlying conditions, for example inflammation, cachexia, obesity, and aging, can alter the TME and affect treatment response. By leveraging this ever-expanding wealth of information, the long-held potential of targeting the TME for the benefit of a much broader population of patients diagnosed with cancer is now a goal that is finally within our reach.

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