Shedding Light on another Dark Side of the Devil in Precision Oncology: Tumor Microenvironment and Targeted Therapies

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ABSTRACT

Tumor formation and development involves the long-term co-evolution of neoplastic cells together with the surrounding tumor microenvironment (TME), the latter including fibroblasts, extracellular matrix (ECM), immune cells, tumor vasculature and various other stromal components. Tumor outgrowth at the primary foci and subsequent metastasis is determined not only by genetic changes and/or epigenetic modifications in cancer cells, but also by multiple TME-associated pathological factors. As such, the dynamic tumor topography poses an increasingly daunting challenge to targeted cancer treatment, as the TME compromises the efficacy of otherwise successful anticancer agents by actively dampening therapeutic response and conferring cancer resistance. Therapeutic strategies that are developed to prevent, attenuate or abolish tumor progression should take advantage of cutting-edge-front technologies, and consider to target both cancer cells and the disease-supporting TME, a conception that should be well incorporated into the fine-tuning steps of clinical oncology.
Keywords: tumor microenvironment, stromal fibroblast, immune cells, tumor vasculature, targeted therapy, combinatorial treatment

INTRODUCTION

Cancer is a systemic disease that engages both malignant cells and host stromal cells. Under physiological conditions, the stroma functions as the first and major barrier against tumorigenesis. However, cancer cells frequently initiate critical changes that transform this environment into one that accelerates disease progression. The orchestration of these changes implicates activation of fibroblasts, remodeling of ECM, recruitment of immune cells, and eventually expansion of vascular frameworks. Although the identification of genetic and epigenetic variability within the same tumor suggests complicated events of whole tumor mass evolution, regional differences in selective pressures such as acidity, hypoxia, and the presence of soluble factors (including but not limited to cytokines, chemokines and growth factors) constitutively exist within a tumor and actively shape pathological development. Further, distinct environmental landscapes within a given tumor foci select for mutations that engender continued survival and unlimited expansion, thereby facilitating disease exacerbation. In this chapter, we provide the updated perspectives and insightful understanding of the cancer cell extrinsic compartments, namely the TME, discuss practical attempts to target the individual TME components, and analyze the challenges that lie ahead before we can achieve durable agent-elicited responses when treating cancer patients in clinical settings.

CANCER-ASSOCIATED FIBROBLASTS

Cell of Origin, Molecular Regulation and Pathological Implications

Molecular events through which reactive stromal cells affect nearby cancer cells can be defined so that biomarkers and therapeutic targets can be identified. In vast majority of human solid organs, fibroblasts represent the most abundant mesenchyme-derived cell type that sustains the structural framework in local tissues and make up the bulk of tumor stroma upon tumorigenesis. Compared with normal fibroblasts which typically suppress tumor formation, cancer-associated fibroblasts (CAFs) have increased proliferation rate, active metabolic turnover, enhanced ECM production and intensive cytokine secretion (such as hepatocyte growth factor, HGF; platelet-derived growth factor, PDGF; stromal cell-derived factor 1, SDF1; vascular endothelial growth factor, VEGF) [1], distinct properties that together continuously promote tumorigenesis. Differences in the physiological behavior and stress response of fibroblasts lead to extensive tissue remodeling, which is mediated by increased expression of proteolytic enzymes including diverse matrix metalloproteinases (MMPs), deposition of ECM proteins and pathogenic angiogenesis via releasing multiple proangiogenic factors within the TME milieu [2]. Specifically, tumor stroma heterogeneity may enhance generation of unique damage signals, to which fibroblasts are frequently exposed [3]. Despite the intra- and inter-tumoral heterogeneity of tumor stroma, CAFs within tumors are clinically relevant in most cases. The abundance of stromal fibroblasts
correlates with poor clinical prognosis for diverse cancer types, including breast, pancreatic and skin malignancies [4-6]. Specifically, elevated expression of MMPs by fibroblasts correlates with enhanced aggressiveness and worse prognosis in certain cancers [7,8].

Although CAFs promote tumor initiation, angiogenesis, invasion and metastasis, their normal partners or the physiological fibroblasts are usually quiescent and tend to be activated in a wound healing-like response [9]. The mechanisms that drive quiescent or resting fibroblasts to become “activated” are not thoroughly raveled yet. Recent data suggests that there might be two types of fibroblast activation pathways: “reversible” and “irreversible”, determined partly by epigenetic programs including promoter hypermethylation [10,11]. Once activated, fibroblasts express α smooth muscle actin (αSMA or ACTA2), a cytoskeletal protein associated with smooth muscle cells, and are also termed myofibroblasts [12,13]. Activated fibroblasts are also a principal component of scars and chronic tissue response upon wound healing, the latter well documented in organ fibrosis and found to be different from acute wound healing [14,15].

Activated fibroblasts are highly heterogeneous, and exhibit distinct expression patterns depending on the types of tissues from which they are derived [16]. The expression signature of homeobox genes in fibroblasts is retained in culture, and fibroblast heterogeneity is correlated with site-fidered tissue-resident mesenchymal stem cells (trMSC), which are different from the bone marrow-derived MSCs (BM-MSCs) in nature [9]. However, how these two types of MSCs differ in physiological function remains largely unclear.

It is now established that quiescent fibroblasts share many features with mesenchymal stem cell (MSC) precursors, and they hold the potential to be activated into MSCs in diverse conditions [9]. However, whether all activated fibroblasts are MSCs or only some exhibit such properties remains to be clarified, and one could argue that most current clinical trials with MSCs can also be considered as clinical examination of activated and cultured fibroblasts. Activated fibroblasts in culture can develop into adipocytes, endothelial or chondrocyte-like cells, and also can be induced into pluripotent stem cells (iPSCs) [17,18].

Generally, activated fibroblasts or CAFs isolated from human tumors show multiple distinct properties in contrast to fibroblasts cultured from normal human organs, and fibroblast activation programs can be induced de novo merely by culture conditions [9]. CAFs enhance invasiveness of not only originally invasive but also non-invasive cancer cells [1,19]. The ability of CAFs to influence tumor growth partly depends on their capacity to induce angiogenesis by the CAF-derived factor SDF1, and recruitment of bone marrow-derived endothelial cells [1]. Besides a wide variety of CAF-secreted molecules that have pro-tumorigenic roles, heat shock factor 1 (HSF1) upregulation in CAFs strengthens the HSF1 driven tumor-promoting program, amplifying the pathological influence exerted by the TME [20,21]. In such a case, tumors are able to co-opt the ancient survival functions of HSF1 to orchestrate malignancy, with far-reaching therapeutic implications.
CAFs also mediate secondary tumor growth at the metastatic sites. Particularly, CAFs facilitate cancer dissemination by releasing soluble factors including growth factors and cytokines into peripheral blood to enhance the growth and invasive features of malignant cells in distant organs [22]. Tumor growth factor beta 1 (TGF-β1) stimulated CAFs produce interleukin (IL) 11 to promote colorectal cancer cell survival and increase organ colonization efficiency [23]. In addition, PDGF-stimulated CAFs enhance the intravasation of colorectal cancer cells and formation of distant metastases through secreted stanniocalcin 1 (STC1) [24]. Interestingly, missing fibroblast specific protein 1 (FSP1) impairs mouse fibroblast motility, eventually reducing metastasis rate [25].

Distinct from quiescent fibroblasts which are usually metabolically indolent, CAFs tend to have active metabolism that rely on aerobic glycolysis, similar to highly proliferating cells [26,27]. Several factors are found to be involved in driving metabolic shifts in fibroblast activation, including TGFβ, PDGF, hypoxia, hypoxia-inducible factor (HIF)1α and reactive oxygen species (ROS)-mediated loss of caveolin 1 (CAV1) [9]. Moreover, increased Warburg effect in CAFs is coupled with enhanced catabolic activity and autophagy [28,29]. CAF metabolic reprogramming is regulated by both paracrine signaling from cancer cells and direct contacts between CAFs and cancer cells [30]. Specifically, cancer cells transfer Warburg metabolism to their nearby corrupted CAFs, exploiting their byproducts and proliferate in an environment of low glucose content, symbiotically adapting with their stromal partners to glucose availability. Furthermore, gene expression profiling revealed that CAFs suppress isocitrate dehydrogenase 3A (IDH3A), stabilize HIF1α and induce pro-glycolytic genes under normoxia [28]. Despite unraveled specific molecular underpinnings that support CAF metabolic reprogramming, CAFs may self-sustain their distinct metabolic status, partially through epigenetic remodeling [9]. The metabolism of CAFs may also generate pro-tumorigenic circuits by changing metabolite availability. Starvation of amino acids including tryptophan and arginine is important for T cell activation and lymphocyte function, whereas CAF metabolic shift can regulate immune cell access to these inflammatory metabolites [31,32]. Checkpoint blockade agents against CTLA-4, PD-1, and PD-L1 restore glucose supply in the TME and resume T cell glycolysis and interferon (IFN)-γ production, implying that tumor-imposed metabolic restrictions can dampen T cell responsiveness in cancer patients [31].

**Targeting Stromal Fibroblasts**

Clinically targeting stromal fibroblasts, particularly CAFs, represents a reasonable approach to control tumor progression. There are several factors that make CAFs an attractive target for therapy. Physically, they compose a large portion of tumor mass in solid malignancies, actively participating in the constant crosstalk with cancer cells. CAFs are also relatively stable in genetics as compared with cancer cells, thus the conventional risk of acquired resistance is minimal. As CAFs secrete a large array of soluble factors to the TME space, targeting the signaling pathways activated by these factors hold the potential of controlling specific TME-generated effects. For
example, Lucitanib is a receptor tyrosine kinase (RTK) inhibitor against fibroblast growth factor receptor (FGFR) isoforms 1 through 3, VEGF receptor (VEGFR) isoforms 1 through 3, and PDGF receptors (PDGFRs) a and b, and is currently in phase II trials in patients with advanced malignancies including metastatic breast cancer and lung cancer (NCT02202746, NCT02053636, NCT02109016 and NCT02747797). In addition, Nintedanib is a non-specific inhibitor of RTKs including VEGFR 1 to 3, FGFR 1 to 3, PDGFR a and b, currently undergoing phase I, II, and III clinical trials either as monotherapy or in combinatorial chemotherapy for lung cancer, metastatic melanoma, colorectal cancer and breast cancer patients (NCT02835833, NCT02225405, NCT02308553, NCT02393755). A phase III trial involving Nintedanib recently demonstrated prominent efficacy in promoting progression-free survival (PFS) of advanced ovarian cancer when combined with carboplatin and paclitaxel, in contrast to carboplatin and paclitaxel alone [33].

Despite such exciting progress, however, the majority of fibroblast-oriented therapies currently in clinical trials are not FGFR-specific and only achieved modest success so far. It is proposed that therapies targeting multiple receptor types may be more effective in circumventing malignant phenotypes particularly cancer resistance frequently encountered in clinics [34]. Alternatively, Hedgehog ligands released by cancer cells can activate the pathway in stromal fibroblasts, which in turn induces the production of secreted factors and promotes tumorigenesis by acting back on cancer cells or other stromal components such as the vasculature [35,36]. However, in stark contrast to cancer cells with mutated Hedgehog pathway, inhibitors to proteins including smothened, vismodegib and saridegib, have largely failed to show a benefit in colorectal, ovarian or pancreatic cancer upon combination with standard-of-care therapies [37-39]. Despite the assumption that Hedgehog pathway inhibition should reduce the desmoplasmic stroma in pancreatic tumors and facilitate drug delivery [40], this effect in preclinical models led only to very limited responses that did not ultimately translate into clinical trials [39].

ENDOTHELIAL CELLS AND TUMOR VASCULATURE

Pathological Functions

In tumors, vessel formation involves multiple steps including degradation and reincorporation of existing vascular basement membranes, usually in a tissue-specific manner [41]. In addition, tumor-co-opted vessels maintain some tissue-specific characteristics, and the host tissue can influence the resulting vascular network organization. Inadequately developed or poorly organized tumor vasculatures generates areas of hypoxia and insufficient nutrient supply, while the distance from cancer cells to vascular beds creates a gradient that can be crucial for the distribution of chemicals to the tumor center [42]. It was reported that microvessel density (MVD) is a significant prognostic index for poor outcome in non-small-cell lung cancer (NSCLC), colorectal cancer and breast cancer [43-45].
Beyond the role of tumor vasculature as a natural barrier to optimal drug delivery [46], recent data indicated that cytoreduction of the stroma can reduce interstitial pressure, while improving vessel patency and flow through enzymatic destruction of hyaluronan [47]. In this case, drug delivery was potently improved, as illustrated by enhanced efficacy of standard-of-care chemotherapy upon combination with hyaluronan depletion in an experimental animal model of pancreatic cancer.

A direct role for endothelial cells in tumor response to therapies through secreted factors was recently reported. Chemotherapeutic agent doxorubicin induces thymic endothelial cell production of the cytokine IL-6 and tissue inhibitor of metalloproteinase (TIMP)-1, rendering the thymus a protective reservoir for cancer cells during chemotherapy [48]. As supporting evidence, increased expression of the soluble factor VEGFA in the TME is associated with a worse prognosis than those of low VEGFA expression in metastatic colorectal, lung and renal cell malignancies [49].

Physical niches in the TME protect cancer cells during drug treatment, with the perivascular space in tumor foci playing a remarkable role in minimizing treatment responses. The observation of tumor re-initiating cells along tumor vessels indicates these locations are functionally protective for the transformed cell population. Paracrine signaling from endothelial cells within such niches can promote chemo resistance via inducing stem-cell-like features in a subset of colorectal cancer cells [50]. Further, hypoxic regions in tumor foci support the survival of colon cancer stem cells between the cycles of chemotherapy [51]. Together, data from these studies highlight the unanticipated effects of tumor vasculature upon therapeutic intervention, which can dramatically limit anticancer efficacy.

**Targeting Tumor Vasculature**

There are some agents that specifically target VEGFA pathways and have exhibited promising values in clinical oncology. The humanized monoclonal anti-VEGFA antibody, Bevacizumab (Avastin; Genentech/Roche), was the first agent approved by the US Food and Drug Administration (FDA) to target tumor VEGFA pathway [52]. Although initially approved in 2004 for the case of metastatic colorectal cancer, Bevacizumab-associated regimes were later proved to be effective therapeutics in both oncology and ophthalmology [53]. The application of Bevacizumab in ophthalmology was based on the fact that VEGFA inhibition could specifically suppress ocular neovascularization in animal models [54,55]. Subsequently, pegaptanib (Macugen; Valeant/Pfizer) and ranibizumab (Lucentis; Novartis/Genentech) received FDA approval to treat neovascular age-related macular degeneration (AMD) in 2004 and 2006, respectively [56,57].

When supplied with other agents as combinational chemotherapy, bevacizumab helps promote disease regression in patients with advanced NSCLC and metastatic colorectal cancer [58,59]. In addition, bevacizumab demonstrated remarkable benefits for metastatic renal cancer when the agent was combined with interferon-α (IFN-α) and recurrent glioblastoma multiforme.
(GBM), rather than the monotherapies alone [60,61]. However, a recent study indicates that
bevacizumab has limited activity in patients with recurrent grade II/III gliomas, and advocates to
better define its therapeutic roles among the limited options in people with recurrent grade II/
III gliomas [62]. Despite the restrained efficacy of bevacizumab in certain cases, improvements in
progression-free survival (PFS) were reported in patients with high-risk ovarian cancer and in
overall survival for patients with advanced metastatic cervical cancer [63,64], providing further
evidence to support the use of bevacizumab in cancer patient treatments. Since then, the FDA
has approved several other small molecule inhibitors such as sunitinib, axitinib, pazopanib,
vandetanib, cabozantinib and sorafenib, which target the VEGF receptor VEGFR2 and alternative
RTKs for multiple cancers [65].

Of note, some preclinical data suggested that small molecule anti-angiogenic treatments
could rather enhance invasiveness and metastasis [66,67]. Further evidence including the data
derived with a VEGFR tyrosine kinase inhibitor (TKI) confirmed the negative effects of these
agents at supraclinical dose levels, largely in line with data from many epithelial malignancies,
including metastatic breast, colorectal, kidney, and pancreatic cancers [66,68]. Moreover, clinical
investigations indicate that inhibiting VEGF in a subset of GBM patients may exacerbate the
invasive phenotype of cancer cells [69], implying that VEGF can function as a negative regulator of
invasive signaling pathways and combination therapy may deserve consideration and exploration.

INFLAMMATORY AND IMMUNE CELLS

Lineages and Tumor-Promoting Activities

In humans, both the innate and adaptive immune systems are implicated in enhancing
and preventing tumor progression. Although a potent immune system can mount anti-tumor
responses, mechanisms of immune suppression do prevent this process and accelerate disease
progression.

Recruitment and localization of immune cells in the TME niche vary widely in neoplastic
lesions, and the tumor immune contexture is subject to influence by various factors including
those secreted by CAFs, the permeability of tumor vasculature, and the characteristics of
neoplastic cells [65]. For instance, the vascular bed in a tumor may substantially influence the
immune contexture since endothelial cells modulate immune cell motility, as evidenced by the
fact that high grade ovarian tumors have more tumor-infiltrating lymphocytes than those that
have low numbers of such immune cells [70].

Activation of T-cells involves both positive and negative checkpoint signals that can together
regulate responses to prevent overt autoimmunity and tissue damage. First, continuous
engagement of inhibitory receptors on T-cells including cytotoxic T-lymphocyte-associated
antigen-4 (CTLA-4) and programmed death 1 (PD-1) via over expression of their ligands
represents a direct way of minimizing cytotoxic T-cell activation in the tumor foci [71]. Second,
dampening antitumor T-cell responses by forming an immunosuppressive environment is an indirect means. Particularly, the myeloid-derived suppressor cell (MDSC) population composed of immature dendritic cells (DCs), monocytes, neutrophils and early myeloid progenitors, actively communicate with the immune system, a major mechanism that is associated with immune suppression in tumors [72]. Recent data indicated that CAFs can attract monocytes by SDF-1a/chemokine CXC motif receptor (CXCR) 4 pathway and induce differentiation of monocytes into MDSCs via IL-6-mediated STAT3 activation, and these MDSCs subsequently interfere with T-cell proliferation and alter T-cell function and/or phenotype in an STAT3-dependent manner [73]. Recruitment of MDSCs to the tumor suppresses adaptive immunity and fosters angiogenesis through the release of soluble factors including VEGFA, basic fibroblast growth factor (bFGF) and TGF-β [74]. In addition, MDSCs inhibit natural killer (NK) cell function, expand the population of immunosuppressive regulatory T-cells, while inhibiting activation, expansion and migration of effector T-cells by changing the TME [75]. In diffuse large B-cell lymphoma (DLBCL), the number of circulating monocytes and neutrophils represents an independent prognostic factor, and T-cell proliferation can be restored after depletion of monocytes [76]. Besides, B cells are able to promote tumor progression by enhancing pro-tumoral inflammation, a function independent of T-cells [77].

Alterations in the receptor repertoire of NK cells and ligand expression at primary tumors or metastatic sites might diminish NK cell activity, causing increased tumor invasion and metastasis [78]. Cancer cells, CAFs and aberrant infiltrates of immune cells such as tolerogenic or suppressive macrophages, DCs and T cells, can either secrete immunosuppressive molecules or modify a complex series of receptors that normally modulate the activation and anticancer function of NK cells. In both the primary tumor and the periphery, regulatory T cells (Treg cells) and MDSCs inhibit the activation and function of NK cells by several mechanisms [79]. Cytokines and metabolites including TGF-β, adenosine, prostaglandin (PGE)2 and idoleamine dioxygenase (IDO) can directly suppress the maturation, proliferation and function of NK cells [80-82]. Particularly, TGF-β signaling affects both the number and anti-metastasis function of NK cells [83].

A recent study indicated that B cells play a detrimental role in anticancer immunity, while targeting B cells can potentially enhance the anticancer response and improve the clinical efficacy of cancer vaccines [84]. In diverse hematological and solid tumors, mast cell accumulation is associated with increased neovascularization, tumor aggressiveness, and metastatic dissemination. Emerging data indicates that mast cells are a new target for cancer therapy via the selective inhibition of angiogenesis and tissue remodeling, together preventing mast cell-mediated immune suppression [85]. Furthermore, Tumor-associated macrophages (TAMs, or M2) also drastically affect disease progression depending on their specific polarization [86]. TAMs affect organization of the ECM and angiogenesis by over expression of legumain, a protein that can promote degradation of fibronectin and collagen, and therefore play a critical role in the progression of DLBCL [87].
Targeting Immune Cells

In the year of 2010, the US FDA approved the cell-based therapy called Provenge (Sipuleucel-T) as a first immune-agent for castration resistant prostate cancer (CRPC) [88]. Sofar, Provenge together with other treatments including chemotherapy (docetaxel and cabazitaxel), radiation (alpharadin) and hormone therapy (abiraterone and enzalutamide) represent a set of standard cares to improve life expectancy of CRPC patients. Later, ipilimumab, a monoclonal antibody targeting the pivotal immune checkpoint protein CTLA-4, was also approved by FDA [89]. Administration of ipilimumab generated significant improvement in the overall survival (OS) of metastatic melanoma patients, with manageable toxic effects in real life [90]. In addition, biological data including lymphocyte and eosinophil counts at the time of the second ipilimumab infusion hold the potential to be early markers that are associated with a better OS. By blocking CTLA-4, ipilimumab enhances anticancer effector T-cell function and inhibits immunosuppressive regulatory T-cell activities [91]. Recent clinical advancement has triggered increasing interest in the development of tumor therapies that either counteract immunosuppressive mechanisms, such as PD-1 and its ligand PD-L1, restore T-cell function or promote immune activities by engaging co-stimulators such as OX40 with agonistic antibodies which enhance priming of antigen-specific CD8(+) T cells [92].

Therapeutically targeting individual components of human innate immune system is also under intensive clinical examination. For example, an anti-CD40 antibody has shown prominent response when administered together with gemcitabine in chemotherapy. In a small cohort of patients with surgically incurable pancreatic ductal adenocarcinoma (PDA), CD40-activated macrophages substantially infiltrated tumors and facilitated the tumor stroma depletion independent of therapy-induced T cells [93]. In addition, Toll-like receptors (TLR) ligands have successfully been employed to improve patient health conditions. However, it is proposed that such TLRs need to be finely tuned to further optimize treatments. Specifically, novel specific ligands with conjugated molecules, nanoparticles, and targeted drug delivery hold eminent promise to effectively optimize the therapy for various cancer etiologies [94].

Remodeling the immunosuppressive tumor microenvironment to restore NK cell function is another attractive therapeutic strategy, mainly because NK cell activities do not rely on antigen specificity [95]. Targeting NK cells are also hopeful in treating the minimal residual disease (MRD). Recent studies have highlighted the capacity of human NK cells to specifically eliminate cancer stem-like cells, a “resting” subpopulation in most solid tumors that is resistant to most conventional anticancer agents [96]. Although NK cells can be derived from various sources including both patients (autologous setting) or healthy donors (allogeneic setting), a predominant approach to adoptive NK cell therapy is to transfer unmodified autologous or allogeneic NK cells [95]. In particular, the human NK cell line NK-92 is highly cytotoxic against a broad spectrum of cancer cells, and infusions of NK-92 cells are demonstrated to be basically safe and well tolerated in
cancer patients [97]. However, since NK-92 cells need to be irradiated before provided to patients as a safety measure, a study has recently evaluated the effects of such irradiation. Experimental data indicated that irradiated NK-92 cells expressing a chimeric antigen receptor (CAR) directed against ErbB2 reserve high and specific cytotoxicity and can protect mice against lung metastasis in a similar efficacy as unprocessed NK-92 cells, supporting the outstanding potential of this methodology for future clinical development [98].

SUMMARY AND FUTURE DIRECTIONS

Successful treatment of human cancer, particularly in the settings of therapy-resistant or drug-refractory conditions, remains a major challenge in contemporary era. Despite the advancements in targeting non-cancerous tissue compartments (Table 1), major barriers and significant challenges stubbornly lie ahead for improving stromal targeting strategies in clinical practice. There are substantial difficulties in thoroughly assessing the composition of the TME, appropriately pinpointing the pathological mechanisms underlying the heterogeneity of observed responses of human cancer patients and effectively correlating such responses with drug resistance and clinical outcome. As well reviewed elsewhere, the intricate and complex crosstalk between the TME and cancer cells can actively shape acquired resistance and ultimately cause treatment failure [65,99-102]. Targeting the TME is obviously advantageous, since stromal components usually have limited genetic mutations or epigenetic aberrations as frequently found in cancer cells. Many of the strategies outlined in this chapter hold significant promise for improving the efficacy of the TME-targeted therapies and enhancing patient treatment indexes.
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Table 1: Typical therapeutic agents targeting the specific TME compartments, either approved or in clinical trials.
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<tr>
<td>Blinatumomab</td>
<td>CD3 and CD19</td>
<td>Bi-specific scFv</td>
<td>Amgen</td>
<td>Phase II (NCT01741792, NCT01466179, NCT01207388, NCT01471782, NCT00560794, NCT01209286)</td>
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<td>AMG 820</td>
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<td>Amgen</td>
<td>Phase I (NCT01444404)</td>
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<td>AMP-224</td>
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<td>TRX-518</td>
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<td>IMC-CS4</td>
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<td>CP-870,893</td>
<td>CD40</td>
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<td>Pfizer</td>
<td>Phase I (NCT00711191, NCT01005827, NCT00607048, NCT01456585, NCT01103635)</td>
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<td>IMC-CS4/LY3022855</td>
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<td>Carlumab</td>
<td>CCL2</td>
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<td>MNRP1685A</td>
<td>Neuropilin-1</td>
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<td>Genentech</td>
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<td>Clazakizumab</td>
<td>IL-6</td>
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<td>Bristol-Myers Squibb</td>
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<td>Olokizumab</td>
<td>IL-6</td>
<td>Antibody</td>
<td>UCB Japan, UCB Pharma</td>
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<tr>
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<td>Cell</td>
<td>Clinical trial (or FDA approved if part of HSCT)</td>
<td>Completed (NCT00187096)</td>
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<tr>
<td>Allogeneic NK cells</td>
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<td>St. Jude Children's Research Hospital</td>
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<td>NantKwest</td>
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<td>Antibody</td>
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<td>Phase I (NCT01775631, NCT02110082, NCT01307267)</td>
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<td>mAbs: to NKG2A</td>
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<td>Antibody</td>
<td>Innate Pharma/ MedImmune LLC</td>
<td>Phase II (NCT02420938)</td>
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**Notes:**
- **FDA approved** indicates that the drug is approved by the FDA.
- **Phase(s)** list the phases of clinical trials completed or ongoing for each drug.
In a long run, combinatorial treatments engaging TME-targeting agents are more effective than conventional anticancer drugs alone. Cancer patients often undergo substantial conventional therapy prior to receiving TME-treatments such as immunotherapy. Future work may focus on understanding the influence of chemotherapy and conditioning agents on immune cell activity for selection of the appropriate clinical settings for immune cell-directed regimens. Further, critical knowledge about immune cell-homing capacities and key insights into their limited infiltration in solid tumors is still lacking [95,103]. Further understanding of the heterogeneity of the TME cell components and their individual implications in human malignancies will definitely allow scientists and clinicians to take better advantage of recent pharmacological advances. Altogether, it is an exciting time for the rapidly developing TME biology and targeting pipelines in drug industry. Fortunately, the tremendous anticancer capacities of various novel agents are increasingly being brought to the cancer clinics, and the results from the many ongoing and prospective trials are eagerly awaited.

**CONFLICT OF INTEREST STATEMENT**

The author declares no conflicts of interest.

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