SMGroup

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

Yu Sun[1,2,3,4]*

[1]Key Lab of Stem Cell Biology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Shanghai Jiaotong University School of Medicine, , Shanghai, China 200031

[2]Collaborative Innovation Center of Systems Biomedicine, Shanghai Jiaotong University School of Medicine, Shanghai, China 200240

[3]VA Seattle Medical Center, Seattle, WA, USA 98108

[4]University of Washington, Department of Medicine, Seattle, WA, USA 98195

***Corresponding author:** Yu Sun, Key Laboratory of Stem Cell Biology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Shanghai Jiaotong University School of Medicine, 320 Yueyang Road, Biological Research Building A, Shanghai 200031, China. Tel: +86 21 54923302; Fax: +86 21 54923302; E-mail: sunyu@sibs.ac.cn

Published Date: October 31, 2016

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\f\bf\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 secret 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 smoothened, 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 metal-loproteinase **(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.

Table 1: Typical therapeutic agents targeting the specific TME compartments, either approved or in clinical trials.

Molecule	Target	Molecular type	Company	Status
ECM/fibroblasts				
Sonidegib	SMO	Small molecule	Novartis	Phase II (NCT01708174, NCT01757327, NCT02195973)
Vasculature				
Bevacizumab	VEGFA	Antibody	Genentech/Roche	FDA-approved ((BLA) 125085)
Vandetanib	VEGFRs, PDGFRs, EGFR	Small molecule	AstraZeneca	FDA-approved ((NDA) 022405)
Sunitinib	VEGFRs, PDGFRs, FLT3, CSF1R	Small molecule	Pfizer	FDA-approved ((NDA) 021938)
Axitinib	VEGFRs, PDGFRs, KIT	Small molecule	Pfizer	FDA-approved ((NDA) 022324)
Sorafenib	VEGFRs, RAF, PDGFRs, KIT	Small molecule	Bayer	FDA-approved ((NDA) 021923)
Pazopanib	VEGFRs, PDGFRs, KIT	Small molecule	GlaxoSmithKline	FDA-approved ((NDA) 022465)
Cabozantinib	VEGFR2, RET, MET	Small molecule	Exelixis	FDA-approved ((NDA) 023756)
Ziv-aflibercept	VEGFA, VEGFB, PIGF	Receptor-Fc fusion	Regeneron	FDA-approved ((BLA) 125418)
AMG 386	ANG2	RP-Fc fusion protein	Amgen	Phase III (NCT01204749, NCT01493505, NCT01281254)
Parsatuzumab	EGFL-7	Antibody	Genentech/Roche	Phase II (NCT01399684, NCT01366131)
Enoticumab	DLL4	Antibody	Regeneron	Phase I (NCT00871559)
Demcizumab	DLL4	Antibody	OncoMed	Phase I (NCT00744562, NCT01189968, NCT01189942, NCT01189929)
Nesvacumab	ANG2	Antibody	Regeneron	Phase I (NCT01271972 NCT01688960 NCT01997164)
Immune				
Ipilimumab	CTLA-4	Antibody	Bristol-Myers Squibb	FDA-approved ((BLA) 125377)
Sipuleucel-T	PAP	DC vaccine	Dendreon	FDA-approved ((BLA) 125197)
Aldesleukin	IL-2	RP	Prometheus	FDA-approved ((BLA) 103293)
IFNα-2b	IFN-α receptor	RP	Merck	FDA-approved ((BLA) 103132)
MK-3475	PD1	Antibody	Merck	Phase III (NCT01866319)
Nivolumab	PD1	Antibody	Bristol-Myers Squibb	Phase III (NCT01642004, NCT01668784, NCT01673867, NCT01721746, NCT01721772, NCT01844505)
Nivolumab	OX40	Antibody	Bristol-Myers Squibb and PPMC	Phase III (NCT01642004, NCT01668784, NCT01673867, NCT01721746, NCT01721772, NCT01844505)
MPDL3280A	PDL1	Antibody	Genentech/Roche	Phase II (NCT01846416)
PLX3397	KIT, CSF1R, FLT3	Small molecule	Plexxikon	Phase II (NCT01349036)
BMS-663513	CD137 (4-1BB)	Antibody	Bristol-Myers Squibb	Phase II (NCT00612664)

Blinatumomab	CD3 and CD19	Bi-specific scFv	Amgen	Phase II (NCT01741792, NCT01466179, NCT01207388, NCT01471782, NCT00560794, NCT01209286)
AMG 820	CSF1R	Antibody	Amgen	Phase I (NCT01444404)
AMP-224	PD1	Antibody	GlaxoSmithKline	Phase I (NCT01352884)
TRX-518	GITR	Antibody	GITR, Inc.	Phase I (NCT01239134)
IMC-CS4	CSR1R	Antibody	ImClone/Eli Lilly	Phase I (NCT01346358)
CP-870,893	CD40	Antibody	Pfizer	Phase I (NCT00711191, NCT01008527, NCT00607048, NCT01456585, NCT01103635)
IMC-CS4/ LY3022855	CSF1R	Antibody	Eli Lilly	Phase I (NCT01346358 NCT02265536)
Carlumab	CCL2	Antibody	Centocor Research & Development	Phase II (NCT00992186)
MNRP1685A	Neuropilin-1	Antibody	Genentech	Phase I (NCT00954642 NCT00747734)
Clazakizumab	IL-6	Antibody	Bristol-Myers Squibb	Phase I (NCT02015520)
Olokizumab	IL-6	Antibody	UCB Japan UCB Pharma	Phase II (NCT01463059
Autologous NK cells	NK cell	Cell	Clinical trial (or FDA approved if part of HSCT)	UMIN000007527
Allogeneic NK cells	NK cell	Cell	St. Jude Children's Research Hospital	Completed (NCT00187096)
NK cell lines	NK cell	Cell	NantKwest	Phase I (NCT00900809) Phase II (NCT02465957)
CAR NK cells	NK cell	Cell	National University Health System, Singapore	Phase II (NCT01974479)
IL-2	NK cell	Cytokine	FDA approved	FDA approved
IL-15	NK cell	Cytokine	University of Minnesota/ National Cancer Institute	Phase II (NCT01385423 NCT01875601)
GSK3 inhibitors	NK cell	Small molecule	Eli Lilly	Phase I (NCT01632306 NCT01287520) Phase II (NCT01632306 NCT01214603)
mAbs to CD137	NK cell	Antibody	Bristol-Myers Squibb/Pfizer/ M.D. Anderson Cancer Center	Phase I (NCT01775631 NCT02110082 NCT01307267) Phase II (NCT02420938)
mAbs: to NKG2A (monalizumab (IPH2201))	NK cell	Antibody	Innate Pharma/ MedImmune LLC	Phase I (NCT02459301 NCT02331875 NCT02557516 NCT02643550 NCT02671435) Phase II (NCT02331875 NCT02557516 NCT02643550 NCT02671435)

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.

ACKNOWLEDGMENTS

We are grateful to members of our laboratory for contributions in this area. Supported by the grants from National Natural Science Foundation of China (81472709, 31671425), and the National 1000 Youth Elites Research Program of China, the U.S. Department of Defense **(DoD)** Prostate Cancer Research Program **(PCRP)** (Idea Development Award PC111703). We also thank the reviewers for valuable insights, comments and suggestions. Our sincere apologies go to numerous scientists and clinicians whose work we are unable to cite due to space limitations.

References

1. Halpin GT and Nelson P. Land development in the south east wheat belt. Perth: Dept. of Agriculture. 1967; 41.
2. Hobson FG. Montgomery County law reporter. Montgomery Bar Association etc: Norristown, Pa.
3. Quante M, Tu SP, Tomita H, Gonda T, Wang SS, et al. Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth. Cancer Cell. 2011; 19: 257-272.
4. Kempsky NP, et al. Representing parolees and prisoners; a two-hour discussion. California Continuing Education of the Bar. CR/PA I, II: Berkeley. 1975.
5. Wing GM, Nelson P. Transport theory, invariant imbedding, and integral equations : proceedings in honor of G.M. Wing's 65th birthday. Lecture notes in pure and applied mathematics. New York: M. Dekker. 1989; 444.
6. Nelson PM. Transportation noise reference book. London; Boston: Butterworth. 1987.
7. Amin N, Moyo NP. Development and crisis in Sub-Saharan Africa: a critical comment. DPP joint Working paper / University of Zimbabwe. Milton Keynes, U.K: Development Policy and Practice Research Group, Technology Faculty, The Open University. 1991; 36.
8. Garcia AB, Gocke RP, Johnson NP. Virtual prototyping: concept to production. Fort Belvoir, VA: Defense Systems Management College Press. 1994; 179.
9. Bard, N.P., Pioneers with web feet. 1st ed. 1970, Solon, Ohio,: Solon Sesquicentennial Committee. 80 p.
10. Benson, N.P., Our government; national, state, local. 1922, Milton, Pa., Philadelphia etc.: Weidenhamer & company. 249 p.

11. Benson NP. Our government, national, state, local. Milton, Pa., Philadelphia etc: Weidenhamer & company. 1921; 249.

12. Bonachea RE, Valde´s NP. Cuba in revolution. Garden City, N.Y: Anchor Books. 1972; 544.

13. Polanska UM, Orimo A. Carcinoma-associated fibroblasts: Non-neoplastic tumour-promoting mesenchymal cells. Journal of Cellular Physiology. 2013; 228: 1651-1657.

14. Castro F, Bonachea RE, Valde´s NP. Revolutionary struggle, 1947-1958. Selected works of Fidel Castro, v 1. Cambridge: MIT Press. 1972; 471.

15. Duffy NP. Useful hints to parents relative to the preservation of the natural teeth. Altoona, Pa. 1886; 15.

16. Estrada NP. From followers to leaders: the Apostles in the ritual of status transformation in Acts 1-2. Journal for the study of the New Testament Supplement series. London; New York: T & T Clark International. 2004; 281.

17. Guidry NP. A graphic summary of world agriculture. Rev. Sept. 1964. ed. Miscellaneous publication / United States Department of Agriculture. Washington, D.C: U.S. Dept. of Agriculture, Economic Research Service, Regional Analysis Division. 1964; 64.

18. Guild NP, Palmer KT. Introduction to politics; essays and readings. New York: Wiley. 1968; 341.

19. Lande NP. Classical logic and its rabbit holes: a first course. 2013; 504.

20. Landman JH, Mead NP. An outline history of the world since 1914. 4th ed. College outline series. New York: Barnes & Noble. 1940; 347.

21. Scherz-Shouval R, Santagata S, Mendillo ML, Sholl LM, Ben-Aharon I, et al. The reprogramming of tumor stroma by HSF1 is a potent enabler of malignancy. Cell. 2014; 158: 564-578.

22. Miller N. A law student's guide: legal education's knowledge, skills, and ethics dimensions. 2010, Durham, N.C.: Carolina Academic Press. xvi, 216 p.

23. Miller N, Eggers V. Teaching law: a framework for instructional mastery. Getzville, N.Y.: Bridge Pub. Group. 2010; 174.

24. Moyo NP, Purcell J, Talintyre D. Industrial relations at establishment level: a statistical survey. CIR study 2. 1973; 74.

25. Moyo NP. Notes on industrial relations at Bamangwato Concessions Limited, Selebi-Pikwe. Working paper - National Institute for Research in Development and African Studies, University of Botswana and Swaziland no 18. Gaborone, Botswana National Institute for Research in Development and African Studies, Documentation Unit, University College of Botswana, University of Botswana and Swaziland. 1978; 38.

26. Sunderlin G, et al. Studies in home canning. Ames, Iowa: Iowa State College of Agriculture and Mechanic Arts. 1928; 189-212, 289-311.

27. Moyo NP. The state, planning, and labour: towards transforming the colonial labour process. Working papers in economics. Harare: Dept. of Economics, University of Zimbabwe. 1988; 31.

28. Sutherland E, Nelson PM. Food preparation, principles and procedures; a laboratory manual for college courses in food manipulation. St. Louis, New York etc: Planographed by John S. Swift co., inc. 1943; 292.

29. Valde´s NP. Ideological roots of the Cuban revolutionary movement. Occasional papers - Institute of Latin American Studies no 15. Glasgow: Institute of Latin American Studies, University of Glasgow. 1975; 5: 49.

30. Valde´s NP. Cuba. Bogota´: Ediciones Tercer Mundo. 1973; 106.

31. Woodburne, L.S., N.P. Meade, and New York (City) Board of Higher Education. [from old catalog], An appraisal of the faculty organization bylaws governing the College of the City of New York. 1950, New York?

32. Ghesquière B, Wong BW, Kuchnio A, Carmeliet P. Metabolism of stromal and immune cells in health and disease. Nature. 2014; 511: 167-176.

33. du Bois A, Kristensen G, Ray-Coquard I, Reuss A, Pignata S, et al. Standard first-line chemotherapy with or without nintedanib for advanced ovarian cancer (AGO-OVAR 12): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet Oncol. 2016; 17: 78-89.

34. Hansen JM, Coleman RL, Sood AK. Targeting the tumour microenvironment in ovarian cancer. Eur J Cancer. 2016; 56: 131-143.

35. Bailey JM, Mohr AM, Hollingsworth MA. Sonic hedgehog paracrine signaling regulates metastasis and lymphangiogenesis in pancreatic cancer. Oncogene. 2009; 28: 3513-3525.

36. Theunissen JW, de Sauvage FJ. Paracrine Hedgehog signaling in cancer. Cancer Res. 2009; 69: 6007-6010.

37. Berlin J, Bendell JC, Hart LL, Firdaus I, Gore I, et al. A randomized phase II trial of vismodegib versus placebo with FOLFOX or FOLFIRI and bevacizumab in patients with previously untreated metastatic colorectal cancer. Clin Cancer Res. 2013; 19: 258-267.

38. Kaye SB, Fehrenbacher L, Holloway R, Amit A, Karlan B, et al. A phase II, randomized, placebo-controlled study of vismodegib as maintenance therapy in patients with ovarian cancer in second or third complete remission. Clin Cancer Res. 2012; 18: 6509-6518.

39. Catenacci DV, Junttila MR, Karrison T, Bahary N, Horiba MN, et al. Randomized Phase Ib/II Study of Gemcitabine Plus Placebo or Vismodegib, a Hedgehog Pathway Inhibitor, in Patients With Metastatic Pancreatic Cancer. J Clin Oncol. 2015; 33: 4284-4292.

40. Olive KP, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science. 2009; 324: 1457-1461.

41. Kalluri R. Basement membranes: structure, assembly and role in tumour angiogenesis. Nat Rev Cancer. 2003; 3: 422-433.

42. Trédan O, Galmarini CM, Patel K, Tannock IF. Drug resistance and the solid tumor microenvironment. J Natl Cancer Inst. 2007; 99: 1441-1454.

43. Meert AP, Paesmans M, Martin B, Delmotte P, Berghmans T, et al. The role of microvessel density on the survival of patients with lung cancer: a systematic review of the literature with meta-analysis. Br J Cancer. 2002; 87: 694-701.

44. Des Guetz G, Uzzan B, Nicolas P, Cucherat M, Morere JF, et al. Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature. Br J Cancer. 2006; 94: 1823-1832.

45. Uzzan B, Nicolas P, Cucherat M, Perret GY. Microvessel density as a prognostic factor in women with breast cancer: a systematic review of the literature and meta-analysis. Cancer Res. 2004; 64: 2941-2955.

46. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science. 2005; 307: 58-62.

47. Provenzano PP, Cuevas C, Chang AE, Goel VK, Von Hoff DD, et al. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. Cancer Cell. 2012; 21: 418-29.

48. Gilbert LA, Hemann MT. DNA damage-mediated induction of a chemoresistant niche. Cell. 2010; 143: 355-366.

49. Hegde PS, Jubb AM, Chen D, Li NF, Meng YG, et al. Predictive impact of circulating vascular endothelial growth factor in four phase III trials evaluating bevacizumab. Clin Cancer Res. 2013; 19: 929-937.

50. Lu J, Ye X, Fan F, Xia L, Bhattacharya R, et al. Endothelial cells promote the colorectal cancer stem cell phenotype through a soluble form of Jagged-1. Cancer Cell. 2013; 23: 171-185.

51. Mao Q, Zhang Y, Fu X, Xue J, Guo W, et al. A tumor hypoxic niche protects human colon cancer stem cells from chemotherapy. J Cancer Res Clin Oncol. 2013; 139: 211-222.

52. Ferrara N, Adamis AP. Ten years of anti-vascular endothelial growth factor therapy. Nat Rev Drug Discov. 2016; 15: 385-403.

53. Holfinger S, Miller AG, Rao LJ, Rowland DY, Hornik JH, et al. Effect of Regulatory Requirement for Patient-Specific Prescriptions for Off-Label Medications on the Use of Intravitreal Bevacizumab. JAMA Ophthalmol. 2016; 134: 45-48.

54. Adamis AP, Shima DT, Tolentino MJ, Gragoudas ES, Ferrara N, et al. Inhibition of vascular endothelial growth factor prevents retinal ischemia-associated iris neovascularization in a nonhuman primate. Arch Ophthalmol. 1996; 114: 66-71.

55. Aiello LP, Pierce EA, Foley ED, Takagi H, Chen H, et al. Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. Proc Natl Acad Sci U S A. 1995; 92: 10457-10461.

56. Ng EW, Shima DT, Calias P, Cunningham ET Jr, Guyer DR, et al. Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. Nat Rev Drug Discov. 2006; 5: 123-132.

57. Chen Y, Wiesmann C, Fuh G, Li B, Christinger HW, et al. Selection and analysis of an optimized anti-VEGF antibody: crystal structure of an affinity-matured Fab in complex with antigen. J Mol Biol. 1999; 293: 865-881.

58. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med. 2006; 355: 2542-2550.

59. Hurwitz HI, Fehrenbacher L, Hainsworth JD, Heim W, Berlin J, et al. Bevacizumab in combination with fluorouracil and leucovorin: an active regimen for first-line metastatic colorectal cancer. J Clin Oncol. 2005; 23: 3502-3508.

60. Yang JC, Haworth L, Sherry RM, Hwu P, Schwartzentruber DJ, et al. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N Engl J Med. 2003; 349: 427-434.

61. Friedman HS, Prados MD, Wen PY, Mikkelsen T, Schiff D, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol. 2009; 27: 4733-4740.

62. Seystahl K, Wiestler B, Hundsberger T, Happold C, Wick W, et al. Bevacizumab alone or in combination with irinotecan in recurrent WHO grade II and grade III gliomas. Eur Neurol. 2013; 69: 95-101.

63. Oza AM, Cook AD, Pfisterer J, Embleton A, Ledermann JA, et al. Standard chemotherapy with or without bevacizumab for women with newly diagnosed ovarian cancer (ICON7): overall survival results of a phase 3 randomised trial. Lancet Oncol. 2015; 16: 928-936.

64. Friedlander ML. Commentary on the clinical trial reported by: Tewari KS, Sill M, Long III HJ, et al. Incorporation of bevacizumab in the treatment of recurrent and metastatic cervical cancer: a phase III randomized trial of the Gynecologic Oncology Group. J Clin Oncol 2013; (suppl; abstr 3). Chin Clin Oncol. 2014. 3: 6.

65. Junttila MR, de Sauvage FJ. Influence of tumour micro-environment heterogeneity on therapeutic response. Nature. 2013; 501: 346-354.

66. Ebos JM, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG, et al. Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. Cancer Cell. 2009; 15: 232-239.

67. Paez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, et al. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell. 2009; 15: 220-231.

68. Miles D, Harbeck N, Escudier B, Hurwitz H, Saltz L, et al. Disease course patterns after discontinuation of bevacizumab: pooled analysis of randomized phase III trials. J Clin Oncol. 2011; 29: 83-88.

69. de Groot JF, Fuller G, Kumar AJ, Piao Y, Eterovic K, et al. Tumor invasion after treatment of glioblastoma with bevacizumab: radiographic and pathologic correlation in humans and mice. Neuro Oncol. 2010; 12: 233-242.

70. Buckanovich RJ, Facciabene A, Kim S, Benencia F, Sasaroli D, et al. Endothelin B receptor mediates the endothelial barrier to T cell homing to tumors and disables immune therapy. Nat Med. 2008; 14: 28-36.

71. Tang H, Wang Y, Chlewicki LK, Zhang Y, Guo J, et al. Facilitating T Cell Infiltration in Tumor Microenvironment Overcomes Resistance to PD-L1 Blockade. Cancer Cell. 2016; 29: 285-96.

72. Sarhan D, Cichocki F, Zhang B, Yingst A, Spellman SR, et al. Adaptive NK Cells with Low TIGIT Expression Are Inherently Resistant to Myeloid-Derived Suppressor Cells. Cancer Res. 2016; 76: 5696-5706.

73. Deng Y, Cheng J, Fu B, Liu W, Chen G, et al., Hepatic carcinoma-associated fibroblasts enhance immune suppression by facilitating the generation of myeloid-derived suppressor cells. Oncogene. 2016.

74. Motz GT, Coukos G. The parallel lives of angiogenesis and immunosuppression: cancer and other tales. Nat Rev Immunol. 2011; 11: 702-711.

75. Komohara Y, Morita T, Annan DA, Horlad H, Ohnishi K, et al. The Coordinated Actions of TIM-3 on Cancer and Myeloid Cells in the Regulation of Tumorigenicity and Clinical Prognosis in Clear Cell Renal Cell Carcinomas. Cancer Immunol Res. 2015; 3: 999-1007.

76. Azzaoui I, Uhel F, Rossille D, Pangault C, Dulong J, et al. T-cell defect in diffuse large B-cell lymphomas involves expansion of myeloid-derived suppressor cells. Blood. 2016; 128: 1081-1092.

77. Ammirante M, Luo JL, Grivennikov S, Nedospasov S, Karin M. B-cell-derived lymphotoxin promotes castration-resistant prostate cancer. Nature. 2010; 464: 302-305.

78. Guillerey C, Smyth MJ. NK Cells and Cancer Immunoediting. Curr Top Microbiol Immunol. 2016; 395: 115-145.

79. Vitale M, Cantoni C, Pietra G, Mingari MC, Moretta L. Effect of tumor cells and tumor microenvironment on NK-cell function. Eur J Immunol. 2014; 44: 1582-1592.

80. Ghiringhelli F, Ménard C, Terme M, Flament C, Taieb J, et al. CD4+CD25+ regulatory T cells inhibit natural killer cell functions in a transforming growth factor-beta-dependent manner. J Exp Med. 2005; 202: 1075-1085.

81. Smyth MJ, Teng MW, Swann J, Kyparissoudis K, Godfrey DI, et al. CD4+CD25+ T regulatory cells suppress NK cell-mediated immunotherapy of cancer. J Immunol. 2006; 176: 1582-1587.

82. Mittal D, Young A, Stannard K, Yong M, Teng MW, et al. Antimetastatic effects of blocking PD-1 and the adenosine A2A receptor. Cancer Res. 2014; 74: 3652-3658.

83. Viel S, Marçais A, Guimaraes FS, Loftus R, Rabilloud J, et al. TGF-Î² inhibits the activation and functions of NK cells by repressing the mTOR pathway. Sci Signal. 2016; 9: ra19.

84. Tang A, Dadaglio G, Oberkampf M. B cells promote tumor progression in a mouse model of HPV-mediated cervical cancer. Int J Cancer. 2016; 139: 1358-1371.

85. Ribatti D. Mast cells as therapeutic target in cancer. Eur J Pharmacol. 2016; 778: 152-157.

86. Mantovani A, Sica A. Macrophages, innate immunity and cancer: balance, tolerance, and diversity. Curr Opin Immunol. 2010; 22: 231-237.

87. Shen L, Li H, Shi Y, Wang D, Gong J, et al. M2 tumour-associated macrophages contribute to tumour progression via legumain remodelling the extracellular matrix in diffuse large B cell lymphoma. Sci Rep, 2016; 6: 30347.

88. Vaishampayan U. Therapeutic options and multifaceted treatment paradigms in metastatic castrate-resistant prostate cancer. Curr Opin Oncol, 2014; 26: 265-73.

89. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010; 363: 711-723.

90. Delyon J, Mateus C, Lefeuvre D, Lanoy E, Zitvogel L, et al. Experience in daily practice with ipilimumab for the treatment of patients with metastatic melanoma: an early increase in lymphocyte and eosinophil counts is associated with improved survival. Ann Oncol, 2013; 24: 1697-703.

91. Yano H, Thakur A, Tomaszewski EN, Choi M, Deol A, et al. Ipilimumab augments antitumor activity of bispecific antibody-armed T cells. J Transl Med. 2014; 12: 191.

92. Fromm G, de Silva S, Giffin L, Xu X, Rose J, et al. Gp96-Ig/Costimulator (OX40L, ICOSL, or 4-1BBL) Combination Vaccine Improves T-cell Priming and Enhances Immunity, Memory, and Tumor Elimination. Cancer Immunol Res, 2016; 4: 766-78.

93. Beatty GL, Chiorean EG, Fishman MP, Saboury B, Teitelbaum UR, et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. Science. 2011; 331: 1612-1616.

94. Batool M, Anwar MA, Choi S. Toll-like receptors targeting technology for the treatment of lymphoma. Expert Opin Drug Discov. 2016; 11: 1047-1059.

95. Guillerey C, Huntington ND, Smyth MJ. Targeting natural killer cells in cancer immunotherapy. Nat Immunol. 2016; 17: 1025-1036.

96. Grossenbacher SK, Canter RJ, Murphy WJ. Natural killer cell immunotherapy to target stem-like tumor cells. J Immunother Cancer. 2016; 4: 19.

97. Tonn T, Schwabe D, Klingemann HG, Becker S, Esser R, et al. Treatment of patients with advanced cancer with the natural killer cell line NK-92. Cytotherapy. 2013; 15: 1563-1570.

98. Schönfeld K, Sahm C, Zhang C, Naundorf S, Brendel C, et al. Selective inhibition of tumor growth by clonal NK cells expressing an ErbB2/HER2-specific chimeric antigen receptor. Mol Ther. 2015; 23: 330-338.

99. Chen F, Qi X, Qian M, Dai Y, Sun Y. Tackling the tumor microenvironment: what challenge does it pose to anticancer therapies? Protein Cell. 2014; 5: 816-826.

100. Chen F, et al. New horizons in tumor microenvironment biology: challenges and opportunities. BMC Med, 2015; 13: 278.

101. Sun Y. Tumor microenvironment and cancer therapy resistance. Cancer Lett. 2016; 380: 205-215.

102. Sun Y. Translational horizons in the tumor microenvironment: harnessing breakthroughs and targeting cures. Med Res Rev. 2015; 35: 408-436.

103. Farkona S, Diamandis EP, Blasutig IM. Cancer immunotherapy: the beginning of the end of cancer? BMC Med. 2016; 14: 73.