Microenvironmental regulation of therapeutic response in cancer

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Abstract

The tumor microenvironment (TME) not only plays a pivotal role during cancer progression and metastasis but also has profound effects on therapeutic efficacy. In the case of microenvironment-mediated resistance this can involve an intrinsic response, including the co-option of pre-existing structural elements and signaling networks, or an adaptive response of the tumor stroma following the therapeutic insult. Alternatively, in other contexts, the TME has a multifaceted ability to enhance therapeutic efficacy. This review examines recent advances in our understanding of the contribution of the TME during cancer therapy and discusses key concepts that may be amenable to therapeutic intervention.

Keywords

The TME orchestrates tumorigenesis and malignant progression

While cancer was long considered a disease defined and driven by genomic instability, chromosomal alterations, and genetic mutations [1], the influence of nonmalignant, stromal cells of the TME is now widely appreciated [2,3]. Tumors are complex tissues comprising not only malignant cells but also genetically stable stromal cells [4], including endothelial cells, fibroblasts, and immune cells among many others (Figure 1), in addition to the extracellular matrix (ECM) they produce. As in healthy organs, the various cellular compartments of the TME are not mere bystanders, but instead critically regulate tumorigenesis [5]. This extends not only to tumor initiation, malignant progression, and metastasis but importantly also to response to therapy. Moreover, the realization that distinct stromal cell types in different contexts can exhibit tumor-promoting or opposing tumoricidal capacities has further complicated our understanding of cancer biology. While the role of the TME during tumorigenesis has recently been reviewed in detail elsewhere [2,3], this review focuses on how the TME regulates therapeutic response, a field that has been rapidly expanding in recent years. As in the context of malignant progression, the TME exhibits a multifaceted ability to influence therapeutic outcome in either a positive or a negative manner. Harnessing this expanding knowledge to improve therapeutic response or even to

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develop new treatment options through normalization and re-education of the TME is increasingly within reach. The recent clinical success of immune checkpoint inhibitors serves as an illustrative example of this goal. A brief overview of the major components of the TME highlighted in Box 1 provides the necessary background to introduce the reader to the different concepts contributing to both TME-intrinsic and -acquired/adaptive resistance with regard to traditional anticancer therapies, molecularly targeted therapies, and agents targeted against the TME itself, which are summarized in Box 2.

Box 1

Major components of the TME

Vasculature

The formation of blood vessels during malignant progression is a critical tumor property acquired at an early stage of tumorigenesis [174] and is therefore considered to be a hallmark of cancer [1]. The activation of this 'angiogenic switch' not only is essential for adequate supply of the tumor with nutrients and oxygen, to allow growth beyond a certain size, but also facilitates subsequent metastatic spread. Compared with normal blood vessels which display a highly organized architecture, the vasculature within a tumor typically exhibits anatomical, structural, and functional properties such as aberrant recruitment of pericytes, increased vascular permeability, and turbulent blood flow [175]. Although angiogenesis can be driven by various molecules, VEGF-A is often considered to be the prototypical angiogenic factor in malignant disease. Accordingly, its expression is inversely correlated with overall survival in various cancers [176–178]. While tumor cells are major contributors of VEGF-A, TME cells represent additional sources [179].

CAFs

CAFs constitute a large proportion of the non-epithelial or stromal cells within the tumor. CAFs are considered to be functionally distinct from normal fibroblasts, which can inhibit malignant transformation [180,181], as they can support progression of premalignant lesions in prostate cancer [182] and enhance metastasis in breast cancer [180]. However, CAFs can have tumor-suppressive functions in pancreatic ductal adenocarcinoma [34,35]. CAFs are stimulated to proliferate by growth factors and cytokines such as TGF-β, FGF-2, and PDGF that are abundant in the TME [183,184]. Following activation, CAFs become a major source of secreted growth factors, including VEGF-A, which promotes angiogenesis and vascular permeability [179]. Additionally, CAFs produce proinflammatory factors that drive leukocyte infiltration [185].

Inflammatory cells

Cells of the innate immune system not only provide an essential nonspecific defense against pathogens but also regulate tissue home-ostasis and wound healing [2]. Their physiological functions therefore predispose them to play a major role in the inflammatory reactions that occur in cancers [5]. Accordingly, many of the immune cells within the TME are of myeloid origin and are either tissue resident or recruited by cancer cells from the BM and the spleen to enhance their survival, growth, invasion, and dissemination [186]. Tumor cells can be nurtured by TAMs through a plethora of signals

[187]. Notable examples include a paracrine loop involving EGF/CSF-1 signaling between TAMs and tumor cells [188–190] and the activation of WNT signaling in TAMs [191,192]. Furthermore, TAMs constitute a major source of proteases, such as cysteine cathepsins, that drive cancer progression [193] via regulation of angiogenesis and tumor growth [194]. Similarly, TAM-secreted MMPs not only degrade the ECM [195,196], but also increase the availability of ECM-bound factors such as VEGF-A [197].

Extracellular matrix

ECM is produced by all of the cell types within the TME, resulting in an intricate fiber network that not only provides structural support but also integrates local signals and regulates cellular movement, proliferation, and differentiation [84]. While collagens and fibronectin provide mechanical strength, proteoglycans contribute growth factor and cytokine-binding properties [198]. TME-associated ECM is fundamentally different from that of the normal tissue stroma [199] and serves as a guiding scaffold for chemotaxis and tumor cell invasion [200]. Paradoxically, increased ECM synthesis [201] and pronounced crosslinking of collagen fibers in the malignant stroma further enable tumor cell invasion [202]. Deregulation of collagen crosslinking also results in alterations of the biomechanical properties of the ECM, significantly increasing tissue rigidity [199,203]. Finally, desmoplastic tumor stroma constitutes a physical barrier for drug delivery and influences the architecture of the tumor vasculature [30].

Box 2

Therapeutic approaches to treating malignancies

Chemotherapy

Conventional chemotherapy, first introduced into the clinic during the 1940s in the form of DNA-alkylating agents and antimetabolites [204], has since been expanded to encompass a wide variety of drug classes and combinations thereof and still constitutes the mainstay of many systemic drug regimens in solid and hematological malignancies. Chemotherapy is often combined with local therapeutic interventions such as surgery and RT in either the adjuvant or neoadjuvant setting. The mechanism of action of many cytotoxic drugs results from their ability to perturb biological pathways that are required for a cell to progress through the cell cycle and maintain its genomic integrity. The major downside of this mode of action is its inability to distinguish between malignant and normal cells, leading to severe systemic short- and long-term side effects. This extends not only to healthy, nonaffected tissues but also to the TME within the tumor. For instance, exposure to chemotherapy leads to a general tissue-damage response that triggers an influx of inflammatory cells into the TME, which can further drive resistance [106,107].

Surgery and RT

Both surgery and ionizing radiation are major cornerstones of most cancer treatment approaches. While these modalities are inherently aimed at local control they result in local and systemic effects that encompass the TME [205,206]. Especially in surgery, the physiological wound-healing response mechanisms exhibit a considerable degree of

similarity to the TME [207,208]. A plethora of proliferative and angiogenic factors, many of which originate in the stromal compartment, are found to be altered in the serum of patients after surgery [209]. Consequently, it has been postulated that the wound's response contributes to the progression of minimal residual disease and distant, dormant micrometastases after surgery [210–212].

RT has profound consequences on the TME, beyond its direct cytotoxic effects on the tumor cells [206], that can ultimately alter therapeutic responses. Experimental studies show a significant difference between the radiosensitivity of GBM cells *in vitro* versus *in vivo* that can be attributed to the TME [213]. Due to the fact that sufficient oxygenation is a major determinant of radiosensitivity, this observation is inherently connected to the physical nature of the tumor vasculature *in vivo*.

Oncogene-targeted therapy

An increasing understanding of the genetic alterations that drive cancer has led to a new era of 'targeted' therapeutic agents. The prototype for this concept is imatinib mesylate, an inhibitor of the fusion tyrosine kinase protein BCR–ABL [214]. For the first time, an oncogenic driver could be targeted with specificity and striking efficacy. This proof of concept spurred the development of a broad range of TKIs. However, the notion of targeted therapy includes not only inhibitors in the form of small molecules but also therapeutic antibodies.

One paradigmatic example of these two strategies is the family of di-/oligomeric human EGFRs (HERs). EGFR (HER1/ErbB1) is constitutively activated due to a mutation in a small subset of non-small-cell lung cancers (NSCLCs), which forms the rationale for treatment with EGFR inhibitors such as erlotinib [215] in these patients. Furthermore, a large proportion of NSCLC and CRC cases exhibit increased EGFR expression. In CRC, the addition of the EGFR-specific antibodies cetuximab or panitumumab confers a clinical benefit [216]. However, reliable predictive biomarkers that identify patients most likely to benefit from this therapy are not equally well established across different tumor types [217]. Modulation of EGFR activity by and within the TME through the provision of alternative EGFR ligands might account for the therapeutic effect observed in EGFR-negative cases [218,219].

Recently, new TKIs have entered the spotlight. One notable example is vemurafinib, which inhibits BRAF, a member of the ERK protein kinase pathway that is frequently mutated in malignant melanoma [220]. While vemurafinib can have an impressive initial response rate [221], this is overshadowed by the emergence of secondary resistance in most cases. There is increasing evidence that the TME contributes in part to this phenomenon [102,104].

TME-targeted therapy

VEGF-A is a major driver of tumor angiogenesis and therefore the VEGF-A-neutralizing antibody bevacizumab can be considered as the first approved therapeutic agent that perturbs a key axis of tumor cell–TME crosstalk (Figure 1). Bevacizumab is effective in metastatic CRC and NSCLC when combined with conventional chemotherapy [222,223]. A similar efficacy was observed with several multi-kinase TKIs (e.g., sorafenib,

sunitinib), which target VEGFR2 and other RTKs and are approved for multiple cancer types including advanced renal carcinoma.

Currently the most advanced approach to therapeutically utilize the antitumor activity within the TME is the blockade of immune checkpoints. This concept has recently had considerable clinical success in patients with advanced malignant melanoma. Both the inhibition of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) with the antibody ipilimumab [224] and the blockade of the programmed death 1 (PD1) receptor via the antibody nivolumab resulted in increased overall survival [225] (Figure 1). This effect appears to be even more pronounced when the two therapeutic agents are combined [226]. Phase III trials investigating whether this can be translated to other malignancies such as lung and renal cancer are currently under way. Another promising approach is the reprogramming rather than depletion of TAMs via CSF-1R inhibition (Figure 1). The feasibility of this concept was recently demonstrated in mouse models of GBM [162] and PDA [163] and it will now be critical to investigate whether this reprogramming can be similarly achieved in patients.

Therapeutic response is significantly influenced by the TME

Although an increasing number of cancers can be treated successfully if detected at an early stage, the presence of disseminated disease or recurrence of the primary tumor still confers a poor patient prognosis [6,7]. This is due in part to the current paucity of effective therapeutic options in this setting [8]. An initial response to treatment is often followed by disease progression, which, accompanied by a diminution of therapeutic options, ultimately leads to treatment failure and death from recurrent or metastatic disease [9]. Intriguingly, at least some of the traits that promote metastasis appear to be intertwined with resistance to chemotherapy [10–12]. In line with a tumor cell-centric view, this lack of a sustained treatment response has been largely attributed to either intrinsic or acquired therapeutic resistance of the malignant cells via a plethora of mechanisms including increased drug efflux, drug inactivation, altered DNA repair machinery, dysregulation or alteration of the drug targets, upregulation of growth factor and survival signaling, and evasion of apoptosis [8,13]. These mechanisms appear to be partially fueled by a pre-existing intratumoral heterogeneity that supports the outgrowth of resistant clones [14].

In addition to tumor cell-intrinsic mechanisms, an increasing number of examples of TME-mediated resistance have been reported, representing an alternative means to interfere with therapeutic efficacy. Early seminal work by Teicher *et al.* elegantly linked the development of resistance to chemotherapy *in vivo* to interactions with the host's normal tissues [15]. The discrepancy they observed between the *in vitro* efficacy of and *in vivo* resistance to a panel of various chemotherapies has subsequently been confirmed and extended by numerous studies [16,17], providing many examples of where TME-mediated resistance may be at play. However, there are also instances where radiotherapy (RT) and certain chemotherapies require an active immune cell response for optimal efficacy, as in the case of 'immunogenic cell death' [18]. Interestingly, a simple quantification of the tumor-to-stroma ratio in breast and colon cancers predicts worse clinical outcome in patients undergoing adjuvant chemotherapy as an independent variable [19,20]. Furthermore, analysis of stromal gene

expression in various cancers not only yielded tumor type-specific prognostic benefit [21,22] but also exhibited predictive value regarding response to neoadjuvant chemotherapy [23]. Thus, analysis of the TME could convey significant clinical information to aid in the evaluation of treatment options.

TME-mediated therapeutic resistance can be broadly separated into two types. Inherent or intrinsic resistance is present before drug or RT exposure and is therefore evident without any selective pressure. This type of resistance is based on the multitude of pre-existing reciprocal interactions between tumor cells and the surrounding TME. This is in contrast to tumor cell-intrinsic resistance, which is due to existing genetic alterations within the biochemical or molecular target [8]. Acquired TME resistance, by contrast, evolves in response to the effects of therapy and is defined by an adaptive host response to therapeutic perturbation. This can result in pronounced changes in the microenvironment and the emergence of new tumor–TME dialogs operating at the local and/or systemic level.

Ultimately, the protective effect of the TME on tumor cells can lead to persistent residual disease that further increases the risk of recurrence [17]. Therefore, deciphering this complex network and introducing targeted perturbations will be critical for improving therapeutic efficacy and ultimately patient survival. However, it is essential to emphasize that these effects are organ, context, and stage dependent, as the TME can also confer a beneficial effect on treatment response. This concept has been demonstrated both in drug screens that incorporate the tumor stroma [16] and in many studies revealing the importance of various immune cell types in modulating therapeutic efficacy (reviewed in [18]).

In the following sections we discuss intrinsic and acquired responses of the TME to traditional, cancer cell-targeted, and microenvironment-targeted therapies.

Effects of pre-existing TME properties on therapeutic efficacy

The intrinsic mechanisms through which the TME modulates drug response involve pre-existing properties of the tumor including a chaotic, frequently inefficient vascular supply, elevated interstitial fluid pressure (IFP), a pronounced desmoplastic stroma, increased tissue rigidity, and the presence of niches within the tumor that protect cancer cells from therapeutic insults. As several of these parameters have been previously reviewed [24–28], we only briefly summarize these topics in the context of drug delivery in the TME and focus in more depth on newly emerging areas of TME-mediated intrinsic resistance including the role of protective niches (Figure 2).

Drug delivery, vascular remodeling, and drug escape are modulated by the TME

An abnormal tumor vasculature, increased IFP, and altered ECM constitute major obstacles that prevent che-motherapeutic drugs from effectively penetrating tumor tissue (Figure 2A). One tumor type in which these properties have been investigated in depth is pancreatic ductal adenocarcinoma (PDA), in which a desmoplastic tumor stroma driven by hedgehog (Hh) signaling is a hallmark feature [29]. In a genetically engineered PDA mouse model, various strategies to improve chemotherapy delivery have been explored, including inhibition of Hh signaling [30] and the exogenous introduction of hyaluronidase to

enzymatically degrade hyaluronic acid in the ECM [31]. However, recent Phase II clinical trials combining gemcitabine with the Hh inhibitor saridegib failed to demonstrate a clinical benefit in PDA [32]. It therefore remains to be seen whether a recently described tumor-suppressive effect of activated Hh signaling in PDA, via downregulation of integrin expression and transforming growth factor beta (TGF-β) activity that in turn diminishes stromal myofibroblast activation, might account for this observation [33]. Two recent studies reported the tumor-suppressive effects of cancer-associated fibroblasts (CAFs) and a Hh-driven stroma in PDA [34,35], again demonstrating the often dichotomous roles of cells in the TME as discussed above.

Drug delivery into the tumor can also be influenced by vessel functionality and vascular leakage [36]. This was elegantly demonstrated by live imaging in a mouse mammary cancer model where vascular permeability, which was highest in intermediate tumor stages, facilitated doxorubicin penetration [37]. When matrix metalloproteinase (MMP) 9 was deleted in the host stroma, vascular leakage was dramatically increased, resulting in improved doxorubicin delivery into the tumor. A counterpoint to these observations is the phenomenon of vessel normalization, in which vessel density and morphology along with pericyte and basement membrane coverage are normalized, resulting in decreased vascular leakiness and enhanced drug delivery [28,38,39]. These findings highlight the complex interplay between intra- and extravascular factors during drug delivery. Angiogenesis inhibitors, such as the anti-vascular endothelial growth factor (VEGF)-A antibody bevacizumab, are thought to act in part through normalization of the tumor vasculature [28,40] (Figure 1). Recent preclinical studies have also shown that immunotherapy efficacy can be enhanced in combination with vessel-normalization strategies [41]. The clinical experience that angiogenesis inhibitors exert their greatest benefit in combination with conventional cytotoxic drugs in certain cancers supports their use within multimodal treatment regimens [42,43]. However, this is often accompanied by the rapid emergence of resistance [43], which is discussed in the following section on acquired resistance in the TME.

Equally as important as drug delivery into the tumor is the rate of clearance of chemotherapeutic agents by the malignant cells. For instance, integrin 'outside-in' signaling via the extracellular signal-related kinase (ERK)/mitogen-activated protein kinase (MAPK) signaling pathway upregulates the multidrug resistance protein ATP-binding cassette, subfamily C (CFTR/MRP), member 1 (ABCC1) in T-cell leukemias, which then actively expels doxorubicin [44]. Tumor-associated macrophages (TAMs) have been shown to mediate gemcitabine resistance in PDA by upregulating cytidine deaminase, the enzyme that metabolizes gemcitabine to its inactive form, in the tumor cells [45].

Another mechanism by which drug availability can be altered is through the expulsion of cytostatic drugs from tumor cells via extracellular vesicles (EVs) [46,47] and the horizontal transfer of multidrug efflux transporters by EVs [48]. Tumor cell-derived EVs can also act as decoys for therapeutic antibodies such as rituximab [49] and trastuzumab [50]. Recent data additionally implicates stromal–tumor EV transfer in promoting chemoresistance. For example, endothelial cell (EC)-derived EVs conferred increased resistance of breast and ovarian cancer cells to doxorubicin and paclitaxel [51]. It has recently been demonstrated

that stromal exosomes, which are rich in non-coding RNA, elicit a signal transducer and activator of transcription (STAT) 1 response mediated by the pattern recognition receptor retinoic acid-inducible gene 1 (RIG-1) in basal-like breast cancer cells. This augments juxtacrine Notch 3 signaling, which ultimately expands tumor-initiating cells (TICs) that are known to exhibit increased resistance to chemotherapy [52]. Interestingly, EVs also exert a protective effect on nonmalignant populations within the TME: prostate cancer cell-derived EVs blunted apoptosis of fibroblasts following treatment with actinomycin D in an ERK-dependent manner [53]. In a similar fashion, EVs carrying TGF-β from injured epithelial kidney cells led to fibroblast activation that ultimately resulted in fibrosis, a process that closely mirrors the tumor stroma [54]. Exchange of cellular contents can also occur via tunneling nanotubes, as recently demonstrated for the transfer of mitochondria between ECs and cancer cells, which led to doxorubicin resistance [55].

Taken together, these representative examples not only provide substantial evidence for a protective effect of the tumor stroma via direct regulation of drug accessibility and turnover, but also highlight the intricate interactions within the TME that go beyond simple bystander effects.

Protective niches in the TME confer survival signals

Several cellular niches exist within tumors, including the perivascular niche and the bone marrow (BM) niche, which are critical for supporting cancer stem cells (CSCs)/TICs [56]. While CSCs/TICs have been postulated to be inherently therapy resistant [57], it is now becoming apparent that pre-existing niches can also confer protective signals non-cell autonomously in the face of therapeutic intervention, enabling a subset of cells to survive and thus reestablish a malignant tumor (Figure 2B). Here we discuss examples of both soluble factor- and ECM-mediated intrinsic resistance to various therapies in different TME niches and how these niches can be altered to support adaptive resistance as a result of therapeutic intervention.

The perivascular niche (PVN), comprising ECs and pericytes, provides an ideal 'microenvironment within the microenvironment' for cancer cells to resist therapeutic insults. To date, this has been most widely shown in brain tumors, which possess a particularly rich PVN comprising multiple cell types that constitute the blood–brain barrier: astrocytes and often microglia in addition to ECs and pericytes [58–62]. In preclinical models of medulloblastoma, for example, CSCs located within the PVN survive irradiation through several mechanisms including enhanced phosphatidylinositide 3-kinase (PI3K)/v-akt murine thymoma viral oncogene homolog 1 (Akt) signaling [63] or via Yes-associated protein (YAP) expression, which drives insulin-like growth factor 2 (IGF2)/Akt activation [64]. This allows tumor cells to enter mitosis with persistent DNA strand breaks and thus contributes to radio-resistance.

It is now becoming evident that PVN-mediated signals can have similarly potent effects in organs other than the brain. PVNs for CSCs have been reported in multiple tumor types including breast, head and neck, and melanoma [65–67] and thus it will be interesting to determine whether PVN factors similarly modulate therapeutic resistance in these organs. In several different mouse models, including B16 melanoma, EC-specific deletion of focal-

adhesion kinase (FAK) was recently shown to sensitize tumors to RT and various chemotherapies, including doxorubicin, due to loss of EC-derived protective cytokines. Interestingly, the authors also studied lymphoma patients and found that low FAK expression in blood vessels at the time of diagnosis correlated with a better response to doxorubicin [68]. Recently, EC-secreted paracrine factors, termed angiocrine factors, that have potent roles in liver regeneration and fibrosis have been identified [69]. Related angiocrine factors were shown to promote chemoresistance in B-cell lymphoma via a paracrine signaling loop in which lymphoma cells cocultured with ECs induced fibroblast growth factor (FGF)-4 expression, leading to activation of FGF receptor 1 (FGFR1) signaling and induction of Jagged 1 in ECs. Consequently, they showed that EC-specific deletion of FGFR1 or Jagged 1 *in vivo* resulted in enhanced sensitivity of lymphoma-initiating cells to doxorubicin [70], supporting consideration of inhibitors targeting these pathways as a means to enhance chemotherapy efficacy. Therefore, the link between CSC maintenance or the induction of 'stemness' by protective niches within the TME and the resulting resistance to therapy, as suggested by several of these studies, warrants further investigation.

Intimate interactions between cancer cells and stromal cells that substantially influence therapeutic outcome have similarly been reported for niches in the BM microenvironment, which can comprise mesenchymal cells, ECs, macrophages, osteoblasts, fibroblasts, and other stromal cell types [71–73]. This is the case for hematological malignancies that develop in the BM [74,75] such as acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and multiple myeloma (MM), and cancers that metastasize to bone. A paracrine interleukin (IL)-6 axis between the BM stroma and MM cells contributes to chemotherapy resistance via STAT3-mediated protection from apoptosis [76]. Stromal IL-6 expression has also been demonstrated in solid tumors such as gastric, lung, and colon cancer [77–79]. In colorectal cancer (CRC), depleting stromal IL-6 showed greater antitumor effects than targeting tumor cell-derived IL-6. However, recent Phase II trials of the anti-IL-6 antibody siltuximab failed to demonstrate substantial clinical benefits in newly diagnosed or relapsed/refractory MM [80,81]. Likewise, in advanced ovarian cancer or renal cancer the use of siltuximab as a single agent predominantly facilitated disease stabilization [82,83]. It therefore remains to be seen whether this therapeutic approach in combination with conventional chemotherapy might provide more beneficial results in solid cancers.

The ECM is an essential component that maintains both primary and metastatic niches in cancer [84,85]. However, interactions between cancer cells and the various components of the ECM go far beyond simple structural support. For example, adhesion of malignant cells to ECM substrates restores cellular polarization of breast cancer cells, which results in resistance to apoptosis on treatment with etoposide [86]. The mechanism of this cell adhesion-mediated drug resistance (CAM-DR) relies on integrin binding to ECM constituents such as collagen, fibronectin, and laminin [11,84,85]. The protective effects of integrin–ECM binding result not only from the upregulation of multidrug resistance transporters [44] but also from the downstream activation of FAK, PI3K/AKT, STAT3, and nuclear factor kappa light chain enhancer of activated B cells (NF-κB) signaling, all of which exhibit antiapoptotic properties [11,87]. While this has been most extensively studied in the context of the BM niche in hematological malignancies, it has also been observed in

breast, lung, and pancreatic cancer [24]. Moreover, the protective effect of integrin ligation extends beyond chemotherapeutic agents; it is also involved in mediating resistance to radiotherapy [88–90] and oncogene-targeting therapies such as small-molecule [91–93] and antibody-based receptor tyrosine kinase (RTK) inhibition [94], which can be attributed to bypassing of the targeted pathways.

Paracrine signaling loops in the TME

Classical paracrine signaling loops between different cell types in the TME have been shown to mediate therapeutic resistance (Figure 2C). These studies typically have either investigated well-known factors using candidate-based approaches or, more recently, expanded to large-scale unbiased screens. One prototypic example of the former approach involves chemokine stromal derived factor (SDF)-1, which is produced by BM stromal cells, and its cognate receptor chemokine (C-X-C motif) receptor 4 (CXCR4), which is expressed on cancer cells [95]. SDF-1 protects AML cells from apoptosis induced by the nucleotide analog cytarabine [96]. Treatment with the CXCR4 inhibitor AMD3465, an analog of the FDA-approved drug plerixafor (Figure 1), blocked stromal SDF1-induced activation of the prosurvival AKT and ERK signaling pathways in AML [97]. A similar antiapoptotic mechanism has been observed *in vitro* for small-cell lung cancer [98], which has a predisposition for bone metastasis. Outside the BM niche, recruitment of circulating BM-derived mesenchymal stem cells (BMMSCs) into solid tumors has been described in preclinical models of breast and lung cancer [99]. Therefore, it is tempting to speculate that these BMMSCs could constitute a local source for SDF-1 in solid tumors that similarly modulates therapeutic response [100].

Interestingly, cytokine-mediated protection can circumvent targeting of RTK-mediated signaling pathways, even in kinase-addicted cancers. In AML cells with fetal liver tyrosine kinase-3 (FLT3) gene mutations, coculture with stromal cells significantly diminished the antileukemic effects of the FLT3-targeting inhibitor sorafenib, while CXCR4 inhibition reversed this effect in a preclinical animal model [97]. In another example, AKT inhibitors effectively override stroma-associated cytoprotection of FLT3-mutated AML cells [101]. Soluble RTK ligands can thus rescue kinase-addicted tumor cell lines from targeted tyrosine kinase inhibitor (TKI) therapies due to a convergence at the level of downstream signaling targets [102]. Combined with the observation that stromal CAF-like cells can attenuate endothelial growth factor (EGF) receptor (EGFR) inhibition at the RTK or downstream level in prostate cancer or lung cancer cells, respectively [103], a substantial body of evidence indicates the TME as a source for these alternative activators. Accordingly, hepatocyte growth factor (HGF) was shown to be a stroma-derived factor that mediates resistance to BRAF inhibition in V600E BRAF mutant melanoma [102,104], as well as HER2 inhibition in HER2^{+} breast cancer [102] and EGFR inhibition in triple-negative breast cancer [105].

Several recent high-throughput screens involving complex matrices of numerous cancer cell lines from various organ sites and molecular subtypes, panels of distinct stromal cell lines, and a broad range of different drugs have demonstrated both the extent and the potency of stromal effects on drug response, which appear to be most pronounced in the context of targeted therapies [75,102,104]. Critically, as shown in these screens, stromal cells are not

always mediators of resistance; in several cases they can sensitize to therapy [16]. These types of large-scale approach have underscored the importance of the TME in modulating therapeutic response to chemotherapeutics and targeted agents, yet remain relatively simple coculture platforms that may not fully recapitulate the *in vivo* TME. In this regard, increasingly sophisticated modeling approaches are becoming more accessible to address these questions, as summarized in Box 3.

Box 3

Model systems for investigating TME-mediated resistance

While cancer drug screening assays were historically performed using transplantable tumor models, this approach has been superseded by tumor cell culture systems in recent decades [227] (Figure I). This tumor cell-focused approach has considerable advantages such as large scale, reproducibility, and time efficiency; however, one significant downside is the loss of information about the TME. Recently, several studies that incorporate 2D coculture with stromal cells in their screens have uncovered an important contribution of the TME to therapeutic response [75,102,104]. Nonetheless, these systems are still far from fully recapitulating the complex spatial and temporal composition of a tumor and its TME. It has been demonstrated that sophisticated 3D culture systems result in significant cytoskeletal reorganization, appropriate cellular polarity, and the generation of hypoxic gradients [24,228,229], which in turn alter chemoresistance [230]. This can be further expanded to multicellular direct [231] and indirect [232] coculture systems. Another level of complexity can be achieved by using organotypic normal tissue slice-culture systems that allow the investigation of microenvironmental interactions and vessel co-option during tumor colonization [233,234]. In a similar fashion, tumor explants can be studied as slice cultures [235,236]. One main advantage of these *in vitro* and *ex vivo* systems is that they are easily amenable to therapeutic intervention and accurate measurement of therapeutic efficacy. Moreover, specialized imaging techniques such as fluorescence recovery after photobleaching (FRAP) and Förster resonance energy transfer (FRET) [229] can be incorporated to analyze the downstream signaling cascades.

Highly sophisticated imaging techniques in animal models, including confocal [37,237] and intravital multiphoton microscopy [238], allow one to unravel tumor–stroma dynamics that potently impact therapeutic responses. Advanced approaches that combine these imaging modalities with various markers and reporters allow investigators to monitor tumor progression, the TME, and the success or failure of anticancer therapy with remarkable spatial and temporal resolution *in vivo* [239]. However, the tumor model needs to be chosen carefully, as the response to therapy differs greatly not only between orthotopically and subcutaneously transplanted tumors [240] but also between primary tumors and postsurgical metastases [241]. From a translational perspective, it could be that patient-derived xenografts (PDXs) will be developed into valuable models to study tumor–stromal interactions. Currently their usefulness is limited by loss of the human stromal compartment [242] and the necessity for immunodeficient recipient mice. However, the transplantation of PDX tumors into humanized tumor stroma [243] or the

utilization of mice with a transplanted, functional human immune system [244,245] is likely to circumvent some of these obstacles.
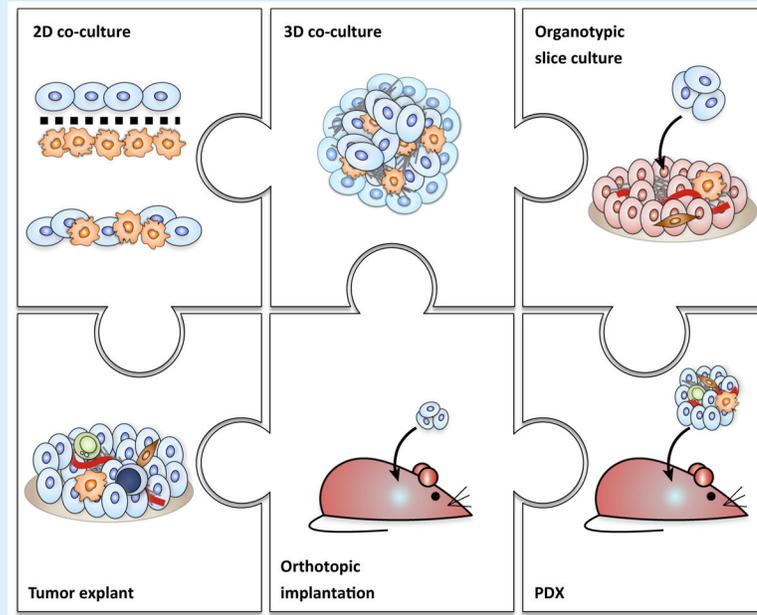

Figure I.
Commonly used indirect and direct coculture techniques have yielded valuable information about the role of stromal cells during therapeutic interventions. These cocultures can be further extended to a 3D system to account for spatial relations within the tumor microenvironment (TME). Organotypic slice cultures allow recapitulation of the microenvironment of different organs *in vitro*, while *ex vivo* explants of tumors retain the original TME. For preclinical *in vivo* studies, orthotopic implantation of tumor cells permits the investigation of tumor cells within the appropriate organ-specific TME. The correlation between therapeutic response in the mouse model and clinical efficacy can potentially be increased when patient-derived xenografts (PDXs) are used. It is important to note that the experimental strategies presented here are neither exhaustive nor in a hierarchical order. They rather represent complementary approaches to investigate the role of the stroma in therapeutic response.

Together, the representative examples discussed here indicate the possibility that a cancer cell (sub)population that successfully co-opts cues from the TME will survive and expand under both conventional and targeted therapies. This interplay between the TME and the malignant cells can result from a pre-existing interaction but, equally importantly, can also be introduced by the TME response to a therapeutic intervention.

Therapy-induced responses and acquired resistance in the TME

In the previous section we discussed intrinsic, pre-existing niches and the physical properties of the TME that contribute to non-cell-autonomous therapeutic resistance. Here we focus on how the TME can also be significantly changed by therapeutic intervention and how this can

lead to acquired resistance (Figure 2). One paradigmatic example of TME alterations following therapy involves the response of the innate and adaptive immune system.

The immune response influences therapeutic outcome

The innate immune system is a crucial component of the altered TME after therapeutic intervention (Figure 2D). Macrophage infiltration, in response to paclitaxel-enhanced expression of colony-stimulating factor 1 (CSF-1) and IL-34, appears to be a major contributor to chemoresistance and immunosuppression [106]. Furthermore, in a mouse model of breast cancer, recruitment of chemokine (C-C motif) receptor 2 (CCR2)-expressing monocytic cells occurs following doxorubicin treatment, via stroma-derived CCL2 [37]. In turn, the presence of these CCR2-expressing cells contributes to suboptimal treatment response and tumor re-emergence. Likewise, treatment of murine breast cancers with various chemotherapies leads to increased TAM accumulation, which then enables cathepsin protease B- and S-mediated chemoresistance to paclitaxel, etoposide, and doxorubicin [107].

Macrophages also constitute an important link to the adaptive immune system. Their increased influx into recurrent tumors leads to a surge of regulatory T cells that is accompanied by impaired recruitment of CD8^{+} cytotoxic T cells [108]. Regulatory T cells also accumulate following RT, as do macrophages, and their transient ablation was shown to enhance RT efficacy in a mouse breast cancer model [109]. A similar observation has been made for myeloid-derived suppressor cells (MDSCs), which drive IL-1β-induced secretion of IL-17 by CD4^{+} T cells that leads to chemoprotection [110]. Interestingly, there is also evidence for a chemoprotective role of B cells involving the education of TAMs toward a tumor-supporting phenotype by the activation of the Fc receptor [111]. Accordingly, B cell depletion resulted in increased recruitment of CD8^{+} cells and an enhanced therapeutic response.

The complexity of the immune system's response to therapeutic intervention is underscored by contradicting observations that support a positive contribution of the immune system to improved chemotherapeutic efficacy (Figure 2H). Interestingly, apoptosis, long thought to be immunologically silent, has been shown to result in the production of proinflammatory cytokines such as IL-8 and CCL2 [112]. Furthermore, several chemotherapeutic agents, such as anthracyclines and platinum derivatives, are able to induce immunogenic cell death that culminates in the release of damage-associated molecular patterns (DAMPs) [113,114]. It has been proposed that these mechanisms ultimately improve the anticancer effects of chemotherapy through increased antigen-presenting ability of dendritic cells and a subsequent T cell response [115]. The latter has been linked to infiltration of innate IL-17-producing γδ T cells that precedes the invasion of CD8^{+} T cells [116]. The importance of a robust CD8^{+} response is evident not only in the setting of conventional chemotherapy but also when mouse mammary tumor virus (MMTV)-neu transgenic mice were treated with the HER2 inhibitor lapatinib [117]. In the context of antibody-dependent cell-mediated cytotoxicity (ADCC), low-dose cyclophosphamide has been shown to promote macrophage-mediated killing of antibody-targeted cells in the bone marrow. This is due to an acute secretory phenotype of the tumor cells after cyclophosphamide exposure that increases the

phagocytic capacity of macrophages through tumor necrosis factor (TNF)-α, VEGF, and CCL4 [118].

In summary, the available evidence indicates that the immune response can both support and obstruct therapeutic efficacy. Therefore, unraveling and exploiting this complexity continues to be a challenging task.

Reactions of the TME beyond the immune response influence therapeutic outcome

An important mechanism that contributes to the altered phenotype of the TME after therapeutic intervention is induction of the senescence-associated secretory phenotype (SASP) (Figure 2G). Originally linked to replicative and genotoxic stress, senescence induction is typically considered a tumor-suppressive mechanism due to the resulting growth arrest. However, in some circumstances the SASP can promote cancer progression [119]. In the context of therapy-induced changes, DNA-damaging agents such as doxorubicin and RT lead to SASP in various tumor types [120]. This is associated with the secretion of various cytokines, proteases, and growth factors including HGF, TGF-β, IL-6, and CCL2 [121–123]. As outlined above, several of these molecules can also dramatically alter therapeutic response. Furthermore, this phenomenon similarly occurs within non-transformed TME cells [124] and may even spread in a paracrine fashion [121]. Thus, SASP induction in the TME can have potentially pleiotropic effects on therapeutic response.

Besides SASP-associated growth factors and cytokines, other signaling molecules have also been implicated in the therapy-altered TME (Figure 2F). For example, both RT and chemotherapy lead to upregulation of wingless-type MMTV integration site family member 16B (WNT16B) in CAFs, which then acts as a paracrine signal that attenuates the effects of anthracycline therapy in prostate cancer cells [125]. The upregulation of WNT16B is mediated via NF-κB, which in turn activates β-catenin-dependent WNT signaling in the cancer cells. The authors demonstrated a similar effect in breast cancer models. CAFs are similarly enriched in CRC during post-therapy tumor recurrence and exhibit upregulation of the cytokine IL-17A, which promotes the maintenance of TICs via NF-κB activation [126].

The BM also constitutes a rich source of cells of varying progeny beyond myeloid cell populations that are mobilized and recruited to the TME in response to therapeutic insult. For instance, BMMSCs have been identified as a source of novel chemoprotective factors: secreted polyunsaturated fatty acids [127]. While apparently only cisplatin-based therapy was capable of inducing their release, these fatty acids were able to confer resistance to various agents and even act on a systemic level.

Marked increases in BM-derived circulating endothelial progenitors (CEPs) in preclinical models and patient samples have been reported following the administration of various chemotherapies including taxanes, doxorubicin, and 5-fluorouracil (5-FU) [128]. In a mouse lung cancer model, the elevated CEPs were subsequently recruited to the tumor and treatment with antibodies targeting VEGF receptor 2 (VEGFR2) or SDF-1 abrogated this accumulation and increased chemotherapeutic efficacy. Interestingly, this synergy did not occur when gemcitabine was used in the combination treatment. Again, this highlights the

varying response patterns of the TME between different tumor types to distinct therapeutic interventions.

With regard to TME-mediated resistance to antiangiogenic therapies, the role of non-VEGF angiogenic pathways such as FGF or SDF-1 as possible escape mechanisms is increasingly appreciated [129,130]. Several cell populations within the TME are able to counteract anti-VEGF therapies: CD11b^{+}Gr1^{+} MDSCs and CEPs both contribute to antiangiogenic resistance [128,131,132]. Interestingly, in the case of MDSCs, this did not predict resistance to conventional chemotherapy [128] and was due to the nonconventional mediator of angiogenesis Bv8 [133]. Subsequent studies showed that murine lymphomas refractory to anti-VEGF therapy cause CAFs to secrete platelet-derived growth factor (PDGF)-C, which stimulates EC migration independent of myeloid cell infiltration [134]. Another illustrative example is the paracrine signaling network between T helper type 17 cells (Th17), the TME, and immature myeloid cells or MDSCs. Tumor-infiltrating T_H17 cells induce the expression of granulocyte CSF (G-CSF) in the stromal compartment via IL-17. This in turn attracts MDSCs, which driveanti-VEGF-A resistance [135].

In the case of the commonly used non-hypofractionated RT, hypoxia-inducible factor 1 (HIF-1) induction in the tumor cells leads to increased VEGF-A and SDF-1 expression [136]. While the former directly promotes EC recovery [137], the latter attracts BM-derived myeloid monocytic cells that contribute to vasculogenesis and tumor regrowth [138,139]. Interestingly, upregulation of CSF-1 in irradiated prostate cancers represents an alternative means to increase myeloid cell numbers after RT [140]. Taken together, the angiogenic network exhibits an impressive degree of plasticity involving numerous cell types within the TME that affect the outcome of both antiangiogenic and RT-based therapies. While VEGF-A inhibition in combination with RT and chemotherapy in glioblastoma (GBM) demonstrated increased progression-free survival [141,142], CSF-1 receptor (CSF-1R) inhibition combined with RT is currently being evaluated in Phase II clinical trials.

Finally, various therapeutic interventions can alter the TME at the level of the various niches (Figure 2E) discussed in the section on intrinsic resistance. For example, in a murine model of Burkitt's lymphoma, DNA-damaging agents induced the secretion of IL-6 and tissue inhibitor of metalloproteinases 1 (TIMP-1) from ECs in the thymus [143]. This resulted in specific protection of lymphoma cells in the thymus but not other lymphoid organs and served as a reservoir of cancer cells that subsequently fueled tumor relapse. Consistently, the increased tumor load in the thymus following doxorubicin administration was blunted in IL-6-deficient mice. A recent study in a mouse model of leukemia showed that a BM niche is created by ALL cells when physiological BM niches have been destroyed due to leukemic dissemination, in response to cytarabine and daunorubicin which are frontline chemotherapies used to treat ALL patients. This leukemia-induced niche evolves in response to therapeutic intervention and withdrawal [144]. Mesenchymal cells, recruited by leukemia cell-secreted CCL3, were found to be the major components that built a therapy-induced niche and evolved from Nestin^{+} cells to α smooth muscle actin (α-SMA)[+] cells under the influence of TGF-β and ultimately developed into fiber residues. The authors also identified furin-cleaved growth differentiation factor (GDF15) as the effector molecule provided by the niche to activate TGF-βII and confer chemoprotection and correspondingly found that

blocking GDF15 or furin enhanced chemotherapy sensitivity. Other examples of niches that either serve as a 'safe haven' from therapeutic intervention or contribute to metastatic escape include a lymphatic vessel niche in melanoma that promotes survival and enrichment of CD133^{+} TICs following dacarbazine treatment [145] and a TME stem cell 'unit' that is altered by RT and subsequently confers radioresistance on glioma cells [146].

Therapy-induced changes in differentiation and activation of TME cells

Altered differentiation represents another cell-intrinsic mechanism for cancer cells to develop resistance; for example, via epithelial–mesenchymal transition (EMT) [8,147]. There have been several recent studies revealing how the TME can also influence this process in a paracrine manner, including examples of how alterations to the polarization state of TME cells can influence therapeutic outcome. A fascinating case of cancer cell dedifferentiation was recently uncovered in a melanoma mouse model following adoptive cell transfer (ACT), which resulted in loss of melanocytic antigens and the subsequent development of ACT resistance [148]. Interestingly, this phenomenon was driven by an inflammatory response in the TME: specifically, TNF-α released by tumor-infiltrating T cells and macrophages that mediated the dedifferentiation. The authors therefore propose that ACT for melanoma, which is currently targeted toward melanocytic antigens, be expanded to simultaneously target tumor-specific mutated non-melanocytic antigens, thus ensuring broad recognition of both differentiated and dedifferentiated melanoma cells.

TME-produced cytokines such as NF-κB and TNF-α have also been shown to promote mesenchymal differentiation in GBM, resulting in enhanced radioresistance [149]. Macrophages/microglia were enriched in pockets within the tumor that were positive for mesenchymal markers, suggesting a potential link between TAMs and changes in cancer cell differentiation. Macrophages themselves can also be altered by distinct therapies including platinum-based agents, which induce an M2/alternatively activated state *in vitro* [150], and low-dose irradiation that promotes an iNOS[+] M1 phenotype [151]. In the case of M1-polarized macrophages, this RT-induced change in polarization toward an immunosuppressive state allows the recruitment of cytotoxic T cells in the tumor and thereby enhances immunotherapy efficacy in animal models [151].

Together, these examples illustrate the diversity of therapies that can induce altered polarization states within the TME and changes in cancer cell differentiation. As this effect is often reversible, this indicates numerous potential targets that could be used in combination with, or following, standard therapeutic interventions.

Concluding remarks

The number of mechanisms by which cancers can develop resistance to various therapeutic interventions inevitably increases as our arsenal of anticancer treatments expands. We can clearly now add TME-mediated resistance to this list and, as indicated by the representative examples we have highlighted here, these mechanisms of resistance are similarly diverse in their nature. While there are an increasing number of TME-targeted approaches to circumvent resistance (Figure 1), there are also evident challenges in considering how to translate this knowledge to the clinic.

One obstacle is our currently limited understanding of the underlying mechanisms through which some these therapeutic interventions affect distinct subpopulations within the TME. This is especially evident for the potential immunomodulatory effects of conventional chemotherapeutic agents (Figure 1), which remain poorly understood and thus cannot be utilized to their full potential.

Another challenge is to fully understand and ultimately target microenvironmental contributions to therapeutic response. It is clear that the stromal and immune composition of tumors is complex and highly dynamic and we currently only have a limited perspective on these alterations in certain human tumors, such as through the analysis of stromal gene expression in breast cancer [21] and the recent 'immune landscaping' in CRC [152]. These types of immune cell analysis are being extended to other tumor types through the 'Immunoscore', the prognostic value of which is currently being evaluated [153], but information on stromal cells such as fibroblasts, ECs, and organ-specific TME cell types is not currently being captured in these large-scale approaches. Thus, where possible, comprehensive landscaping of the entire TME before and after therapeutic intervention would significantly enhance these efforts.

Alternative strategies available at present include using computational deconvolution of gene expression data from whole-tumor samples from patients to capture gene signatures of stromal and immune cells [154]. Advanced bioinformatics tools that can discern subpopulations in complex tissues [155,156] and identify factors that could be targeted concomitantly in tumor cells and the TME [157] may provide new insights into potential 'double-hit' therapeutic strategies. Similarly, profiling the RTK ligands present in the TME(for example, via ELISA of tumor biopsies, represents another attractive approach, given their potent roles in mediating resistance [102,104]. These examples serve as a reminder that complementary technological approaches will be required to dissect the TME response to therapy that relies in part on physiological tissue-specific survival pathways.

It is also critical to remember that the TME plays a multifaceted role and can exhibit tumoricidal capacities, often through interplay with the immune system. A recent study revealed a surprising, tumor-promoting effect of CAF depletion in PDA [34]. Taken together with the observation that the tumor stroma can also enhance therapeutic efficacy [16,104] and the examples discussed here where immune cells are required for optimal therapeutic response, it is reasonable to propose that re-education of the TME rather than elimination of an entire cellular compartment will be substantially more beneficial [3,158–163] (Figure 1).

The idea of re-education raises a currently unmet need: how to translate the 'activation state' of the TME, which encompasses the cellular and molecular composition of the TME and can be envisioned as an intricate and dynamic network, into predictive biomarkers. This applies to both conventional cytotoxic and targeted therapeutic interventions. Initial studies that have highlighted the ability of tumor stroma-related gene signatures to predict therapeutic response to chemotherapy demonstrate that this is a feasible concept [23,164]. However, the fact that putative markers are very likely not dichotomous variables, but rather gradual and multivariable, make this a challenging task. This difficulty is illustrated by the current lack of a definite predictive biomarker for anti-VEGF therapies, although this treatment modality

has been in use for many years [165]. Nonetheless, there is a critical need to integrate these questions into future trial designs to ultimately confer clinical benefit and shield patients from unnecessary harm. Achieving this objective will thus be a major goal in the coming years and will necessitate a close dialog between investigators working on the TME and clinical oncologists (Box 4).

Box 4

Emerging areas in TME-mediated resistance: the microbiome and the metabolome

In this review, we focus on how different cellular and extracellular components of the TME can profoundly impact therapeutic response and outcome. However, it is becoming increasingly evident that bacteria in the host can modulate the TME to influence the initiation and progression of cancers in the gastrointestinal (GI) tract [246]. Moreover, there are examples in which the gut microbiota can promote disease progression in other organ sites, including the liver [247] and breast [248,249]. Thus, the microbiome is emerging as a major player in many diseases including cancer, obesity, and metabolic syndromes and is likely to influence both local and systemic therapeutic response. Two recent studies showed that the response to chemotherapy or immunotherapy could be negatively impacted by the use of broad-spectrum antibiotics that disrupted the intestinal microbiota [250,251]. Cyclophosphamide chemotherapy, which has immune-modulating properties, was found to increase intestinal permeability resulting in an altered bacterial composition in the gut and the entry of certain Gram-positive bacteria into the spleen and lymph nodes [251]. Interestingly, cyclophosphamide converted naïve $CD4^+$ T cells in these organs to Th17 cells, which contributed to the immune response against the tumor. This beneficial effect could be blocked by antibiotic treatment or by using germ-free mice, demonstrating the critical role of these bacterial species in promoting the cyclophosphamide response. Iida *et al.* also examined immunotherapies in their study and found an impaired antitumor response in animals pretreated with antibiotics, which they attributed to decreased TNF production by tumor-infiltrating myeloid cells. Together, these studies demonstrated that the microbiome can have a potent effect on the efficacy of distinct therapies through interactions with different immune cell types in the TME.

Given that gut microbiota can be profoundly disrupted by chemotherapy and radiotherapy [252], often resulting in a substantial decrease in microbial diversity, strategies to restore microbial balance could have a profound impact not only on the health of the patient but also on therapeutic response. 'Beneficial' bacteria have been shown to stimulate host immune cells (regulatory $CD4^+CD25^+$ lymphocytes) to suppress mammary cancer in mice [253], raising the intriguing possibility that altering the microbiome could similarly be considered as a novel strategy to circumvent TME-mediated therapeutic resistance in GI malignancies and possibly other organs.

Another previously underappreciated layer of complexity that needs to be incorporated into the multifaceted network of tumor–TME interactions is the role of the metabolome [254,255]. There are emerging data demonstrating a 'reverse' Warburg effect beyond the malignant cells; that is, anabolic, aerobic glycolysis occurring in the tumor stroma that

provides the metabolite lactate to tumor cells [256]. Interestingly, the metabolic reprogramming of CAFs within the TME appears to be linked to their production of tumor-promoting IL-6 and TGF-β [257]. Initial preclinical studies aimed at disrupting the synergistic metabolism between tumor compartments proved to be feasible and thus might constitute a promising avenue to pursue [258,259].

As indicated by these examples, and the emergence of additional important stromal cell types in the TME including adipocytes and nerves [3], the complexity of the TME extends far beyond the local environment to involve a multitude of systemic interactions within the host. Thus, targeting the TME in the context of evading or overcoming therapy resistance will increasingly require a sophisticated understanding of the physiology of the patient.

Acknowledgments

References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144:646–674. [PubMed: 21376230]

2. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell. 2012; 21:309–322. [PubMed: 22439926]

3. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med. 2013; 19:1423–1437. [PubMed: 24202395]

4. Campbell I, et al. Clonal mutations in the cancer-associated fibroblasts: the case against genetic coevolution. Cancer Res. 2009; 69:6765–6769. [PubMed: 19706773]

5. Egeblad M, et al. Tumors as organs: complex tissues that interface with the entire organism. Dev Cell. 2010; 18:884–901. [PubMed: 20627072]

6. Edwards BK, et al. Annual Report to the Nation on the status of cancer, 1975–2010, featuring prevalence of comorbidity and impact on survival among persons with lung, colorectal, breast, or prostate cancer. Cancer. 2014; 120:1290–1314. [PubMed: 24343171]

7. Siegel R, et al. Cancer statistics, 2013. CA Cancer J Clin. 2013; 63:11–30. [PubMed: 23335087]

8. Holohan C, et al. Cancer drug resistance: an evolving paradigm. Nat Rev Cancer. 2013; 13:714–726. [PubMed: 24060863]

9. Roché H, Vahdat LT. Treatment of metastatic breast cancer: second line and beyond. Ann Oncol. 2011; 22:1000–1010. [PubMed: 20966181]

10. Acharyya S, et al. A CXCL1 paracrine network links cancer chemoresistance and metastasis. Cell. 2012; 150:165–178. [PubMed: 22770218]

11. Alexander S, Friedl P. Cancer invasion and resistance: interconnected processes of disease progression and therapy failure. Trends Mol Med. 2012; 18:13–26. [PubMed: 22177734]

12. Hu G, et al. MTDH activation by 8q22 genomic gain promotes chemoresistance and metastasis of poor-prognosis breast cancer. Cancer Cell. 2009; 15:9–20. [PubMed: 19111877]

13. Wilson TR, et al. Chemoresistance in solid tumours. Ann Oncol. 2006; 17:x315–x324. [PubMed: 17018746]

14. Turner NC, Reis-Filho JS. Genetic heterogeneity and cancer drug resistance. Lancet Oncol. 2012; 13:e178–e185. [PubMed: 22469128]

15. Teicher BA, et al. Tumor resistance to alkylating agents conferred by mechanisms operative only *in vivo*. Science. 1990; 247:1457–1461. [PubMed: 2108497]

16. McMillin DW, et al. The role of tumour–stromal interactions in modifying drug response: challenges and opportunities. Nat Rev Drug Discov. 2013; 12:217–228. [PubMed: 23449307]

17. Meads MB, et al. Environment-mediated drug resistance: a major contributor to minimal residual disease. Nat Rev Cancer. 2009; 9:665–674. [PubMed: 19693095]

18. Zitvogel L, et al. Mechanism of action of conventional and targeted anticancer therapies: reinstating immunosurveillance. Immunity. 2013; 39:74–88. [PubMed: 23890065]

19. Dekker TJA, et al. Prognostic significance of the tumor–stroma ratio: validation study in node-negative premenopausal breast cancer patients from the EORTC perioperative chemotherapy (POP) trial (10854). Breast Cancer Res Treat. 2013; 139:371–379. [PubMed: 23709090]

20. Park JH, et al. The relationship between tumour stroma percentage, the tumour microenvironment and survival in patients with primary operable colorectal cancer. Ann Oncol. 2014; 25:644–651. [PubMed: 24458470]

21. Finak G, et al. Stromal gene expression predicts clinical outcome in breast cancer. Nat Med. 2008; 14:518–527. [PubMed: 18438415]

22. Planche A, et al. Identification of prognostic molecular features in the reactive stroma of human breast and prostate cancer. PLoS ONE. 2011; 6:e18640. [PubMed: 21611158]

23. Farmer P, et al. A stroma-related gene signature predicts resistance to neoadjuvant chemotherapy in breast cancer. Nat Med. 2009; 15:68–74. [PubMed: 19122658]

24. Correia AL, Bissell MJ. The tumor microenvironment is a dominant force in multidrug resistance. Drug Resist Updat. 2012; 15:39–49. [PubMed: 22335920]

25. Heldin CH, et al. High interstitial fluid pressure – an obstacle in cancer therapy. Nat Rev Cancer. 2004; 4:806–813. [PubMed: 15510161]

26. Provenzano PP, Hingorani SR. Hyaluronan, fluid pressure, and stromal resistance in pancreas cancer. Br J Cancer. 2013; 108:1–8. [PubMed: 23299539]

27. Wiig H, et al. Interstitial fluid: the overlooked component of the tumor microenvironment? Fibrogenesis Tissue Repair. 2010; 3:12. [PubMed: 20653943]

28. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science. 2005; 307:58–62. [PubMed: 15637262]

29. Bailey JM, et al. Sonic hedgehog promotes desmoplasia in pancreatic cancer. Clin Cancer Res. 2008; 14:5995–6004. [PubMed: 18829478]

30. Olive KP, et al. Inhibition of hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science. 2009; 324:1457–1461. [PubMed: 19460966]

31. Provenzano PP, et al. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. Cancer Cell. 2012; 21:418–429. [PubMed: 22439937]

32. Madden JI. Infinity Reports Update from Phase 2 Study of Saridegib Plus Gemcitabine in Patients with Metastatic Pancreatic Cancer. Infinity Pharmaceuticals. 2012

33. Moutasim KA, et al. Suppression of hedgehog signalling promotes pro-tumourigenic integrin expression and function. J Pathol. 2014; 233:196–208. [PubMed: 24573955]

34. Özdemir BC, et al. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. Cancer Cell. 2014; 25:719–734. [PubMed: 24856586]

35. Rhim AD, et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. Cancer Cell. 2014; 25:735–747. [PubMed: 24856585]

36. Stylianopoulos T, Jain RK. Combining two strategies to improve perfusion and drug delivery in solid tumors. Proc Natl Acad Sci USA. 2013; 110:18632–18637. [PubMed: 24167277]

37. Nakasone ES, et al. Imaging tumor–stroma interactions during chemotherapy reveals contributions of the microenvironment to resistance. Cancer Cell. 2012; 21:488–503. [PubMed: 22516258]

38. Leite de Oliveira R, et al. Gene-targeting of Phd2 improves tumor response to chemotherapy and prevents side-toxicity. Cancer Cell. 2012; 22:263–277. [PubMed: 22897855]

39. Tong RT, et al. Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. Cancer Res. 2004; 64:3731–3736. [PubMed: 15172975]

40. Goel S, et al. Normalization of the vasculature for treatment of cancer and other diseases. Physiol Rev. 2011; 91:1071–1121. [PubMed: 21742796]

41. Huang Y, et al. Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. Proc Natl Acad Sci USA. 2012; 109:17561–17566. [PubMed: 23045683]

42. Jayson GC, et al. Antiangiogenic therapy – evolving view based on clinical trial results. Nat Rev Clin Oncol. 2012; 9:297–303. [PubMed: 22330688]

43. Sennino B, McDonald DM. Controlling escape from angiogenesis inhibitors. Nat Rev Cancer. 2012; 12:699–709. [PubMed: 23001349]

44. El Azreq MA, et al. Collagen/β1 integrin signaling upregulates the ABCC1/MRP-1 transporter in an ERK/MAPK-dependent manner. Mol Biol Cell. 2012; 23:3473–3484. [PubMed: 22787275]

45. Weizman N, et al. Macrophages mediate gemcitabine resistance of pancreatic adenocarcinoma by upregulating cytidine deaminase. Oncogene. 2014; 33:3812–3819. [PubMed: 23995783]

46. Safaei R, et al. Abnormal lysosomal trafficking and enhanced exosomal export of cisplatin in drug-resistant human ovarian carcinoma cells. Mol Cancer Ther. 2005; 4:1595–1604. [PubMed: 16227410]

47. Shedden K, et al. Expulsion of small molecules in vesicles shed by cancer cells: association with gene expression and chemosensitivity profiles. Cancer Res. 2003; 63:4331–4337. [PubMed: 12907600]

48. Jaiswal R, et al. Microparticle-associated nucleic acids mediate trait dominance in cancer. FASEB J. 2012; 26:420–429. [PubMed: 21965597]

49. Aung T, et al. Exosomal evasion of humoral immunotherapy in aggressive B-cell lymphoma modulated by ATP-binding cassette transporter A3. Proc Natl Acad Sci USA. 2011; 108:15336–15341. [PubMed: 21873242]

50. Ciravolo V, et al. Potential role of HER2-overexpressing exosomes in countering trastuzumab-based therapy. J Cell Physiol. 2012; 227:658–667. [PubMed: 21465472]

51. Pasquier J, et al. Microparticles mediated cross-talk between tumoral and endothelial cells promote the constitution of a pro-metastatic vascular niche through Arf6 up regulation. Cancer Microenviron. 2014; 7:41–59. [PubMed: 24424657]

52. Boelens MC, et al. Exosome transfer from stromal to breast cancer cells regulates therapy resistance pathways. Cell. 2014; 159:499–513. [PubMed: 25417103]

53. Castellana D, et al. Membrane microvesicles as actors in the establishment of a favorable prostatic tumoral niche: a role for activated fibroblasts and CX3CL1-CX3CR1 axis. Cancer Res. 2009; 69:785–793. [PubMed: 19155311]

54. Borges FT, et al. TGF-β1-containing exosomes from injured epithelial cells activate fibroblasts to initiate tissue regenerative responses and fibrosis. J Am Soc Nephrol. 2013; 24:385–392. [PubMed: 23274427]

55. Pasquier J, et al. Preferential transfer of mitochondria from endothelial to cancer cells through tunneling nanotubes modulates chemoresistance. J Transl Med. 2013; 11:94. [PubMed: 23574623]

56. Cabarcas SM, et al. The cancer stem cell niche – there goes the neighborhood? Int J Cancer. 2011; 129:2315–2327. [PubMed: 21792897]

57. Maugeri-Saccà M, et al. Cancer stem cells and chemosensitivity. Clin Cancer Res. 2011; 17:4942–4947. [PubMed: 21622723]

58. Brooks MD, et al. Hitting them where they live: targeting the glioblastoma perivascular stem cell niche. Curr Pathobiol Rep. 2013; 1:101–110. [PubMed: 23766946]

59. Calabrese C, et al. A perivascular niche for brain tumor stem cells. Cancer Cell. 2007; 11:69–82. [PubMed: 17222791]

60. Charles NA, Holland EC. The perivascular niche microenvironment in brain tumor progression. Cell Cycle. 2010; 9:3084–3093.

61. Filatova A, et al. The cancer stem cell niche(s): the crosstalk between glioma stem cells and their microenvironment. Biochim Biophys Acta. 2013; 1830:2496–2508. [PubMed: 23079585]

62. Hjelmeland AB, et al. Twisted tango: brain tumor neurovascular interactions. Nat Neurosci. 2011; 14:1375–1381. [PubMed: 22030548]

63. Hambardzumyan D, et al. PI3K pathway regulates survival of cancer stem cells residing in the perivascular niche following radiation in medulloblastoma *in vivo*. Genes Dev. 2008; 22:436–448. [PubMed: 18281460]

64. Fernandez-L A, et al. Oncogenic YAP promotes radioresistance and genomic instability in medulloblastoma through IGF2-mediated Akt activation. Oncogene. 2012; 31:1923–1937. [PubMed: 21874045]

65. Ghajar CM, et al. The perivascular niche regulates breast tumor dormancy. Nat Cell Biol. 2013; 15:807–817. [PubMed: 23728425]

66. Lai C-Y, et al. CD133$^+$ melanoma subpopulations contribute to perivascular niche morphogenesis and tumorigenicity through vasculogenic mimicry. Cancer Res. 2012; 72:5111–5118. [PubMed: 22865455]

67. Ritchie KE, Nör JE. Perivascular stem cell niche in head and neck cancer. Cancer Lett. 2013; 338:41–46. [PubMed: 22842095]

68. Tavora B, et al. Endothelial-cell FAK targeting sensitizes tumours to DNA-damaging therapy. Nature. 2014; 514:112–116. [PubMed: 25079333]

69. Ding BS, et al. Divergent angiocrine signals from vascular niche balance liver regeneration and fibrosis. Nature. 2014; 505:97–102. [PubMed: 24256728]

70. Cao Z, et al. Angiocrine factors deployed by tumor vascular niche induce B cell lymphoma invasiveness and chemoresistance. Cancer Cell. 2014; 25:350–365. [PubMed: 24651014]

71. Cukierman E, Bassi DE. The mesenchymal tumor microenvironment: a drug-resistant niche. Cell Adh Migr. 2012; 6:285–296. [PubMed: 22568991]

72. Ehninger A, Trumpp A. The bone marrow stem cell niche grows up: mesenchymal stem cells and macrophages move in. J Exp Med. 2011; 208:421–428. [PubMed: 21402747]

73. Meads MB, et al. The bone marrow microenvironment as a tumor sanctuary and contributor to drug resistance. Clin Cancer Res. 2008; 14:2519–2526. [PubMed: 18451212]

74. Konopleva M, et al. Stromal cells prevent apoptosis of AML cells by up-regulation of anti-apoptotic proteins. Leukemia. 2002; 16:1713–1724. [PubMed: 12200686]

75. McMillin DW, et al. Tumor cell-specific bioluminescence platform to identify stroma-induced changes to anticancer drug activity. Nat Med. 2010; 16:483–489. [PubMed: 20228816]

76. Hodge DR, et al. The role of IL-6 and STAT3 in inflammation and cancer. Eur J Cancer. 2005; 41:2502–2512. [PubMed: 16199153]

77. Kinoshita H, et al. Interleukin-6 mediates epithelial–stromal interactions and promotes gastric tumorigenesis. PLoS ONE. 2013; 8:e60914. [PubMed: 23593346]

78. Nagasaki T, et al. Interleukin-6 released by colon cancer-associated fibroblasts is critical for tumour angiogenesis: anti-interleukin-6 receptor antibody suppressed angiogenesis and inhibited tumour-stroma interaction. Br J Cancer. 2014; 110:469–478. [PubMed: 24346288]

79. Song LMS, et al. Antitumor efficacy of the anti-interleukin-6 (IL-6) antibody siltuximab in mouse xenograft models of lung cancer. J Thorac Oncol. 2014; 9:974–982. [PubMed: 24922005]

80. Voorhees PM, et al. A Phase 2 multicentre study of siltuximab, an anti-interleukin-6 monoclonal antibody, in patients with relapsed or refractory multiple myeloma. Br J Haematol. 2013; 161:357–366. [PubMed: 23432640]

81. San-Miguel J, et al. Phase 2 randomized study of bortezomib–melphalan–prednisone with or without siltuximab (anti-IL-6) in multiple myeloma. Blood. 2014; 123:4136–4142. [PubMed: 24833354]

82. Coward J, et al. Interleukin-6 as a therapeutic target in human ovarian cancer. Clin Cancer Res. 2011; 17:6083–6096. [PubMed: 21795409]

83. Rossi JF, et al. A Phase I/II study of siltuximab (CNTO 328), an anti-interleukin-6 monoclonal antibody, in metastatic renal cell cancer. Br J Cancer. 2010; 103:1154–1162. [PubMed: 20808314]

84. Hynes RO. The extracellular matrix: not just pretty fibrils. Science. 2009; 326:1216–1219. [PubMed: 19965464]

85. Lu P, et al. The extracellular matrix: a dynamic niche in cancer progression. J Cell Biol. 2012; 196:395–406. [PubMed: 22351925]

86. Weaver VM, et al. β4 integrin-dependent formation of polarized three-dimensional architecture confers resistance to apoptosis in normal and malignant mammary epithelium. Cancer Cell. 2002; 2:205–216. [PubMed: 12242153]

87. Desgrosellier JS, Cheresh DA. Integrins in cancer: biological implications and therapeutic opportunities. Nat Rev Cancer. 2010; 10:9–22. [PubMed: 20029421]

88. Eke I, et al. β1 Integrin/FAK/cortactin signaling is essential for human head and neck cancer resistance to radiotherapy. J Clin Invest. 2012; 122:1529–1540. [PubMed: 22378044]

89. Goel HL, et al. β1 integrins mediate resistance to ionizing radiation *in vivo* by inhibiting c-Jun amino terminal kinase 1. J Cell Physiol. 2013; 228:1601–1609. [PubMed: 23359252]

90. Park CC, et al. β1 integrin inhibition dramatically enhances radiotherapy efficacy in human breast cancer xenografts. Cancer Res. 2008; 68:4398–4405. [PubMed: 18519702]

91. Huang C, et al. β1 integrin mediates an alternative survival pathway in breast cancer cells resistant to lapatinib. Breast Cancer Res. 2011; 13:R84. [PubMed: 21884573]

92. Kanda R, et al. Erlotinib resistance in lung cancer cells mediated by integrin β1/Src/Akt-driven bypass signaling. Cancer Res. 2013; 73:6243–6253. [PubMed: 23872583]

93. Seguin L, et al. An integrin β3–KRAS–RalB complex drives tumour stemness and resistance to EGFR inhibition. Nat Cell Biol. 2014; 16:457–468. [PubMed: 24747441]

94. Lesniak D, et al. β1-integrin circumvents the antiproliferative effects of trastuzumab in human epidermal growth factor receptor-2-positive breast cancer. Cancer Res. 2009; 69:8620–8628. [PubMed: 19887601]

95. Möhle R, et al. The chemokine receptor CXCR-4 is expressed on CD34^{+} hematopoietic progenitors and leukemic cells and mediates transendothelial migration induced by stromal cell-derived factor-1. Blood. 1998; 91:4523–4530. [PubMed: 9616148]

96. Chen Y, et al. CXCR4 downregulation of let-7a drives chemoresistance in acute myeloid leukemia. J Clin Invest. 2013; 123:2395–2407. [PubMed: 23676502]

97. Zeng Z, et al. Targeting the leukemia microenvironment by CXCR4 inhibition overcomes resistance to kinase inhibitors and chemotherapy in AML. Blood. 2009; 113:6215–6224. [PubMed: 18955566]

98. Hartmann TN, et al. CXCR4 chemokine receptor and integrin signaling co-operate in mediating adhesion and chemoresistance in small cell lung cancer (SCLC) cells. Oncogene. 2005; 24:4462–4471. [PubMed: 15806155]

99. Bergfeld SA, et al. Bone marrow-derived mesenchymal stromal cells promote survival and drug resistance in tumor cells. Mol Cancer Ther. 2014; 13:962–975. [PubMed: 24502925]

100. Singh S, et al. CXCL12–CXCR4 signalling axis confers gemcitabine resistance to pancreatic cancer cells: a novel target for therapy. Br J Cancer. 2010; 103:1671–1679. [PubMed: 21045835]

101. Weisberg E, et al. Selective Akt inhibitors synergize with tyrosine kinase inhibitors and effectively override stroma-associated cytoprotection of mutant FLT3-positive AML cells. PLoS ONE. 2013; 8:e56473. [PubMed: 23437141]

102. Wilson TR, et al. Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors. Nature. 2012; 487:505–509. [PubMed: 22763448]

103. Mink S, et al. Cancer-associated fibroblasts derived from EGFR–TKI resistant tumors reverse EGFR pathway inhibition by EGFR–TKIs. Mol Cancer Res. 2010; 8:809–820. [PubMed: 20530582]

104. Straussman R, et al. Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion. Nature. 2012; 487:500–504. [PubMed: 22763439]

105. Mueller KL, et al. Fibroblast-secreted hepatocyte growth factor mediates epidermal growth factor receptor tyrosine kinase inhibitor resistance in triple-negative breast cancers through paracrine activation of Met. Breast Cancer Res. 2012; 14:R104. [PubMed: 22788954]

106. DeNardo DG, et al. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discov. 2011; 1:54–67. [PubMed: 22039576]

107. Shree T, et al. Macrophages and cathepsin proteases blunt chemotherapeutic response in breast cancer. Genes Dev. 2011; 25:2465–2479. [PubMed: 22156207]

108. Predina J, et al. Changes in the local tumor microenvironment in recurrent cancers may explain the failure of vaccines after surgery. Proc Natl Acad Sci USA. 2013; 110:E415–E424. [PubMed: 23271806]

109. Bos PD, et al. Transient regulatory T cell ablation deters oncogene-driven breast cancer and enhances radiotherapy. J Exp Med. 2013; 210:2435–2466. [PubMed: 24127486]

110. Bruchard M, et al. Chemotherapy-triggered cathepsin B release in myeloid-derived suppressor cells activates the Nlrp3 inflammasome and promotes tumor growth. Nat Med. 2013; 19:57–64. [PubMed: 23202296]

111. Affara NI, et al. B cells regulate macrophage phenotype and response to chemotherapy in squamous carcinomas. Cancer Cell. 2014; 25:809–821. [PubMed: 24909985]

112. Cullen SP, et al. Fas/CD95-induced chemokines can serve as "find-me" signals for apoptotic cells. Mol Cell. 2013; 49:1034–1048. [PubMed: 23434371]

113. Kroemer G, et al. Immunogenic cell death in cancer therapy. Annu Rev Immunol. 2013; 31:51–72. [PubMed: 23157435]

114. Krysko DV, et al. Immunogenic cell death and DAMPs in cancer therapy. Nat Rev Cancer. 2012; 12:860–875. [PubMed: 23151605]

115. Bracci L, et al. Immune-based mechanisms of cytotoxic chemotherapy: implications for the design of novel and rationale-based combined treatments against cancer. Cell Death Differ. 2014; 21:15–25. [PubMed: 23787994]

116. Ma Y, et al. Contribution of IL-17-producing γδ T cells to the efficacy of anticancer chemotherapy. J Exp Med. 2011; 208:491–503. [PubMed: 21383056]

117. Hannesdóttir L, et al. Lapatinib and doxorubicin enhance the Stat1-dependent antitumor immune response. Eur J Immunol. 2013; 43:2718–2729. [PubMed: 23843024]

118. Pallasch CP, et al. Sensitizing protective tumor microenvironments to antibody-mediated therapy. Cell. 2014; 156:590–602. [PubMed: 24485462]

119. Pare R, et al. The significance of the senescence pathway in breast cancer progression. J Clin Pathol. 2013; 66:491–495. [PubMed: 23539738]

120. Saretzki G. Cellular senescence in the development and treatment of cancer. Curr Pharm Des. 2010; 16:79–100. [PubMed: 20214620]

121. Acosta JC, et al. A complex secretory program orchestrated by the inflammasome controls paracrine senescence. Nat Cell Biol. 2013; 15:978–990. [PubMed: 23770676]

122. Bavik C, et al. The gene expression program of prostate fibroblast senescence modulates neoplastic epithelial cell proliferation through paracrine mechanisms. Cancer Res. 2006; 66:794–802. [PubMed: 16424011]

123. Rodier F, et al. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. Nat Cell Biol. 2009; 11:973–979. [PubMed: 19597488]

124. Sun Y, Nelson PS. Molecular pathways: involving microenvironment damage responses in cancer therapy resistance. Clin Cancer Res. 2012; 18:4019–4025. [PubMed: 22619305]

125. Sun Y, et al. Treatment-induced damage to the tumor microenvironment promotes prostate cancer therapy resistance through WNT16B. Nat Med. 2012; 18:1359–1368. [PubMed: 22863786]

126. Lotti F, et al. Chemotherapy activates cancer-associated fibroblasts to maintain colorectal cancer-initiating cells by IL-17A. J Exp Med. 2013; 210:2851–2872. [PubMed: 24323355]

127. Roodhart JML, et al. Mesenchymal stem cells induce resistance to chemotherapy through the release of platinum-induced fatty acids. Cancer Cell. 2011; 20:370–383. [PubMed: 21907927]

128. Shaked Y, et al. Rapid chemotherapy-induced acute endothelial progenitor cell mobilization: implications for antiangiogenic drugs as chemosensitizing agents. Cancer Cell. 2008; 14:263–273. [PubMed: 18772115]

129. Casanovas O, et al. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. Cancer Cell. 2005; 8:299–309. [PubMed: 16226705]

130. Orimo A, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell. 2005; 121:335–348. [PubMed: 15882617]

131. Priceman SJ, et al. Targeting distinct tumor-infiltrating myeloid cells by inhibiting CSF-1 receptor: combating tumor evasion of antiangiogenic therapy. Blood. 2010; 115:1461–1471. [PubMed: 20008303]

132. Shojaei F, et al. Tumor refractoriness to anti-VEGF treatment is mediated by $CD11b^+Gr1^+$ myeloid cells. Nat Biotechnol. 2007; 25:911–920. [PubMed: 17664940]

133. Shojaei F, et al. Bv8 regulates myeloid-cell-dependent tumour angiogenesis. Nature. 2007; 450:825–831. [PubMed: 18064003]

134. Crawford Y, et al. PDGF-C mediates the angiogenic and tumorigenic properties of fibroblasts associated with tumors refractory to anti-VEGF treatment. Cancer Cell. 2009; 15:21–34. [PubMed: 19111878]

135. Chung AS, et al. An interleukin-17-mediated paracrine network promotes tumor resistance to anti-angiogenic therapy. Nat Med. 2013; 19:1114–1123. [PubMed: 23913124]

136. Meijer TWH, et al. Targeting hypoxia, HIF-1, and tumor glucose metabolism to improve radiotherapy efficacy. Clin Cancer Res. 2012; 18:5585–5594. [PubMed: 23071360]

137. Moeller BJ, et al. Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors: role of reoxygenation, free radicals, and stress granules. Cancer Cell. 2004; 5:429–441. [PubMed: 15144951]

138. Kioi M, et al. Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. J Clin Invest. 2010; 120:694–705. [PubMed: 20179352]

139. Kozin SV, et al. Recruitment of myeloid but not endothelial precursor cells facilitates tumor regrowth after local irradiation. Cancer Res. 2010; 70:5679–5685. [PubMed: 20631066]

140. Xu J, et al. CSF1R signaling blockade stanches tumor-infiltrating myeloid cells and improves the efficacy of radiotherapy in prostate cancer. Cancer Res. 2013; 73:2782–2794. [PubMed: 23418320]

141. Chinot OL, et al. Bevacizumab plus radiotherapy–temozolomide for newly diagnosed glioblastoma. N Engl J Med. 2014; 370:709–722. [PubMed: 24552318]

142. Gilbert MR, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. N Engl J Med. 2014; 370:699–708. [PubMed: 24552317]

143. Gilbert LA, Hemann MT. DNA damage-mediated induction of a chemoresistant niche. Cell. 2010; 143:355–366. [PubMed: 21029859]

144. Duan CW, et al. Leukemia propagating cells rebuild an evolving niche in response to therapy. Cancer Cell. 2014; 25:778–793. [PubMed: 24937459]

145. Kim M, et al. CXCR4 signaling regulates metastasis of chemoresistant melanoma cells by a lymphatic metastatic niche. Cancer Res. 2010; 70:10411–10421. [PubMed: 21056990]

146. Mannino M, Chalmers AJ. Radioresistance of glioma stem cells: intrinsic characteristic or property of the 'microenvironment–stem cell unit'? Mol Oncol. 2011; 5:374–386. [PubMed: 21659010]

147. Shang Y, et al. Roles of epithelial–mesenchymal transition in cancer drug resistance. Curr Cancer Drug Targets. 2013; 13:915–929. [PubMed: 24168191]

148. Landsberg J, et al. Melanomas resist T-cell therapy through inflammation-induced reversible dedifferentiation. Nature. 2012; 490:412–416. [PubMed: 23051752]

149. Bhat KPL, et al. Mesenchymal differentiation mediated by NF-κB promotes radiation resistance in glioblastoma. Cancer Cell. 2013; 24:331–346. [PubMed: 23993863]

150. Dijkgraaf EM, et al. Chemotherapy alters monocyte differentiation to favor generation of cancer-supporting M2 macrophages in the tumor microenvironment. Cancer Res. 2013; 73:2480–2492. [PubMed: 23436796]

151. Klug F, et al. Low-dose irradiation programs macrophage differentiation to an $iNOS^+$/M1 phenotype that orchestrates effective T cell immunotherapy. Cancer Cell. 2013; 24:589–602. [PubMed: 24209604]

152. Bindea G, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity. 2013; 39:782–795. [PubMed: 24138885]

153. Galon J, et al. Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. J Pathol. 2014; 232:199–209. [PubMed: 24122236]

154. Yoshihara K, et al. Inferring tumour purity and stromal and immune cell admixture from expression data. Nat Commun. 2013; 4:2612. [PubMed: 24113773]

155. Erkkilä T, et al. Probabilistic analysis of gene expression measurements from heterogeneous tissues. Bioinformatics. 2010; 26:2571–2577. [PubMed: 20631160]

156. Shoemaker JE, et al. CTen: a web-based platform for identifying enriched cell types from heterogeneous microarray data. BMC Genomics. 2012; 13:460. [PubMed: 22953731]

157. Meng W, et al. A systems biology approach identifies effective tumor–stroma common targets for oral squamous cell carcinoma. Cancer Res. 2014; 74:2306–2315. [PubMed: 24556718]

158. Beatty GL, et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. Science. 2011; 331:1612–1616. [PubMed: 21436454]

159. Bowman RL, Joyce JA. Therapeutic targeting of tumor-associated macrophages and microglia in glioblastoma. Immunotherapy. 2014; 6:663–666. [PubMed: 25041027]

160. Coussens LM, et al. Neutralizing tumor-promoting chronic inflammation: a magic bullet? Science. 2013; 339:286–291. [PubMed: 23329041]

161. Hagemann T, et al. Re-educating" tumor-associated macrophages by targeting NF-κB. J Exp Med. 2008; 205:1261–1268. [PubMed: 18490490]

162. Pyonteck SM, et al. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. Nat Med. 2013; 19:1264–1272. [PubMed: 24056773]

163. Zhu Y, et al. CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. Cancer Res. 2014; 74:5057–5069. [PubMed: 25082815]

164. Marchini C, et al. Mesenchymal/stromal gene expression signature relates to basal-like breast cancers, identifies bone metastasis and predicts resistance to therapies. PLoS ONE. 2010; 5:e14131. [PubMed: 21152434]

165. Maru D, et al. Predictive biomarkers for bevacizumab: are we there yet? Clin Cancer Res. 2013; 19:2824–2827. [PubMed: 23549876]

166. Albrengues J, et al. LIF mediates proinvasive activation of stromal fibroblasts in cancer. Cell Rep. 2014; 7:1664–1678. [PubMed: 24857661]

167. Vonderheide RH, Glennie MJ. Agonistic CD40 antibodies and cancer therapy. Clin Cancer Res. 2013; 19:1035–1043. [PubMed: 23460534]

168. Sharma N, et al. A Phase 1b study to assess the safety of PLX3397, a CSF-1 receptor inhibitor, and paclitaxel in patients with advanced solid tumors. J Clin Oncol. 2014; 32:5s.

169. Ugel S, et al. Therapeutic targeting of myeloid-derived suppressor cells. Curr Opin Pharmacol. 2009; 9:470–481. [PubMed: 19616475]

170. Germano G, et al. Role of macrophage targeting in the antitumor activity of trabectedin. Cancer Cell. 2013; 23:249–262. [PubMed: 23410977]

171. Suzuki E, et al. Gemcitabine selectively eliminates splenic Gr-1$^+$/CD11b$^+$ myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. Clin Cancer Res. 2005; 11:6713–6721. [PubMed: 16166452]

172. Vincent J, et al. 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. Cancer Res. 2010; 70:3052–3061. [PubMed: 20388795]

173. Alvarez R, et al. Stromal disrupting effects of nab-paclitaxel in pancreatic cancer. Br J Cancer. 2013; 109:926–933. [PubMed: 23907428]

174. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell. 1996; 86:353–364. [PubMed: 8756718]

175. Abramsson A, et al. Endothelial and nonendothelial sources of PDGF-B regulate pericyte recruitment and influence vascular pattern formation in tumors. J Clin Invest. 2003; 112:1142–1151. [PubMed: 14561699]

176. Des Guetz G, 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. [PubMed: 16773076]

177. Gasparini G. Prognostic value of vascular endothelial growth factor in breast cancer. Oncologist. 2000; 5:37–44. [PubMed: 10804090]

178. Zhan P, et al. Prognostic value of vascular endothelial growth factor expression in patients with lung cancer: a systematic review with meta-analysis. J Thorac Oncol. 2009; 4:1094–1103. [PubMed: 19687765]

179. Fukumura D, et al. Tumor induction of VEGF promoter activity in stromal cells. Cell. 1998; 94:715–725. [PubMed: 9753319]

180. Dumont N, et al. Breast fibroblasts modulate early dissemination, tumorigenesis, and metastasis through alteration of extracellular matrix characteristics. Neoplasia. 2013; 15:249–262. [PubMed: 23479504]

181. Kalluri R, Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer. 2006; 6:392–401. [PubMed: 16572188]

182. Olumi AF, et al. Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. Cancer Res. 1999; 59:5002–5011. [PubMed: 10519415]

183. Shao ZM, et al. Human breast carcinoma desmoplasia is PDGF initiated. Oncogene. 2000; 19:4337–4345. [PubMed: 10980609]

184. Löhr M, et al. Transforming growth factor-β1 induces desmoplasia in an experimental model of human pancreatic carcinoma. Cancer Res. 2001; 61:550–555. [PubMed: 11212248]

185. Erez N, et al. Cancer associated fibroblasts express pro-inflammatory factors in human breast and ovarian tumors. Biochem Biophys Res Commun. 2013; 437:397–402. [PubMed: 23831470]

186. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell. 2010; 141:39–51. [PubMed: 20371344]

187. Mantovani A, et al. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol. 2002; 23:549–555. [PubMed: 12401408]

188. Wyckoff J, et al. A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. Cancer Res. 2004; 64:7022–7029. [PubMed: 15466195]

189. Pollard JW. Macrophages define the invasive microenvironment in breast cancer. J Leukoc Biol. 2008; 84:623–630. [PubMed: 18467655]

190. Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. Nat Rev Cancer. 2009; 9:239–252. [PubMed: 19279573]

191. Ojalvo LS, et al. Gene expression analysis of macrophages that facilitate tumor invasion supports a role for Wnt-signaling in mediating their activity in primary mammary tumors. J Immunol. 2010; 184:702–712. [PubMed: 20018620]

192. Pukrop T, et al. Wnt 5a signaling is critical for macrophage-induced invasion of breast cancer cell lines. Proc Natl Acad Sci USA. 2006; 103:5454–5459. [PubMed: 16569699]

193. Gocheva V, et al. IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion. Genes Dev. 2010; 24:241–255. [PubMed: 20080943]

194. Gocheva V, et al. Distinct roles for cysteine cathepsin genes in multistage tumorigenesis. Genes Dev. 2006; 20:543–556. [PubMed: 16481467]

195. Huang S, et al. Contributions of stromal metalloproteinase-9 to angiogenesis and growth of human ovarian carcinoma in mice. J Natl Cancer Inst. 2002; 94:1134–1142. [PubMed: 12165638]

196. Roderfeld M, et al. Innovative immunohistochemistry identifies MMP-9 expressing macrophages at the invasive front of murine HCC. World J Hepatol. 2010; 2:175–179. [PubMed: 21160992]

197. Du R, et al. HIF1α induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion. Cancer Cell. 2008; 13:206–220. [PubMed: 18328425]

198. Kim S-H, et al. Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. J Endocrinol. 2011; 209:139–151. [PubMed: 21307119]

199. Paszek MJ, et al. Tensional homeostasis and the malignant phenotype. Cancer Cell. 2005; 8:241–254. [PubMed: 16169468]

200. Friedl P, Alexander S. Cancer invasion and the microenvironment: plasticity and reciprocity. Cell. 2011; 147:992–1009. [PubMed: 22118458]

201. Kauppila S, et al. Aberrant type I and type III collagen gene expression in human breast cancer *in vivo*. J Pathol. 1998; 186:262–268. [PubMed: 10211114]

202. Levental KR, et al. Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell. 2009; 139:891–906. [PubMed: 19931152]

203. Lopez JI, et al. *In situ* force mapping of mammary gland transformation. Integr Biol (Camb). 2011; 3:910–921. [PubMed: 21842067]

204. Chabner BA, Roberts TG. Timeline: chemotherapy and the war on cancer. Nat Rev Cancer. 2005; 5:65–72. [PubMed: 15630416]

205. Ceelen W, et al. Surgery, wound healing, and metastasis: recent insights and clinical implications. Crit Rev Oncol Hematol. 2014; 89:16–26. [PubMed: 23958676]

206. Thompson, RF., Maity, A. Radiotherapy and the tumor microenvironment: mutual influence and clinical implications. In: Koumenis, C., et al., editors. Tumor Microenvironment and Cellular Stress. Springer; 2014. p. 147-165.

207. Dvorak HF. Tumors: wounds that do not heal. N Engl J Med. 1986; 315:1650–1659. [PubMed: 3537791]

208. Schäfer M, Werner S. Cancer as an overhealing wound: an old hypothesis revisited. Nat Rev Mol Cell Biol. 2008; 9:628–638. [PubMed: 18628784]

209. Curigliano G, et al. Systemic effects of surgery: quantitative analysis of circulating basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF-β) in patients with breast cancer who underwent limited or extended surgery. Breast Cancer Res Treat. 2005; 93:35–40. [PubMed: 16184456]

210. Demicheli R, et al. Tumor dormancy and surgery-driven interruption of dormancy in breast cancer: learning from failures. Nat Clin Pract Oncol. 2007; 4:699–710. [PubMed: 18037874]

211. Demicheli R, et al. The effects of surgery on tumor growth: a century of investigations. Ann Oncol. 2008; 19:1821–1828. [PubMed: 18550576]

212. Park Y, et al. Does surgery for breast cancer induce angiogenesis and thus promote metastasis? Oncology. 2011; 81:199–205. [PubMed: 22067898]

213. Jamal M, et al. The brain microenvironment preferentially enhances the radioresistance of CD133^{+} glioblastoma stem-like cells. Neoplasia. 2012; 14:150–158. [PubMed: 22431923]

214. Druker BJ, et al. Efficacy and safety of a specific inhibitor of the BCR–ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med. 2001; 344:1031–1037. [PubMed: 11287972]

215. Rosell R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised Phase 3 trial. Lancet Oncol. 2012; 13:239–246. [PubMed: 22285168]

216. Bokemeyer C, et al. Addition of cetuximab to chemotherapy as first-line treatment for KRAS wild-type metastatic colorectal cancer: pooled analysis of the CRYSTAL and OPUS randomised clinical trials. Eur J Cancer. 2012; 48:1466–1475. [PubMed: 22446022]

217. Khambata-Ford S, et al. Analysis of potential predictive markers of cetuximab benefit in BMS099, a Phase III study of cetuximab and first-line taxane/carboplatin in advanced non-small-cell lung cancer. J Clin Oncol. 2010; 28:918–927. [PubMed: 20100958]

218. Chung KY, et al. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. J Clin Oncol. 2005; 23:1803–1810. [PubMed: 15677699]

219. Hecht JR, et al. Lack of correlation between epidermal growth factor receptor status and response to panitumumab monotherapy in metastatic colorectal cancer. Clin Cancer Res. 2010; 16:2205–2213. [PubMed: 20332321]

220. Curtin JA, et al. Distinct sets of genetic alterations in melanoma. N Engl J Med. 2005; 353:2135–2147. [PubMed: 16291983]

221. Chapman PB, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med. 2011; 364:2507–2516. [PubMed: 21639808]

222. Hurwitz HI, 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. [PubMed: 15908660]

223. Sandler A, et al. Paclitaxel–carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med. 2006; 355:2542–2550. [PubMed: 17167137]

224. Hodi FS, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010; 363:711–723. [PubMed: 20525992]

225. Topalian SL, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol. 2014; 32:1020–1030. [PubMed: 24590637]

226. Wolchok JD, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013; 369:122–133. [PubMed: 23724867]

227. DeVita VT, Chu E. A history of cancer chemotherapy. Cancer Res. 2008; 68:8643–8653. [PubMed: 18974103]

228. Infanger DW, et al. Engineered culture models for studies of tumor-microenvironment interactions. Annu Rev Biomed Eng. 2013; 15:29–53. [PubMed: 23642249]

229. Rubashkin MG, et al. Deconstructing signaling in three dimensions. Biochemistry. 2014; 53:2078–2090. [PubMed: 24649923]

230. Longati P, et al. 3D pancreatic carcinoma spheroids induce a matrix-rich, chemoresistant phenotype offering a better model for drug testing. BMC Cancer. 2013; 13:95. [PubMed: 23446043]

231. Li L. Optimizing a 3D culture system to study the interaction between epithelial breast cancer and its surrounding fibroblasts. J Cancer. 2011; 2:458–466. [PubMed: 21915190]

232. Fang X, et al. Novel 3D co-culture model for epithelial–stromal cells interaction in prostate cancer. PLoS ONE. 2013; 8:e75187. [PubMed: 24073251]

233. Valiente M, et al. Serpins promote cancer cell survival and vascular co-option in brain metastasis. Cell. 2014; 156:1002–1016. [PubMed: 24581498]

234. Chuang HN, et al. Carcinoma cells misuse the host tissue damage response to invade the brain. Glia. 2013; 61:1331–1346. [PubMed: 23832647]

235. Hovinga KE, et al. Inhibition of Notch signaling in glioblastoma targets cancer stem cells via an endothelial cell intermediate. Stem Cells. 2010; 28:1019–1029. [PubMed: 20506127]

236. Vaira V, et al. Preclinical model of organotypic culture for pharmacodynamic profiling of human tumors. Proc Natl Acad Sci USA. 2010; 107:8352–8356. [PubMed: 20404174]

237. Orth JD, et al. Analysis of mitosis and antimitotic drug responses in tumors by *in vivo* microscopy and single-cell pharmacodynamics. Cancer Res. 2011; 71:4608–4616. [PubMed: 21712408]

238. Ricard C, Debarbieux FC. Six-color intravital two-photon imaging of brain tumors and their dynamic microenvironment. Front Cell Neurosci. 2014; 8:57. [PubMed: 24605087]

239. Alexander S, et al. Preclinical intravital microscopy of the tumour–stroma interface: invasion, metastasis, and therapy response. Curr Opin Cell Biol. 2013; 25:659–671. [PubMed: 23896198]

240. Devaud C, et al. Tissues in different anatomical sites can sculpt and vary the tumor microenvironment to affect responses to therapy. Mol Ther. 2014; 22:18–27. [PubMed: 24048441]

241. Guerin E, et al. A model of postsurgical advanced metastatic breast cancer more accurately replicates the clinical efficacy of antiangiogenic drugs. Cancer Res. 2013; 73:2743–2748. [PubMed: 23610448]

242. Reyal F, et al. Molecular profiling of patient-derived breast cancer xenografts. Breast Cancer Res. 2012; 14:R11. [PubMed: 22247967]

243. Ma CX, et al. Targeting Chk1 in p53-deficient triple-negative breast cancer is therapeutically beneficial in human-in-mouse tumor models. J Clin Invest. 2012; 122:1541–1552. [PubMed: 22446188]

244. Wege AK, et al. Humanized tumor mice – a new model to study and manipulate the immune response in advanced cancer therapy. Int J Cancer. 2011; 129:2194–2206. [PubMed: 21544806]

245. Rongvaux A, et al. Development and function of human innate immune cells in a humanized mouse model. Nat Biotechnol. 2014; 32:364–372. [PubMed: 24633240]

246. Elinav E, et al. Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. Nat Rev Cancer. 2013; 13:759–771. [PubMed: 24154716]

247. Dapito DH, et al. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. Cancer Cell. 2012; 21:504–516. [PubMed: 22516259]

248. Rao VP, et al. Innate immune inflammatory response against enteric bacteria *Helicobacter hepaticus* induces mammary adenocarcinoma in mice. Cancer Res. 2006; 66:7395–7400. [PubMed: 16885333]

249. Rao VP, et al. Breast cancer: should gastrointestinal bacteria be on our radar screen? Cancer Res. 2007; 67:847–850. [PubMed: 17283110]

250. Iida N, et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science. 2013; 342:967–970. [PubMed: 24264989]

251. Viaud S, et al. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science. 2013; 342:971–976. [PubMed: 24264990]

252. Touchefeu Y, et al. Systematic review: the role of the gut microbiota in chemotherapy- or radiation-induced gastrointestinal mucositis – current evidence and potential clinical applications. Aliment Pharmacol Ther. 2014; 40:409–421. [PubMed: 25040088]

253. Lakritz JR, et al. Beneficial bacteria stimulate host immune cells to counteract dietary and genetic predisposition to mammary cancer in mice. Int J Cancer. 2014; 135:529–540. [PubMed: 24382758]

254. Icard P, et al. The metabolic cooperation between cells in solid cancer tumors. Biochim Biophys Acta. 2014; 1846:216–225. [PubMed: 24983675]

255. Martinez-Outschoorn UE, et al. Catabolic cancer-associated fibroblasts (CAFs) transfer energy and biomass to anabolic cancer cells, fueling tumor growth. Semin Cancer Biol. 2014; 25:47–60. [PubMed: 24486645]

256. Carito V, et al. Metabolic remodeling of the tumor microenvironment: migration stimulating factor (MSF) reprograms myofibroblasts toward lactate production, fueling anabolic tumor growth. Cell Cycle. 2012; 11:3403–3414. [PubMed: 22918248]

257. Valencia T, et al. Metabolic reprogramming of stromal fibroblasts through p62–mTORC1 signaling promotes inflammation and tumorigenesis. Cancer Cell. 2014; 26:121–135. [PubMed: 25002027]

258. Martinez-Outschoorn UE, et al. Anti-estrogen resistance in breast cancer is induced by the tumor microenvironment and can be overcome by inhibiting mitochondrial function in epithelial cancer cells. Cancer Biol Ther. 2011; 12:924–938. [PubMed: 22041887]

259. Sotgia F, et al. Cancer metabolism: new validated targets for drug discovery. Oncotarget. 2013; 4:1309–1316. [PubMed: 23896568]
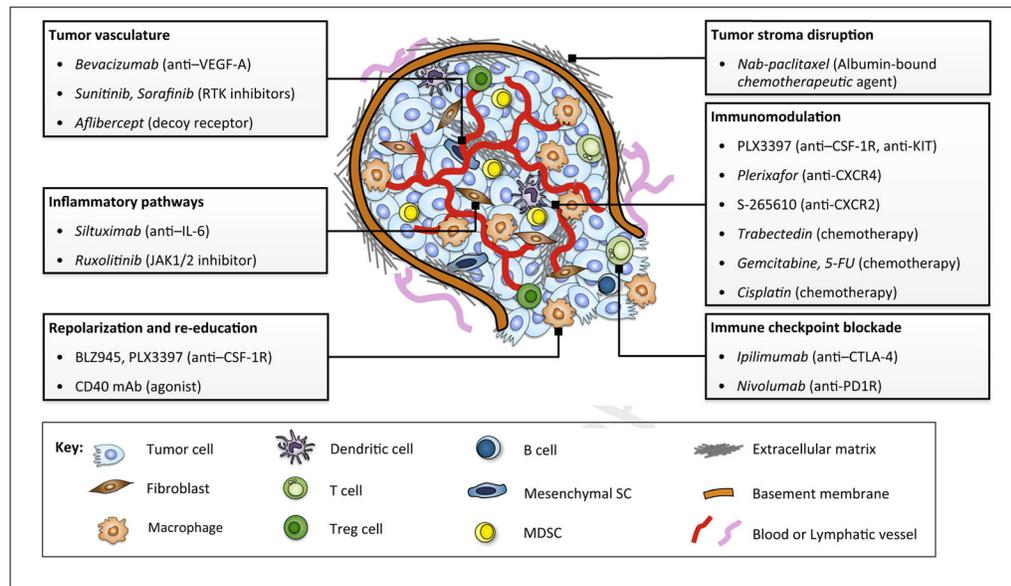
Figure 1. Major constituents of the tumor microenvironment (TME) and TME-targeted therapies

The TME comprises various cell types that modulate treatment response and are putative candidates for therapeutic intervention. The tumor vasculature can be targeted with various drugs such as the vascular endothelial growth factor (VEGF)-A antibody bevacizumab, the multitarget receptor tyrosine kinase (RTK) inhibitors sunitinib and sorafenib, and the decoy VEGF receptor aflibercept. Inflammatory pathway activation can be inhibited by the interleukin-6 (IL-6) antibody siltuximab [79] or the pan-JAK inhibitor ruxolitinib [166]. Cancer-associated fibroblasts are activated by multiple growth factors and cytokines within the TME and in turn acquire a proinflammatory phenotype and become a major source of soluble mediators that drive angiogenesis and enhance tumor cell survival. The immune cell compartment within the TME exhibits extraordinary plasticity: tumor-associated macrophages (TAMs) and myeloid derived suppressor cells (MDSCs) orchestrate an immunosuppressive and protective phenotype that extends to T cells, T regulatory (T_{reg}) cells and B cells. Repolarization or re-education of macrophages or other myeloid cells can be achieved by colony-stimulating factor 1 receptor (CSF-1R) inhibition (for example, BLZ945) [162] or agonistic CD40 antibodies that activate antigen-presenting cells (e.g., dendritic cells) to process and present tumor-associated antigens to local cytotoxic T lymphocytes [158,167]. This immune landscape within the tumor can be sculpted by inhibition of critical cytokine axes such as CSF-1R and/or KIT (PLX3397) [168], chemokine (C-X-C motif) receptor (CXCR) 4 (plerixafor), and CXCR2 (S-265610) [169]. The chemotherapeutic agent trabectedin has been proposed to selectively deplete monocytes and/or macrophages [170]. Both gemcitabine and 5-fluorourocil (5-FU) have been shown to deplete MDSCs [171,172]. Platinum-based cytostatic drugs can not only alter macrophage polarization but also induce increased antigen-presenting ability of dendritic cells. The blockade of immune checkpoints is another promising avenue of therapeutic intervention. This can be achieved through blockade of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) (ipilimumab) or the programmed death 1 (PD1) receptor (nivolumab). Finally,

several extracellular properties also shape the therapeutic response, such as high interstitial fluid pressure and changes in the composition of the extracellular matrix (ECM). Albumin-bound nab-paclitaxel has been postulated to disrupt the stromal composition [173]. FDA-approved drugs are indicated in italics while agents in preclinical or clinical trials are non-italicized.
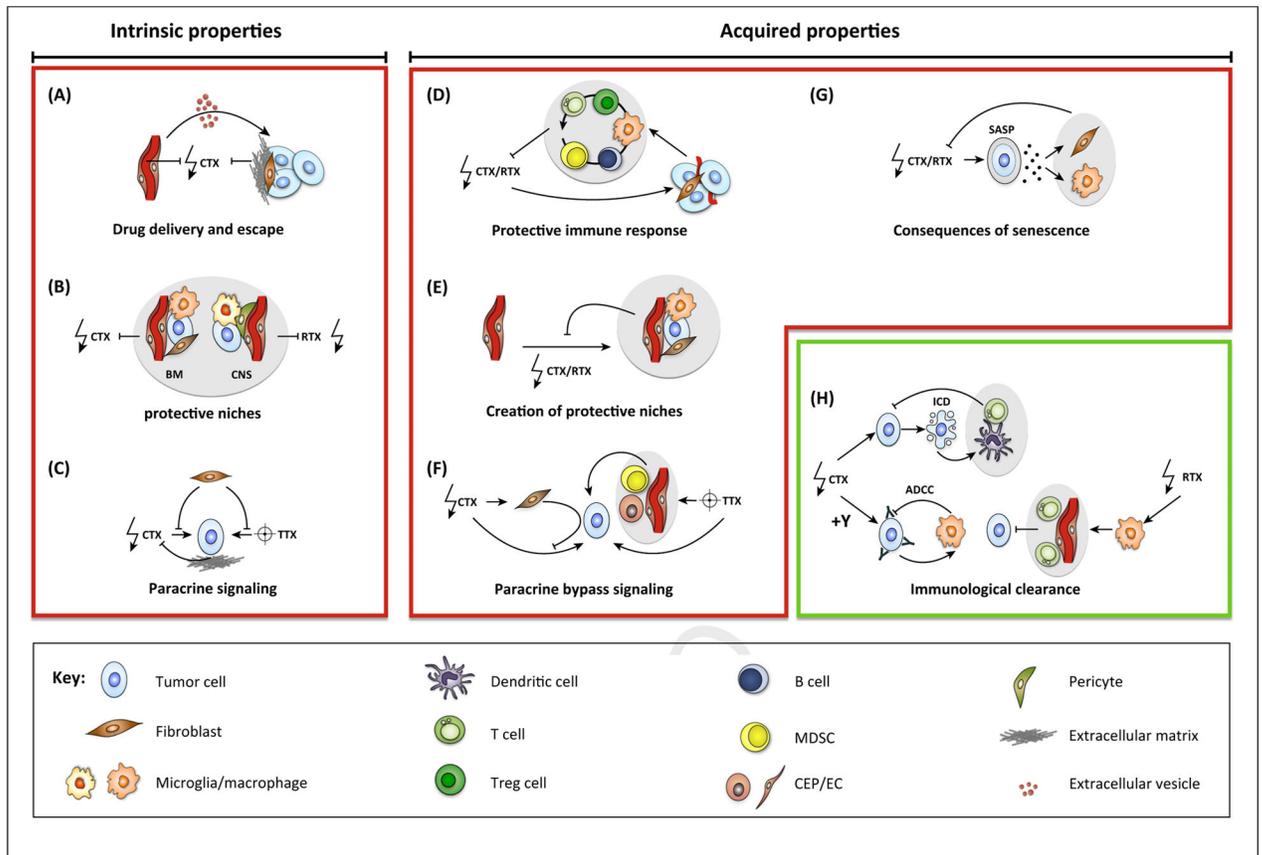
Figure 2. Intrinsic and acquired contributions of the tumor microenvironment (TME) to therapeutic response

The TME alters therapeutic efficacy through both intrinsic traits and properties acquired after exposure to therapy. This applies to chemotherapy (CTX), radiotherapy (RTX), and targeted therapies (TTX). The intrinsic properties of the TME that modulate therapeutic response include: **(A)** the alteration of drug delivery and clearance; **(B)** the utilization of pre-existing protective niches within the bone marrow (BM) and central nervous system (CNS) to shield malignant cells from therapeutic insult; and **(C)** the co-option of prewired paracrine signaling loops within the stroma to counteract therapeutic interventions. **(D)** In response to therapy the TME can orchestrate a protective immune response that is defined by a plethora of multidirectional interactions between different immune cell populations. **(E)** Furthermore, therapeutic interventions can lead to the emergence of newly created protective niches within the TME that function as safe havens. **(F)** In addition, paracrine bypass signaling pathways can override the effects of both conventional and targeted therapies, while **(G)** the senescence-associated secretory phenotype (SASP) can dramatically change the signaling equilibrium within the TME toward a therapy-attenuating state. **(H)** However, the TME can also substantially augment therapeutic efficacy by several mechanisms that ultimately result in an increased immunological response. This can result from immunogenic cell death (ICD) of tumor cells that activates antigen-presenting dendritic cells and cytotoxic T cells, promotion of increased antibody-dependent cell-mediated cytotoxicity (ADCC) by macrophages through cyclophosphamide, and the reprogramming of macrophages by low-

dose radiation to facilitate normalization of the tumor vasculature and recruitment of cytotoxic T cells. Mechanisms that attenuate the therapeutic response are highlighted in red; therapy-ameliorating effects are marked in green. Arrow-headed lines indicate a positive or activating connection and bar-headed lines illustrate an antagonizing function.