Clinical Cancer Research

Molecular Pathways: Targeting the Microenvironment of Liver Metastases

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Abstract

Curative treatment for metastatic solid cancers remains elusive. The liver, which is nourished by a rich blood supply from both the arterial and portal venous systems, is the most common site of visceral metastases, particularly from cancers arising in the gastrointestinal tract, with colorectal cancer being the predominant primary site in Western countries. A mounting body of evidence suggests that the liver microenvironment (LME) provides autocrine and paracrine signals originating from both parenchymal and nonparenchymal cells that collectively create both pre- and prometastatic niches for the development of hepatic metastases. These resident cells and their molecular mediators represent potential therapeutic targets for the prevention and/or treatment of liver metastases (LM). This review summarizes: (i) the current therapeutic options for treating LM, with a particular focus on colorectal cancer LM; (ii) the role of the LME in LM at each of its phases; (iii) potential targets in the LME identified through preclinical and clinical investigations; and (iv) potential therapeutic approaches for targeting elements of the LME before and/or after the onset of LM as the basis for future clinical trials. *Clin Cancer Res; 23(21); 6390–9. ©2017 AACR.*

Background

Metastases remain the primary source of morbidity and mortality from solid tumors, and the liver is the dominant site of metastases from gastrointestinal malignancies such as colorectal cancer. Systemic treatments directed at cancer cells have had limited success, in large part due to the presence of numerous malignant clones, which allow rapid selection of resistant clones in the face of cytotoxic and "targeted" therapies. Our recent recognition that the liver microenvironment (LME) is also critical for facilitating access and fostering the growth of cancer cells within the liver has led to the concept of targeting both cells and molecules within the LME as a strategy for preventing and treating liver metastases (LM). This strategy has many potential advantages over targeting only the cancer cells, including the sheer number of potential targets and the potential to engage the immune system, an approach recently shown to be a highly effective and durable therapeutic modality. In this review, we utilize colorectal cancer as a paradigm to discuss the rationale for targeting the microenvironment as a strategy for the prevention and treatment of LM.

Origins of liver metastases

LMs are tumors that have spread to the liver from other malignant sites. Secondary hepatic malignancies are reportedly 18 to 40 times more common than primary hepatic malignancies in Western countries (1). Approximately half of all patients afflicted with LM have primary colorectal cancer, whereas other primary gastrointestinal cancers, such as esophageal (≈1%–2%) and gastric carcinomas (≈5%–9%), pancreatic and intestinal neuroendocrine tumors (≈1%), biliary tract cancers (≈5%–10%), as well as pancreatic ductal adenocarcinomas (PDAC; ≈14%) and gastrointestinal stromal tumors (<1%), also give rise to LM. LMs from nongastrointestinal cancers are less common but include breast (<1%–2%), lung (12%–20%), and kidney (1%–2%) cancers and melanoma (<1%; refs. 2, 3).

The liver has a dual blood supply, with two thirds to three fourths of the blood supply derived from the portal vein and the remaining from the hepatic artery. Dissemination of tumors from the gastrointestinal tract to the liver is thought to originate from cancer cells that have gained access to the portal venous circulation. On the other hand, dissemination of tumors from outside the gastrointestinal tract may originate from cancer cells that have gained access to the systemic arterial circulation. For instance, lung cancer cells may enter via the pulmonary vein and then embolize the liver via the hepatic artery (4).

These processes of liver metastasis are facilitated by two critical niches, namely the premetastatic niche driven by factors secreted by the primary tumor that in turn, recruit nonparenchymal cells, including Kupffer cells (KC), hepatic stellate cells (HepSC), myeloid-derived suppressor cells (MDSC), and neutrophils, and the post-tumor invasion niche, which develops following tumor cell entry into the liver and can be

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characterized by four key phases: (i) a microvascular phase, (ii) an extravascular preangiogenic phase, (iii) an angiogenic phase, and (iv) the growth phase (detailed below and reviewed extensively in refs. 5–7). With the exception of the angiogenic phase, the potential therapeutic benefit of targeting the microenvironment at each of these phases has not been adequately explored.

Traditional systemic therapy for colorectal LMs

Approximately 20% to 34% of patients with colorectal cancer present with synchronous LM (8, 9), and up to 50% to 60% will develop LM at some point in their disease course (10, 11). At present, the estimated 5-year overall survival (OS) for all patients with stage IV colorectal cancer is 13% (12). Treatment goals for patients with metastatic colorectal cancer (mCRC) can be classified as: (i) curative or potentially curative; this identifies a group of patients where LM may be resectable; (ii) noncurative with active treatment intent (most patients fall into this group); or (iii) palliative intent (13). Cytotoxic systemic chemotherapy is the mainstay of treatment for most advanced malignancies, including colorectal cancer (Table 1). The National Comprehensive Cancer Network (NCCN) guidelines consider fluorouracil (5-FU) combined with leucovorin and oxaliplatin (i.e., FOLFOX) or irinotecan (i.e., FOLFIRI) to be standard-of-care (SOC), first-line chemotherapy regimens for patients with unresectable colorectal cancer liver metastasis (CRCLM; Table 1; refs. 14, 15). These recommendations are based on the results of phase II and III trials that demonstrated improved median OS and progression-free survival (PFS) with combination therapy versus 5-FU and leucovorin alone. A recent meta-analysis found, however, that the response rates in these trials averaged only 68% (16). First-line regimens may also include the combination of FOLFOX or FOLFIRI with bevacizumab, cetuximab, or panitumumab. These three biologic agents are humanized, chimeric mouse/human, and human antibodies, respectively. Bevacizumab targets VEGF-A, whereas the latter two target the EGFR and downstream signaling, including the MAPK pathway (Table 1). At present, only bevacizumab and three additional therapies (i.e., ramucirumab, regorafenib, ziv-aflibercept), which are approved for later lines of therapy, target angiogenesis (15). Perhaps the modest improvements in PFS achieved with these agents are due to their utilization too late in the disease course to affect outcome. Alternatively, VEGF-independent angiogenesis may occur that renders them ineffective, as shown and discussed elsewhere (6, 17, 18). Across the various regimens containing these agents, response rates are generally modest, with improvements in OS ranging from only 1.4 to 2.5 months (15). Thus, for patients with CRCLM who are not resection candidates, the prognosis remains poor, highlighting the need for novel therapeutic approaches.

Clinical–Translational Advances

The role of the microenvironment in the different phases of liver metastasis

The process of liver metastasis has been divided into several phases based on the location of the cancer cells within the liver and the phase-specific interactions between the cancer cells and the LME. These phases were extensively reviewed elsewhere (5–7) and are briefly summarized here as background for subsequent sections.

The "premetastatic niche." Although still contentious, accumulating evidence supports the concept that the microenvironment of secondary organ sites can be rendered permissive to metastatic outgrowth in advance of tumor cell entry (5). For the liver, this was recently demonstrated in a murine model of aggressive PDAC, where tumor-derived exosomes were shown to activate KCs and set in motion a series of events leading to increased TGFβ production, HepSC activation, and extracellular matrix (ECM) deposition (19). Macrophage migration-inhibitory factor (MIF) was implicated in this process, and intriguingly, exosomal MIF levels were associated with an increased risk of relapse in the liver among patients with stage I PDAC. Collectively, the data identified MIF and the level of circulating α_v-bearing exosomes as potential early biomarkers of LMs in this disease, with possible therapeutic implications.

The microvascular phase. Once in the liver microvasculature, cancer cells encounter diverse cell types, including liver sinusoidal endothelial cells (LSEC), KCs, and hepatic natural killer (NK) cells (pit cells; ref. 20; Fig. 1 and ref. 2). They may be rapidly eliminated through KC-mediated phagocytosis and NK cell–derived perforin and granzymes or through apoptosis induced by reactive oxygen species (ROS), nitric oxide (NO), IFNγ, and TNFα. However, cancer cells can escape these tumoricidal mechanisms by attaching to cytokine-induced endothelial CAM and transmigrating into the space of Disse if they express the corresponding counter receptors (5). This may be facilitated via neutrophil extracellular (DNA) traps (NET; ref. 21). Cancer–LSEC adhesion alters gene expression in both cell types, triggering the process of diapedesis and extravasation (reviewed in ref. 22; see Fig. 1 for depiction of the different cell types and mediators of cell–cell communication in the LME).

The preangiogenic phase. In response to proinflammatory cytokines unleashed during the microvascular stage, quiescent HepSCs in the space of Disse are activated (aHepSC) and deposit type I and IV collagen and fibronectin, providing scaffolding for endothelial cell migration, angiogenesis, and the establishment of extravascular micrometastases (23, 24). TNFα and TGFβ are major drivers of this process, and it can be accelerated by KC and neutrophil-derived matrix metalloproteinases MMP-9 and MMP-14 and neutrophil elastase that enhance tumor cell invasion into and expansion within the hepatic parenchyma (5).

The angiogenic phase. Within the liver, parenchyma metastatic cells can co-opt existing vessels to establish a blood supply. This is thought to result in a histologic growth pattern (GP) termed the "replacement GP" (6, 18, 25). Alternatively, they can trigger a process of neovascularization driven by VEGF and basic FGF (bFGF). KCs, newly recruited tumor-associated macrophages (TAM) that are polarized to the M2 phenotype in response to TGFβ and IL10, tumor-associated neutrophils (TAN) that acquire the N2 phenotype in response to TGFβ (26, 27), and aHepSCs also produce VEGF and therefore can contribute to neovascularization that is accelerated by MMPs produced by cancer and LME cells (28, 29).

The growth phase. Once cancer cells gain access to a blood supply, proliferation and expansion can ensue. However, their presence in the liver can activate specific, T-cell–mediated immune responses that may curtail metastatic expansion

Table 1. FDA-approved drugs and drug combinations for metastatic colorectal adenocarcinoma. Shown are the current standard-of-care drugs and drug combinations for metastatic colorectal cancer

FDA-approved drugs												
Generic name	**Capecitabine**	**5-FU**	**Leucovorin**	**Oxaliplatin**	**Irinotecan**	**Bevacizumab**	**Cetuximab**	**Panitumumab**	**Ramucirumab**	**Regorafenib**	**Ziv-aflibercept**	**Trifluridine + tipiracil**
Trade name	Xeloda (Genentech)	Adrucil (Teva Parenteral Medicines)	Wellcovorin (GlaxoSmithKline)	Eloxatin (Sanofi-Aventis)	Camptosar (Pfizer)	Avastin (Genentech)	Erbitux (ImClone)	Vectibix (Amgen)	Cyramza (Eli Lilly)	Stivarga (Bayer)	Zaltrap (Sanofi-Aventis)	Lonsurf (Taiho Oncology)
Class	Antimetabolite; pyrimidine analogue	Antimetabolite; pyrimidine analogue	Vitamer of folic acid	Cytotoxic	Cytotoxic	Humanized antibody	Chimeric mouse/human antibody	Human antibody	Humanized antibody	Tyrosine kinase inhibitor	Recombinant fusion protein	Cytotoxic
Target	Thymidylate synthase (prodrug of 5-FU)	Thymidylate synthase	Purine/pyrimidine synthesis	DNA cross-links	Topoisomerase 1	VEGF-A	EGFR	EGFR	VEGFR2	VEGFR2-TIE2	VEGF	Nucleoside analogue + thymidine phosphorylase
Pathway	DNA replication	DNA replication	Preserves DNA replication (in normal cells)	DNA replication	DNA replication	Angiogenesis	EGFR/MAPK signaling	EGFR/MAPK signaling	Angiogenesis	Angiogenesis	Angiogenesis	DNA replication

Drug combinations with FDA-approved drugs												
	Capecitabine	**5-FU**	**Leucovorin**	**Oxaliplatin**	**Irinotecan**	**Bevacizumab**	**Cetuximab**	**Panitumumab**	**Ramucirumab**	**Regorafenib**	**Ziv-aflibercept**	**Trifluridine + tipiracil**
FU-LV		■	■									
CAPOX (XELOX)[a]	■			■								
CAPOX + bevacizumab[a]	■					■						
FOLFOX[a]		■	■	■								
FOLFOX + bevacizumab[a]		■	■	■		■						
FOLFOX + cetuximab[a]		■	■	■			■					
FOLFOX + panitumumab[a]		■	■	■								
XELIRI	■				■							
FOLFIRI[a]		■	■		■							
FOLFIRI + bevacizumab[a]		■	■		■	■						
FOLFIRI + cetuximab[a]		■	■		■		■					
FOLFIRI + panitumumab[a]		■	■		■							
FOLFOXIRI		■	■	■	■							

[a]Recommended drug combinations according to Colon Cancer, Version 1.2017, NCCN Clinical Practice Guidelines in Oncology (15).

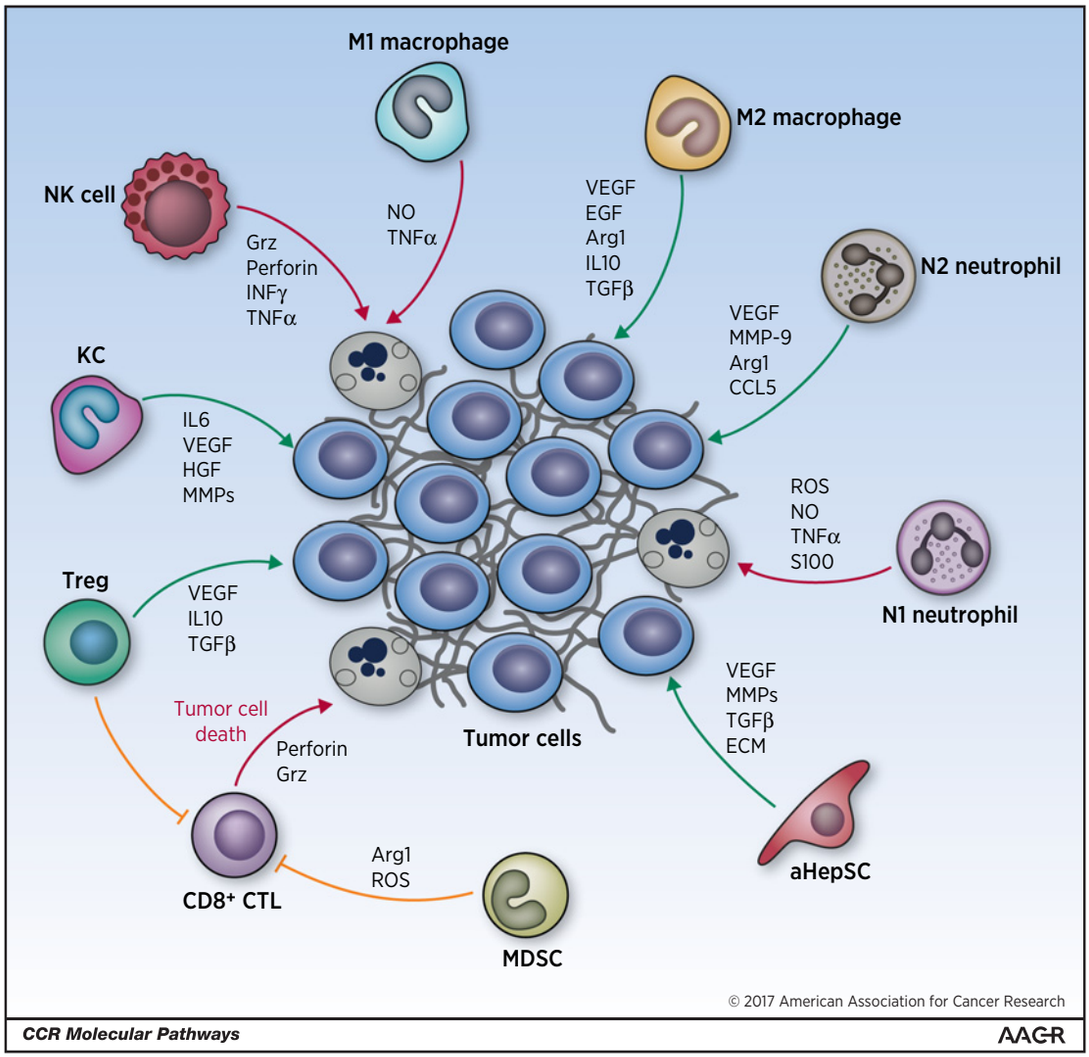

Figure 1.

Cell–cell interactions in the LME. Shown is a diagrammatic representation of the interactions between the cancer cells, the various hepatic cell types that regulate progression of metastasis, and the soluble factors mediating these interactions. Green arrows, interactions that favor metastatic expansion; red arrows, interactions that are detrimental to cancer cell growth; blunt-end yellow arrows, interactions that impede antitumor immunity. aHepSC, activated HepSC; CTL, cytotoxic T lymphocyte; Grz, granzyme.

through different cytolytic mechanisms (for review, see ref. 30). Cancer cells can evade $CD4^+$ T helper cell and $CD8^+$ cytotoxic T lymphocyte (CTL)–mediated kill via coinhibitory molecules, such as death protein 1 (PD-1) that binds ligands PD-L1 or PD-L2 on the cancer cell and the CTL-associated protein 4 (CTLA-4), resulting in the inhibition of T effector cell functions. This state of immune tolerance can be further enhanced by the recruitment of immunosuppressive lymphoid and myeloid subsets, including MDSC and regulatory T cells (Treg) to the liver. The MDSCs, a heterogeneous population of granulocytic and monocytic precursors, can be recruited by LSEC-, KC-, and/or HepSC-derived chemokines, such as CXCL1 and CXCL2 (31–33), and inhibit T-cell activation by producing arginase, ROS and CCL5, a Treg chemoattractant. Naïve T cells can also be polarized into inducible Treg (iTreg) in the presence of TGFβ and IL2 and inhibit $CD8^+$ T-cell activation through the release of TGFβ, IL10, perforin, and granzymes (34) and the upregulation of the coinhibitory receptor CTLA-4 (Figs. 1 and 2). In the TGFβ-rich tumor microenvironment (TME), TAMs and TANs can acquire immunosuppressive (M2 and N2) phenotypes (reviewed in refs. 35, 36). Adding to the protumorigenic microenvironment at this phase are growth factors, such as the type I insulin-like growth factor (IGF-I), EGF, and HGF produced by hepatocytes, M2 TAMs, and aHepSCs, respectively, that activate survival and mitogenic signaling via their respective receptors on the cancer cells (5).

Targeting the TME to impede tumor progression: Preclinical and clinical studies identify potential targets

Recent advances in cancer immunotherapy have heightened interest in targeting the TME as a strategy to prevent and treat metastatic disease, including LM (13). Targeting the

Cell type	Premetastatic niche formation	Phase I Microvascular phase	Phase II Extravascular, preangiogenic phase	Phase III Angiogenic phase	Phase IV Growth phase
KCs	**Contributions:** Produce TGFβ, S100P, S100A ***Potential therapeutic strategy:*** S100 protein inhibition	**Contributions:** Activate LSEC (TNF) CAM, kill tumor cells ***Potential therapeutic strategy:*** Stimulate tumoricidal activities with INFγ, GM-CSF, or muramyl-D.P., TNF inhibition	**Contributions:** Produce MMP-9, MMP-14 ***Potential therapeutic strategy:*** ASK1 blockade, TNFα siRNA, galectin-3 inhibition	**Contributions:** Produce VEGF ***Potential therapeutic strategy:*** VEGF inhibition, gadolinium chloride–mediated depletion, CSF-1R inhibition	**Contributions:** Produce HGF
Macrophages		**Contributions:** Produce NO, TNFα, INFγ		**Contributions:** Produce VEGF, bFGF, TGFβ ***Potential therapeutic strategy:*** Block M2 polarization with anti-TGF, anti-MARCO or anti-CCL5 Ab	**Contributions:** Produce EGF, TGFβ, IL10, arginase, indoleamine-2,3-dioxygenase (IDO) ***Potential therapeutic strategy:*** CCR2 and CCR5 blockade
Neutrophils	**Contributions:** Produce S100A8 ***Potential therapeutic strategy:*** S100 protein inhibition	**Contributions:** Produce TNFα, ROS, defensins, perforins, elastase ***Potential therapeutic strategy:*** N1 induction, DNase to eliminate NETs		**Contributions:** Produce MMP-8, MMP-9, VEGF ***Potential therapeutic strategy:*** VEGF inhibition	**Contributions:** Produce CCL5, arginase ***Potential therapeutic strategy:*** N1 induction, blocking N2 polarization with anti-TGF
MDSCs	**Contributions:** Produce S100A8 ***Potential therapeutic strategy:*** S100 protein inhibition				**Contributions:** Produce ROS, arginase, CCL5 ***Potential therapeutic strategy:*** TNFR2 blockade, anti-Gr1 Ab, VEGF and STAT3 blockade, 5-FU
Tregs				**Contributions:** Produce VEGF ***Potential therapeutic strategy:*** VEGF inhibition	**Contributions:** Produce TGFβ, IL10 ***Potential therapeutic strategy:*** TNFR2 blockade, CD25 blockade, CCR5 antagonism, CCL22 inhibition
aHepSCs	**Contributions:** Produce fibronectin ***Potential therapeutic strategy:*** Inducing HSC apoptosis via IGF-I neutralization (e.g., IGF-Trap)		**Contributions:** ECM deposition, produce MCP-1, CCL5, CCL21, TGFβ ***Potential therapeutic strategy:*** TGFβRII blockade (IQGAP1), IGF-I neutralization, VDR activation	**Contributions:** Produce VEGF; angiopoietin-1; MMP-2, -9, and -13 ***Potential therapeutic strategy:*** VEGF inhibition, IGF-I neutralization	
LSECs		**Contributions:** Produce ROS, NO, INFγ, TNFα express CAMs ***Potential therapeutic strategy:*** Inflammatory cytokine inhibition, E-selectin blockade, TNFR1 blockade		**Contributions:** Produce fibronectin, MIF, vascularize the tumor ***Potential therapeutic strategy:*** VEGF inhibition, Notch1 induction, Ang2 blockade	
Platelet		**Contributions:** Enhances cancer cell adhesion to LSEC, facilitates transmigration, evasion of NK cell surveillance ***Potential therapeutic strategy:*** ASA (COX-1 blockade)			

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Figure 2.
Stromal and immune cells of the LME and their contribution to progression of metastasis. Listed are the cells constituting the hepatic microenvironment and their tumor-promoting contributions in each phase of the metastatic process. Also listed are potential therapeutic strategies aimed at inhibiting protumorigenic stromal cell functions and inflammatory responses. Ab, antibody; aHepSC, activated HepSC; muramyl-D.P., muramyl dipeptide; Treg, regulatory T cell.

TME alone or in combination with chemotherapy and/or tumor-directed biological therapy is appealing for several reasons: (i) cancer cells depend on a supportive microenvironment for survival and growth; (ii) unlike cancer cells, the microenvironment consists of cells that are genetically stable, and their properties and responses are more predictable; and (iii) targeting the microenvironment may be beneficial across tumor types, particularly for tumors that metastasize primarily to the same secondary site, such as the liver. Indeed, antiangiogenic drugs and immunotherapeutics that overcome T-cell tolerance now form the SOC in several malignancies. Several lines of evidence, based mainly on preclinical models, provide a strong rationale for also targeting prometastatic elements of the LME.

LSECs are the first barrier to cancer cell intravasation and become a source of immune cell–recruiting cytokines upon activation by invading cancer cells. Several studies suggest that modulation of endothelial CAM expression may provide a useful strategy to prevent LM. Targeting E-selectin by antibodies or by pretreatment with C-raf antisense oligonucleotides was shown to decrease tumor cell adhesion and reduce LM of lung and colon carcinoma cells, respectively (37–39). Inhibition of VCAM-1 expression by antibody-mediated blockade of IL1β, TNFα, and IL18 also impaired the retention of cancer

cells in the liver sinusoids and reduced LM (40), and this was also seen in TNFR1-deficient mice (41). These studies identified LSEC CAM and their inducers as potential targets during the microvascular phase of LM. As discussed below, endothelial cells are also a source of blood supply for metastatic cells, and antiangiogenic drugs are already part of current SOC for mCRC.

Targeting KCs may also represent an effective strategy to preventing the growth of incipient LM, as shown when gadolinium chloride was used to deplete KCs, resulting in decreased liver tumor burden (42). This was associated with decreased VEGF production and increased iNOS expression and $CD3^+$ lymphocyte infiltration. More recently, Ries and colleagues found that a mAb (RG7155) that inhibits CSF-1 receptor (CSF-1R) activation reduced the numbers of $F4/80^+$ TAMs in animal models, and this was associated with increased $CD8^+$ to $CD4^+$ T-cell ratios. When administered into patients (NCT01494688, phase I), the antibody caused marked reductions in CSF-$1R^+CD163^+$ macrophages in tumor tissues and had antitumor effects (43). It may therefore be effective in blocking TAM recruitment into sites of LM. Another promising approach currently under study is the use of IFNγ, GM-CSF, antibodies, or muramyl dipeptide to increase the tumoricidal activities of KCs (44). This approach may be relevant as a strategy to prevent development of CRCLM if it succeeds in converting anti-inflammatory M2 macrophages to tumoricidal M1 macrophages. KC-targeting strategies are also being assessed for indications other than LM, but their success could have implications for the management of malignant disease. For example, KCs play an important role in the inflammatory processes that lead to HepSC activation and fibrosis. Ongoing phase III clinical trials STELLAR 3 and STELLAR 4 (NCT03053050 and NCT03053063) are currently assessing selonsertib, an inhibitor of the inflammatory signal transducer ASK1 expressed in macrophages and hepatocytes (45), as an antifibrotic agent in cirrhotic patients. This class of inhibitors could potentially be useful in blocking the transient fibrogenic response characteristic of the preangiogenic phase of LM (see review in ref. 44) if applied within the critical time window. However, KC inactivation by anti-inflammatory agents was also shown to accelerate collagen production in a rat model of fibrosis (46), highlighting the complexity of KC functions and the potential risks in KC targeting. Finally, the specific delivery of anti-inflammatory drugs to KC has been investigated as a strategy for blocking KC-driven inflammation. For instance, oral delivery of nanoparticles carrying TNFα siRNA was shown to inhibit TNFα production in macrophages *in vivo*, protecting mice from LPS/d-GalN–induced hepatic injury and lethality (47). Similarly, galectin-3 inhibitors were used to limit KC-mediated inflammation and shown to resolve cirrhosis and portal inflammation in experimental models (48, 49). The utility of these approaches in the context of LM prevention has not been assessed.

As discussed, TGFβ is a major driver of the immunosuppressive and fibrogenic microenvironments essential for the angiogenic and growth phases of LM (reviewed in refs. 50, 51). Several phase II clinical trials based on targeting the TGFβ axis are, in fact, currently in progress. For example, the NCT01373164 trial (phase I/II, completed) evaluated the effect of galunisertib, an inhibitor of the TGFβ receptor I kinase, with a favorable toxicity profile in humans (52) in combination with gemcitabine on the OS of patients with unresectable, metastatic PDAC. In the NCT02423343 trial (phase I/II, recruiting), the effect of this inhibitor is being evaluated in combination with the PD-1 inhibitor nivolumab in patients with recurrent hepatocellular carcinoma. Blockade of TGFβR signaling could render the LME less favorable to metastatic expansion by altering key elements in the prometastatic niche. For example, it could inhibit the polarization of tumoricidal M1 TAMs to the M2 phenotype. In addition, TGFβ signaling blockade can also increase the cytotoxic activities of $CD11b^+Ly6G^+$ TANs by increasing expression of the proinflammatory cytokines TNFα, IFNγ, IL12, and CCL5. This was shown in three different mice strains, two different tumor types (non–small cell lung cancer and mesothelioma), and in both flank and orthotopic models of lung adenocarcinoma (26). Moreover, blockade of TGFβRII signaling can also prevent HepSC activation, thereby disrupting the angiogenic phase of LM, as was recently shown in a murine colorectal cancer model and confirmed in surgical specimens (53). Vitamin D receptor (VDR)-conveyed signals were also implicated in blockade of TGFβ-mediated HepSC activation. Calcipotriol, a low-calcemic analogue of calcitriol and agonist of VDR, was shown to restrict the fibrogenic response of aHepSC by reducing SMAD3 occupancy at profibrotic target genes via chromatin remodeling (54). Although validated in the context of experimental fibrosis, these and other inhibitors that target the process of HepSC activation (55–58) could potentially have beneficial antimetastatic effects by blocking early events in LM, and their evaluation in this context is therefore warranted.

TGFβ blockade can also potentially inhibit $CD4^+$ T-cell differentiation into Tregs. Several other Treg-targeting strategies are currently in development, including the use of daclizumab, a CD25-neutralizing antibody, and the blockade of CCL22. These strategies recently showed promise in preclinical models and in breast cancer clinical trials (59–61) but have not yet been tested in LM models. Clinical trials with combination checkpoint inhibitors tremelimumab (anti–CTLA-4) and MEDI4736 (anti–PD-L1) have recently been initiated for patients with resectable CRCLM, and are aimed at reactivating immunosurveillance in the TME (NCT02754856, phase I, recruiting). However, the benefit of immunotherapy, either as monotherapy or in combination with other modalities for mCRC, remains to be confirmed.

MDSCs are another potential target for immune modulation. Recently, we have shown that MDSC and Treg recruitment into CRCLM was TNFR2 dependent and that treatment of tumor-bearing mice with TNFR2-targeting antisense oligonucleotide significantly reduced experimental LM (62). Other potential strategies include (i) induction of MDSC differentiation into mature, nonimmunosuppressive myeloid cells; (ii) prevention of their expansion from bone marrow precursors; and (iii) impairment of their accumulation or function. STAT3 (63) and VEGF inhibitors (64), chemotherapeutic drugs (e.g., 5-FU and gemcitabine), and chemokine receptor antagonists have already been shown to reduce the accumulation of $CD11b^+GR\text{-}1^+$ cells in peripheral immune organs and the tumor stroma (extensively reviewed in ref. 64; see Fig. 2), although their specific effects on MDSC recruitment to LM remain to be verified.

The case for targeting the prometastatic niche for therapeutic management of hepatic metastases

Our increased understanding of the biological mediators of the four phases of LM suggests multiple opportunities to disrupt both incipient and established disease through a variety of therapeutic strategies. In fact, there is already proof of principle, albeit unappreciated, for the utility of this concept because an effective "chemoprevention" strategy for CRCLM already exists, namely the use of low-dose aspirin. Aspirin (ASA), a negative regulator of prostaglandin E2 signaling via its inhibitory effects on the COX1 and COX2 enzymes has now been shown to significantly reduce CRCLM in several epidemiologic studies (66). Although not completely understood, it is believed that the mechanism of action (MOA) relates to the effect of ASA on COX1 signaling in platelets (67). In the microvascular phase of LM, platelets may promote metastases by enhancing cancer cell adhesion to endothelial cells and leukocytes, thereby facilitating transmigration (66). Platelets may also aid in the evasion of NK cell surveillance. Given the demonstrated positive effects of ASA, significant efforts should be directed to more clearly decipher the underlying MOA, so that additional agents that target the same mechanism(s) can be developed and optimized.

As discussed above, numerous agents targeting the angiogenic phase of metastasis have already been approved for colorectal cancer, including bevazucimab, ziv-aflibercept, and regorafenib, each with a distinct MOA against VEGF-mediated signaling. Additional agents targeting VEGF and other proangiogenic molecules are presently in clinical trials (e.g., NCT02350530, NCT00055692, and NCT00767468). For example, the NCT00055692 trial (phase II, completed) sought to determine the biological effects of bevacizumab in unresectable hepatocellular carcinoma. Although significant clinical and biologic activity was observed in the treated arm, grade 3 or higher hemorrhages occurred in 11% of patients. The MOA of these agents remains incompletely understood, however, and thus there is ample opportunity for optimization of therapies directed at this phase of the metastatic cascade.

Chemokine and growth factor receptors are among the most interesting putative targets within the microenvironment because they are "druggable" by small molecule and antibody-based strategies, and compelling preclinical data exists supporting their utility as targets for treatment of LM. Furthermore, chemokines have been shown to influence numerous phases of LM, particularly the angiogenic and growth phases. CCL5 is produced by colorectal cancer cells and by T cells at the margin of CRCLM (68). CCL5/CCR5 signaling has pleiotropic effects, including recruitment of monocytes and M2 polarization, promoting the expansion of cancer-associated fibroblasts and enhancing TGFβ-mediated killing of $CD8^+$ T cells by Tregs (68, 69). A recent phase I trial with a CCR5 antagonist demonstrated activity against advanced refractory CRCLM, identifying it as a target worthy of further clinical investigation (68). Although no CCR5-targeting drugs are currently approved for the management of liver diseases, repositioning the clinically approved CCR5 antagonist maraviroc, used against CCR5-tropic HIV strains, may be of clinical interest in this context. In addition, a recent report demonstrated that the growth factor IGF-1 participates in recruitment and activation of HepSC to enhance the growth of CRCLM (70). IGF-1 was shown to prevent apoptosis in HepSC exposed to TNFα. Importantly, stromal cells from resected CRCLM expressed activated IGF-IR, and an IGF-Trap markedly reduced IGF-IR activation in HepSC in a murine model of mCRC (70). Together, these data identify IGF signaling as another rational target within the LME.

Although compelling evidence exists that the immune response to primary colorectal cancer correlates with patient prognosis, until recently, there was little clinical evidence to suggest that the immune cell infiltrate within the metastatic niche was of similar clinical impact. A recent study examined gene expression profiles in CRCLM resections from 96 patients (71). Genes involved in T-cell proliferation were significant predictors of OS, whereas genes involved in T-cell proliferation and activation were predictive of relapse-free survival. Analysis of an independent set of tumors by IHC validated these findings, showing that an increased lymphocytic infiltrate and increased expression of the TNFSF14/LIGHT protein were associated with improved OS and relapse-free survival. Another recent report demonstrated that MDSCs expand within the LME of CRCLM and can inhibit responses to CAR T-cell therapy (72). These findings demonstrate that the immune cell infiltrate within the LME may be highly relevant to patient outcomes and manipulating these responses may be of therapeutic benefit.

Although collectively, these studies suggest that LME targeting holds promise as a therapeutic strategy, this approach is not without its challenges. For example, targeting HepSC activation could inhibit metastatic expansion by reducing ECM deposition (53). However, HepSC-derived angiogenesis and ECM remodeling are essential to the liver response to injury (73), and this approach may therefore have deleterious effects in patients undergoing hepatic resections. Our recent understanding of the processes governing immunosuppression in the TME has greatly increased interest in targeting immune cell polarization to improve tumor cell surveillance and clearance. However, as alluded to earlier, both proinflammatory and anti-inflammatory factors contribute to liver colonization by cancer cells. Given that the process of LM is dynamic and the different phases may temporally overlap, the window of opportunity for administrating pro- or anti-inflammatory agents may be limited and difficult to define. TGF axis targeting is also problematic because of its central physiologic role in wound healing and tissue repair. Moreover, limiting downstream effects of TGFβ may potentially contribute to metastatic progression because TGFβ can also have potent tumor cytostatic effects (74). For example, TGFβR signaling was shown to induce the expression of cyclin-dependent kinase (CDK) inhibitors, arresting cell-cycle progression at the G_1 phase (75). The SMAD2/3–SMAD4 complex was also shown to upregulate SH2 domain–containing inositol-5-phosphatase (SHIP) expression, an inhibitor of AKT (76). Further studies are therefore crucial to determine whether the immunomodulatory effects of TGF axis blockers can override their potential protumorigenic activities.

Finally, the use of angiogenesis inhibitors, such as bevacizumab, may not benefit all patients. This was documented in a recent study showing that CRCLMs with a replacement GP resulting from vessel co-option are resistant and respond poorly to antiangiogenic therapy (77). Patient stratification based on the histologic GP of their LMs may therefore be essential to optimize the benefit from antiangiogenic therapies (18, 78). However, at present, biomarkers to predict either the

vascular response or the type of immune microenvironment engendered by individual LM are lacking, limiting the potential to personalize the clinical management of liver metastatic disease.

Conclusions

The LME consists of a diverse group of cells that are co-opted by cancer cells to enable the establishment and growth of metastases. These varying cell types, along with the cytokines/chemokines and growth factors they secrete, represent putative targets to prevent and treat LM. An increased understanding of drivers of the four phases of LM should improve our ability to rationally select and combine therapeutic approaches for clinical investigation. The demonstrated importance of immune modulation during the evolution of LM provides particularly attractive therapeutic opportunities given the recent revelations regarding the power of immunotherapy in different malignancies. It is now recognized that the dominant cytokines and chemokines that modulate immune function within a particular TME differ between tumor types and (we hypothesize) may even differ between patients afflicted by the same cancer (79). Thus, we propose that in the future, it will be optimal to develop personalized panels of immune biomarkers from a patient's tumor to understand the dominant signals driving local immunosuppression and how combination therapies might best engender an antitumor immune response. Targeting the LME will no doubt present new and unique challenges, including the probability of unforeseen toxicities. In addition, the identification of these numerous putative targets and accompanying therapeutic agents engenders a new set of challenges, namely how to efficiently move this vast array of agents through clinical trials. This requires more widespread integration of biomarkers and adaptive trials that utilize accumulating outcome data to rapidly discard less active agents and rapidly integrate new treatment arms (81). Finally, studying these novel approaches earlier in patients' disease course, rather than relegating the study of new agents to second, third, and fourth lines may be an important step toward subverting issues of intra- and intertumoral heterogeneity and drug resistance.

Disclosure of Potential Conflicts of Interest

J.K. Sicklick reports receiving commercial research grants from Foundation Medicine, Inc. and Novartis and is a consultant/advisory board member for Biotheranostics. A.M. Lowy reports receiving commercial research grants from Syros and Tanabe and is a consultant/advisory board member for Halozyme. No potential conflicts of interest were disclosed by the other authors.

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