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Melanoma and the Tumor Microenvironment

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Abstract

Melanoma arises through the accrual of mutations in genes critical for proliferation and survival. Although melanoma had been traditionally conceptualized as a cell-autonomous event, increasing evidence supports the notion that these tumors are not isolated entities but rather depend, interact with, and react to the adjacent microenvironment. Melanoma is composed of not only the malignant cells but also the supporting stroma, which includes fibroblasts, endothelial cells, immune cells, soluble molecules, and the extracellular matrix (ECM). Tumor cells actively interact with the microenvironment in a bidirectional manner through molecular signals that modulate the malignant phenotype. This article briefly reviews the molecular basis of melanomagenesis as well as the interplay of melanoma with other cells of the tumor microenvironment and components of the ECM. It also discusses the influence of the microenvironment on therapeutic targeting of melanoma, highlighting recent studies that propose novel strategies to target tumor–microenvironment interactions.

Introduction

Malignant melanoma constitutes the most rapidly increasing cancer in the United States and its incidence continues to rise worldwide. Melanoma accounts for 4% of all diagnosed forms of skin cancers, but it is responsible for more than 70% of deaths from such cancers. The American Cancer Society estimates that in 2008, close to 60,000 new cases of melanoma will be diagnosed and approximately 8000 patients will die of metastatic disease. The risk factors are both genetic and environmental; the major environmental risk factor is intermittent sun exposure. While research has led to significant progress in the understanding of the biology and genetics of melanoma, no effective treatment is currently available. The best management continues to be early detection and surgical resection; metastatic disease is highly refractory to treatment and 5-year survival rate remains at a dismal 15%. The only US Food and Drug Administration (FDA)-approved chemotherapeutic agent for metastatic melanoma is dacarbazine (DTIC-Dome; Bayer Corp., West Haven, CT), an alkylating agent associated with temporary objective response rates below 10% according to the most recent multicenter trials [1•].

Since the recent discovery of activating BRAF mutations in a large subset of melanomas, special interest in studying the BRAF/mitogen-activated protein kinase (MAPK) pathway

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and developing targeted therapies has emerged. While many BRAF and MEK inhibitors have been developed and have shown promise in preclinical settings, to date they have been disappointing in the clinical setting. Many factors may contribute to this apparent lack of success. Melanoma is a complex and heterogeneous disease; a tumor may be composed of distinct melanoma cell populations with distinct molecular profiles and responses to treatment. It is also important to note that most basic research is performed in vitro by growing isolated melanoma cells in monocultures on plastic culture dishes, under conditions that do not accurately reflect the appropriate tumor microenvironment. New technologies such as three-dimensional organotypic cultures and collagen-implanted spheroids, aimed at modeling the three-dimensional tumor microenvironment, offer an opportunity for the in vitro evaluation of drug response under conditions that may more closely mirror the in vivo behavior of melanoma [2].

The Origin of Melanoma: An Interplay Between Genes and the Environment

The development of melanoma is a multistep process involving the interaction of environmental, genetic, and host factors. Melanoma originates in the melanocytes, which are embryologically derived from the neural crest. Under normal tissue homeostasis, melanocytes in the skin dwell on the basement membrane and in the hair follicles in close contact with keratinocytes that direct their behavior and growth through an intricate system of growth factors and cell adhesion molecules [3]. Melanocytes transform into melanoma in a stepwise manner. The process starts with an initial phase of proliferation leading to the development of benign nevi, representing the earliest hyperplastic melanocytic lesion, followed by aberrant growth and dysplasia. Dysplastic nevi display atypia with enlarged, hyperchromatic nuclei. The first certain malignant stage is the radial growth phase (RGP), in which tumor cells attain the ability to proliferate intraepidermally. In the vertical growth phase (VGP), tumor cells acquire the ability to grow vertically and invade the dermis and subcutaneous tissue. In the final step, tumor cells acquire the ability to metastasize to distant organs, representing the most advanced stage in tumorigenesis.

The molecular events leading to transformation of normal melanocytes into melanoma are not fully understood, although mutations in genes critical for growth and survival have been associated with initiation and progression of melanoma. Mutations in the protein kinase BRAF are by far the most common, being found in 40% to 70% of melanoma patients. The most frequent mutation in BRAF results from a glutamic acid for valine substitution at position 600 (V600E) within the kinase domain of the protein, leading to constitutive activation of the MAPK pathway and stimulation of growth, survival, and angiogenesis. The MAPK pathway can also be activated by mutations in N-Ras or persistent activation of growth factor receptors.

The phosphatidylinositol 3′ kinase (PI3K) pathway also appears to have a major role in melanoma progression [4]. PI3K is generally activated through loss or silencing of the tumor suppressor PTEN or through amplification of AKT. Genes implicated in cell cycle progression such as cyclin D1, the cyclin-dependent kinases cdk4/cdk6, and cdk2 are commonly amplified in sporadic melanoma, whereas deletion of the cyclin-dependent kinase inhibitor 2a (CDKN2A or p16INK4a) is frequently associated with familial melanoma.

Although these genetic alterations are required for melanomagenesis, they are clearly not sufficient. Microenvironmental factors can influence the outcome of the transformation process, either favoring or preventing melanomagenesis.

Within the skin, keratinocytes exert tight control of melanocyte proliferation through paracrine growth factors and intercellular communication via cell adhesion molecules. Deregulated proliferation occurs when melanocytes escape the control imposed by keratinocytes through downregulation of cell adhesion molecules such as E-cadherin, P-cadherin, desmoglein, and connexins [3,5]. Cadherins are a family of transmembrane proteins that promote calcium-dependent cell–cell adhesion. The extracellular domain of cadherins mediates homotypic interactions with similar cadherins in adjacent cells. The cytoplasmic domain of E-cadherin links to the cytoskeleton through interactions with cytoplasmic protein complexes that include β-catenin.

Melanoma cells escape keratinocyte control by downregulation of E-cadherin and upregulation of N-cadherin. This cadherin switch allows melanoma cells to interact with other N-cadherin–expressing cells such as fibroblasts and endothelial cells. Cell adhesion through N-cadherin also allows for increased motility due to less stringent interactions among cells. In addition, N-cadherin can confer survival advantages through repression of proapoptotic factors (eg, Bad) in a PI3K-dependent manner [6]. Overexpression of E-cadherin in melanoma cells restores keratinocyte control over proliferation and precludes invasion. While cell–cell adhesion contributes to keratinocyte-mediated control of proliferation and tumor suppression, E-cadherin can also prevent tumor progression by downregulating β-catenin–mediated signaling [7,8], as β-catenin promotes proliferation by inducing the transcription of growth-regulatory and survival genes such as c-Myc, cyclin D1, and MITF [9,10]. Recently, Delmas et al. [11] showed that β-catenin immortalizes primary skin melanocytes by silencing the p16Ink4a promoter and cooperating with activated N-Ras to promote melanoma in mice.

E-cadherin can be downregulated by several mechanisms. The repressors Slug and Snail inhibit E-cadherin expression at the transcriptional level in melanoma cells [12–14]. During melanoma progression, the autocrine secretion of hepatocyte growth factor/stem cell factor also promotes downregulation of E-cadherin and desmoglein I [15]. Additionally, Smit et al. [16] showed that enforced expression of the matricellular protein osteonectin led to E-cadherin downregulation, upregulation of osteopontin, and increased phosphorylation of focal adhesion kinase ($p125^{FAK}$). Conversely, small interfering (si) RNA–mediated inhibition of osteonectin in highly invasive melanoma cells overexpressing the matricellular protein resulted in upregulation of E-cadherin and decreased invasive potential.

Concurrently with E-cadherin loss and progression of melanoma to an invasive phenotype, alterations in integrin expression allow melanoma cells to dissociate from the primary site, alter the cytoskeleton, migrate through the contiguous stroma, and eventually disseminate through lymphatic or vascular vessels to distant organs. Integrins are heterodimeric transmembrane proteins that not only mediate cell adhesion to the extracellular matrix (ECM) but also play important roles in proliferation, migration, invasion, angiogenesis, and survival [17•]. There is increasing evidence of aberrant expression of integrins in many types

of cancer; thus, these ECM adhesion receptors constitute an appealing therapeutic target for several malignancies, including melanoma. Integrins are heterodimers composed of two subunits: α and β. The extracellular domain of integrins mediates cell–ECM interactions, whereas the intracellular domain links to the actin cytoskeleton and intracellular proteins. Ligand binding to the extracellular domain induces conformational changes and integrin clustering, leading to the activation of signaling cascades through recruitment of protein complexes to focal adhesions (outside-in signaling). Integrins lack kinase activity and hence depend on intracellular kinases and adaptor molecules, such as integrin-linked kinase (ILK), $p125^{FAK}$, small Rho-GTPases, Src-family kinases (SFKs), talin, paxillin, Crk-DOCK180, and p130Cas, to exert their functions. Through these interactions, integrins activate intracellular signaling cascades such as MAPK, PI3K, and nuclear factor kappa B (NF-κB). Conversely, intracellular signaling molecules can also induce conformational changes in the integrin heterodimers, leading to altered affinity for ECM ligands (inside-out signaling) and contributing to the dynamic interaction of tumor cells with their microenvironment.

The protein tyrosine kinase FAK, a central player of integrin-mediated signaling, is a key mediator of melanoma invasion, migration, and vasculogenic mimicry. Indeed FAK phosphorylation appears to correlate with a more invasive phenotype in cell lines and patient tumors. Interestingly, inhibition of FAK signaling decreases melanoma cell invasion, migration, and vasculogenic mimicry in vitro [18]. Integrins can also modulate signaling through growth factor receptors such as epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), and platelet-derived growth factor receptor (PDGFR) by acting as coreceptors through direct association with growth factor receptors or by indirectly modulating their function. Integrins, for example, cooperate with growth factor receptors to sustain MAPK activation into mid-G1 when it is required for cyclin D1 expression, downregulation of cyclin-dependent kinase inhibitors, and cell cycle progression [19]. Modulation of growth factor signaling by integrins can also lead to changes in the activity of survival factors such as bcl-2, bax, and p53 [20]. Transition from RGP to VGP in melanoma is associated with increased expression of $\alpha_v\beta_3$ integrin, leading to increased production of the antiapoptotic factor bcl-2 and matrix metalloproteinase-2 (MMP-2), an endopeptidase that degrades collagen in the basement membrane [21–23].

It has been postulated that integrins may act as biosensors, regulating cell behavior in response to signals from the microenvironment. This is especially important when tumor cells grow within a constraining stroma and during the process of invasion and metastasis. Several studies suggest that pressure or shear forces can activate endogenous intracellular pathways such as FAK, Src, and the adaptor protein paxillin, leading to changes in the binding affinity and adhesive properties of matrix receptors, thus allowing the tumor cells to strive in new microenvironmental conditions [24–26].

The Role of the Tumor Stroma in Melanoma

It has long been recognized that the tumor microenvironment is an active participant in tumorigenesis. In 1889, Paget hypothesized that if the environment where secondary tumors arise were passive, metastasis would be an arbitrary event. Instead, he noticed that some environments were more receptive for growth and establishment of secondary tumors or

metastasis, leading to the proposal of the “seed and soil” theory. More than 30 years ago, Hart and Fidler [27] demonstrated that when B16 melanoma cells were injected into mice, tumor growths developed preferentially in the lungs and in grafts of pulmonary or ovarian tissue implanted either subcutaneously or intramuscularly, but not in renal tissue. Their studies led to the conclusion that metastasis and development of secondary tumors is dependent on both tumor cell properties and host factors and not merely a random process.

The tumor stroma is complex and includes the ECM, growth factors and cytokines, the microvasculature, infiltrating inflammatory cells, and fibroblasts [28]. The composition of the stroma varies from tumor to tumor. In cutaneous melanoma, the stroma appears desmoplastic (fibroblasts and fibrocytes with extensive accumulation of fibrillar ECM components) or myxoid (atypical spindle cells with major proteoglycan accumulation) [28]. In the dermis, the mayor ECM component is collagen I, which is predominantly synthesized by fibroblasts. The papillary layer of the dermis contains a thin arrangement of vertical collagen fibers whereas the reticular layer is made of thick collagen fibers that are arranged parallel to the skin surface. Processes involved in the stromal reaction in melanoma include proteolysis of collagen and elastin at the tumor’s invasive edge as well as infiltration of lymphocytes and variable angiogenesis [29].

Melanoma cells actively interact with their microenvironment, not only through direct cell–cell and cell–matrix interactions but also through secreted growth factors and cytokines. During the initial invasive stages, melanoma cells need to activate a mechanism that allows them to migrate, invade, and survive outside their original niche under new microenvironmental conditions and successfully establish residence in a new location. During tumor progression, melanomas activate growth factor loops that regulate cell adhesive properties favoring survival under otherwise nonfavorable environmental conditions.

Secretion of basic fibroblast growth factor (bFGF) promotes proliferation and survival of melanoma in an autocrine manner. While almost all melanomas produce and secrete bFGF, normal human primary melanocytes require exogenous bFGF for growth and survival. In fact, secretion of bFGF appears to correlate with tumor progression. We have previously demonstrated that over-expression of bFGF in human melanocytes enhances proliferation and anchorage-independent growth [30]. Moreover, melanocytes overexpressing bFGF can grow and proliferate in the absence of insulin or insulin-like growth factor (IGF-1) and melanocortin-stimulating hormone. Importantly, melanoma-secreted bFGF stimulates proliferation of stromal cells in a paracrine manner [31].

The EGFR is a membrane-spanning glycoprotein involved in cell proliferation, growth, migration, invasion, and survival. Although evidence for EGFR contribution in melanoma biology is limited, selective expression of EGFR in advanced melanomas is associated with chromosome 7 loss [32]. Recently, Bardeesy et al. [33] demonstrated the existence of an epidermal growth factor signaling loop essential for H-RASV12G–mediated tumorigenesis using an inducible transgenic mouse model.

Melanoma-secreted growth factors such as bFGF, PDGF, and transforming growth factor (TGF)-β also exercise paracrine functions in angiogenesis and stroma formation by inducing proliferation and activation of fibroblasts and endothelial cells. Besides the mitogenic effect of PDGF, this melanoma-derived growth factor stimulates neighboring fibroblasts to produce ECM proteins such as collagen, fibronectin, and laminin [34]. Tumor-associated fibroblasts play a major role in promoting and sustaining tumorigenesis. In melanoma, tumor-associated fibroblasts actively participate in ECM production and the secretion of paracrine growth factors such as bFGF, IGF-1, and TGF-β into the tumor microenvironment. Fibroblasts can also differentiate into pericytes, promoting vasculature formation and influencing angiogenesis by interacting with endothelial cells. Stromal fibroblasts are recruited from either the local tissue environment or circulating mesenchymal precursors/stem cells derived from the bone marrow. Evidence suggests that once recruited to the tumor site, tumor-associated fibroblasts are activated by the tumor cells and start expressing myofibroblastic markers such as α-smooth muscle actin [35].

Melanoma and stromal cells carry on a continuous cross-talk. Indeed, melanoma-secreted PDGF stimulates fibroblasts to produce and secrete IGF-1, which then stimulates proliferation of melanoma cells in a paracrine fashion. Activated fibroblasts also release bFGF and endothelin, thus promoting melanoma growth [28]. This reciprocal activation between melanoma cells and stromal fibroblasts is a paradigm of how tumor cells and the microenvironment maintain a continuous and bidirectional communication. Furthermore, tumor-associated fibroblasts can play a dual role in tumorigenesis: in early melanoma lesions, fibroblasts appear to repress growth of RGP cells, whereas at later stages of tumorigenesis, tumor-associated fibroblasts enhance proliferation of metastatic cells [34].

Melanomas also produce and secrete TGF-β. TGF-β signaling plays a major role in tumorigenesis and metastasis, exerting either direct or indirect effects on the tumor cell itself or the tumor microenvironment [36••]. Melanoma-derived TGF-β has a growth inhibitory effect on epithelial cells and melanocytes, but melanoma cells themselves are resistant to these inhibitory effects. Some studies have suggested that resistance to TGF-β–mediated growth inhibition in melanoma is accomplished by expression of repressors of TGF-β/SMAD signaling such as Ski and Sno, endoglin, filamin, and follistatin; however, the specific mechanism of resistance is not yet fully understood (reviewed in Hussein [37]). The paracrine secretion of TGF-β promotes increased deposition of ECM, angiogenesis, survival, immunosuppression, and transition to more aggressive phenotypes. TGF-β may also influence melanomagenesis; melanoma cells activate fibroblasts to produce stromal components—such as collagen and tenascin—that are essential for tumor survival and metastatic potential [38].

The development of neovasculature or angiogenesis is critical for guaranteeing the supply of oxygen and nutrients to rapidly growing tumors and allowing dissemination of tumor cells to secondary sites [39•]. Tumor vasculature is derived from sprouting of local vessels (angiogenesis) and from bone marrow–derived circulating cells (vasculogenesis). Melanomas have a proclivity to metastasize via the lymphatic vasculature to distant organs such as the lung, liver, bones, and brain. Angiogenesis in melanoma is stimulated by autocrine and paracrine growth factors such as VEGF, bFGF, PDGF, and TGF-α and TGF-β.

During melanoma progression, tumor cells secrete increased levels of VEGF and bFGF. VEGF is induced by hypoxia, acidic pH, hypoglycemia, among other factors. Hypoxia and increased VEGF levels enhance expression of the tyrosine kinase receptors VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1) on the endothelial and melanoma cells. Moreover, significant amounts of VEGF and bFGF are associated with the ECM; secretion of matrix metalloproteinases (MMPs) by melanomas, tumor-associated fibroblasts, or endothelial cells induces release of these angiogenic factors and promotes endothelial cell proliferation and vasculogenesis [40•].

Placental growth factor (PlGF) is a member of the VEGF family produced by melanoma cells. PlGFs bind to neuropilin-1 and -2 receptors on endothelial cells and exert their angiogenic action by synergizing with VEGF. Furthermore, levels of PlGF correlate with tumor progression and patient prognosis. Recently, Fischer et al. [41•] showed that a monoclonal antibody against PlGF blocks tumor angiogenesis, growth, and metastasis without affecting normal tissues, suggesting that PlGF could be a potential target for the development of novel anticancer therapies.

Additionally, endothelial cells secrete interleukin-8 (IL-8), which stimulates melanoma and endothelial cell migration and modulates vascular permeability [40•]. TGF-β1, ultraviolet B light (UVB; 290–320 nm), and hypoxia can induce expression of IL-8 in human melanoma cells increasing metastatic potential. Clinical studies suggest that increased levels of IL-8 in serum from melanoma patients correlates with advanced disease and overall patient survival [42].

In addition to proangiogenic growth factors, integrins can directly or indirectly modulate the effects of proangiogenic or antiangiogenic factors and thus regulate endothelial cell behavior. In particular, $\alpha_v\beta_3$ and its ligands vitronectin and osteopontin can modulate VEGF and bFGF-induced angiogenesis [43].

MMPs are specialized proteolytic enzymes that play crucial roles in tissue remodeling both during normal development and disease. MMPs promote melanoma progression by modulating intracellular signaling and by promoting tumor growth, survival, angiogenesis, metastasis, and genomic instability. Melanoma cells express a number of MMPs, including MMP-1, -2, -9, -13, and -14, as well as inhibitors of MMPs such as TIMP-1, -2, and -3, which regulate tumor growth, metastasis, and angiogenesis [40•,44]. Stromal cells also produce and secrete MMPs in large quantities, further underscoring the role of the microenvironment in tumor progression.

Targeting the Melanoma Microenvironment: New Opportunities for Therapeutic Intervention

Increasing evidence supports the argument that the tumor microenvironment plays a major role in all phases of tumorigenesis including initiation, progression, maintenance, and metastasis and may also influence the outcome of therapy in several cancers including melanoma. Therefore, understanding the contribution of each component of the tumor microenvironment to melanomagenesis may open new avenues for therapeutic intervention.

A particularly attractive approach is the use of multitargeted strategies aimed at disrupting both melanoma cells and components of the tumor microenvironment.

Interfering with matrix remodeling

Several studies have shown that MMP expression correlates with malignant transformation, metastasis, and decreased patient survival, thus making MMPs attractive therapeutic targets in melanoma (reviewed in Mahabeleshwar and Byzova [40•]). Whereas a large number of broad-spectrum matrix remodeling inhibitors have been developed over the past decade, MMP inhibitors such as marimastat (BB-2516; British Biotech, Oxford, England and Schering-Plough, Madison, NJ), and its analogue batimastat (BB-94) have not met expectations in clinical trials when used as single agents [45]. The apparent clinical failure of MMP inhibitors may be in part due to the fact that MMPs are also required to release antiangiogenic factors such as endostatin and angiostatin. In these trials, MMP inhibitors were tested in patients with advanced disease, although preclinical studies had consistently indicated that they were effective only against early stages of cancer [46•]. These MMP inhibitors resulted in considerable toxicity, thus requiring administration of reduced doses that were not sufficient for target inhibition [47]. Moreover, patients were not pre-screened for upregulated MMP expression and neither pre- nor posttreatment tissue samples were collected to determine efficacy of the MMP inhibitors. In addition to MMP inhibitors, inhibitors of other proteases such as cathepsins and urokinase-type plasminogen activator (uPA) are currently under investigation [48•], although none of these has entered clinical trials for melanoma. Considering that cathepsins B, K, and L as well as uPA and its receptor uPAR, appear to be upregulated in melanoma and stromal cells and to contribute to cell invasion, further investigation into the role of these proteases in melanomagenesis is warranted [49,50].

Targeting tumor angiogenesis

Angiogenesis and lymphangiogenesis play a central role in melanoma progression; thus, endothelial cells as well as growth and angiogenic factors and their receptors constitute attractive targets for therapeutic intervention. Development of antiangiogenic agents may be a complex enterprise. On one side, tumor vessels are relatively genetically stable, and less prone to accrue mutations allowing them to develop drug resistance in the short term. On the other side, tumors can develop resistance to antiangiogenic agents by upregulating other factors (angiogenic rescue); therefore, the use of broad-spectrum antiangiogenic agents may be beneficial. Angiogenic inhibitors with a broad spectrum of anticancer activity and few side effects have been developed (Table 1). Interference with the tumor vasculature can be achieved through different strategies including direct inhibition of proangiogenic factors (eg, bevacizumab, a humanized anti–VEGF-A monoclonal antibody) and their receptors (eg, BAY 43-9006, a multikinase inhibitor that targets VEGFR).

Inhibition of matrix remodeling through protease inhibitors is also expected to have a significant effect on vasculogenesis. Other targets for antiangiogenic therapy include components of the ECM and their receptors and inhibitors of growth factors that promote endothelial cell proliferation. Some of these compounds (Table 1) have shown significant antiangiogenic activity in preclinical models and offer a novel approach for the treatment of

melanoma, particularly if used in combination with other antitumor agents [40•]. Clinical studies in patients with metastatic melanoma using antiangiogenic drugs as single agents or in combination are listed in Table 1 and Table 2, respectively.

Conclusions

Although melanoma is sometimes regarded as a cell-autonomous process that results only from genetic and epigenetic alterations within the transformed cells, many steps in the transformation process (proliferation, invasion, angiogenesis, and metastasis) are modulated by microenvironmental factors such as growth factors and proteolytic enzymes produced by stromal cells. It is the dynamic interaction between tumor and stromal cells that determines the outcome of the transformation process. In addition, stromal cells take part in immune evasion mechanisms of cancer. Considering the influence of the stromal cells and the components of the tumor microenvironment in melanomagenesis, a clear understanding of the role of each component is necessary. Tumor stromal cells and their products are promising targets for cancer therapy. Several strategies to target tumor–stroma interactions are currently under active investigation. Approaches targeting several pathways in the tumor itself and the microenvironment that support melanoma growth and invasion would be more likely to control tumor expansion and dissemination, which we hope will lead to the development of effective clinical treatments.

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Table 1

Single agents targeting different components of the melanoma tumor microenvironment in clinical trials

Target	Compound	Mechanism	Phase	Comments
Vasculature	AG-013736	VEGFR and PDGFR inhibitor	2	Starting dose 5 mg twice daily ± 20% according to toxicity
	ABT-510	Synthetic analogue of thrombospondin-1/ angiogenesis inhibitor	2	100 mg twice daily in patients with metastatic melanoma did not demonstrate definite clinical efficacy
	AZD2171	ATP-competitive inhibitor of VEGFR family	2	May also inhibit Kit and (less potently) PDGFR-A and PDGFR-B
	VEGF Trap (aflibercept)	Fusion protein that binds VEGF-A and placental growth factor	2	Effectively suppresses tumor growth and vascularization in vivo
	Bevacizumab	Anti-VEGF humanized monoclonal antibody binds to VEGF and inhibits VEGF receptor binding	1/2	Treatment with intravitreal bevacizumab for large uveal melanomas; efficacy of bevacizumab monotherapy being tested in trial in Norway
	Vorinostat (FR901228, romidepsin)	Histone deacetylase inhibitor	2	Proapoptotic in preclinical studies; blocks hypoxia-induced angiogenesis and depletes Hsp90-dependent oncoproteins
Integrins	Volociximab (M200)	$\alpha_5\beta_1$ integrin	2	Will also inhibit angiogenesis
	MEDI-522 (abergrin)	$\alpha_v\beta_3$ integrin	2	Humanized monoclonal antibody potent in vivo inhibitor of tumor growth and metastasis
	EMD 121974 (cilengitide)	$\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins	2	Cyclic Arg-Gly-Asp (RGD) peptide; inhibiting endothelial cell–cell interactions, endothelial cell–ECM interactions, and angiogenesis
	CNTO 95	α_v integrins	1/2	Human monoclonal antibody; inhibits melanoma cell adhesion, migration, and invasion
Matrix	COL-3 (NSC-683551)	MMP-2 and MMP-9	1	Completed
	Marimastat	MMP	2	Limited activity in melanoma; trials in Canada and Europe
	GC1008	Human anti-TGF-β monoclonal antibody	1	
Other kinase inhibitors	RAF-265	MAPK inactivation/BRAF and VEGFR-2 inhibitor	1	Inhibits the RAF/MAPK pathway as well as VEGF and thus angiogenesis
	Sunitinib (SU11248)	Multikinase inhibitor including Kit, PDGFR, VEGFR	2	FDA approved for GIST and metastatic kidney cancer

ECM—extracellular matrix; FDA—US Food and Drug Administration; GIST—gastrointestinal stromal tumor; Hsp—heat shock protein; MAPK—mitogen-activated protein kinase; MMP—matrix metalloproteinase; PDGFR—platelet-derived growth factor receptor; TGF—transforming growth factor; VEGFR—vascular endothelial growth factor receptor.

(All data from http://www.clinicaltrials.gov.)

Table 2

Select combination therapies targeting different components of the melanoma tumor microenvironment in clinical trials

Drug combination	Mechanism	Phase	Comments
Bevacizumab and BAY 43-9006	Anti-VEGF antibody; multikinase inhibitor	1/2	Response to MTD evaluated by biochemical changes in Ras-Raf-MAPK and VEGF signaling in tumor lysates by microarray and proteomics
PTK787 (valatanib) and Rad-001	Binds to and inhibits the protein kinase domain of VEGFR; rapamycin analogue and mTOR inhibitor	1	Binds to and inhibits PDGFR, c-Kit, and c-Fms
BAY 43-9006 and CCI-779 or R115777	Multikinase inhibitor; mTOR inhibitor; farnesyltransferase inhibitor	2	Inhibits c-Raf, PDGF, VEGR, c-Kit; an ester analogue of rapamycin
Vorinostat and FR901228 (romidepsin)	Histone deacetylase inhibitors	2	Proapoptotic in preclinical studies; FR901228 blocks hypoxia-induced angiogenesis and depletes several Hsp90-dependent oncoproteins
NP10052 and vorinostat	Proteosome inhibitor; histone deacetylase inhibitor	1	Enhanced potency in preclinical models
Bortezomib, paclitaxel, and carboplatin	Reversibly inhibits the 26S proteosome; mitotic inhibitor; DNA alkylating agent	2	Chemosensitization/potentiation therapy
MEDI-522 with or without dacarbazine	Monoclonal antibody anti-$\alpha_v\beta_3$ integrin; alkylating agent	2	Combination is well tolerated; preliminary overall survival results suggest potential clinical activity
Volociximab and dacarbazine	Anti-$\alpha_5\beta_1$ integrin antibody; alkylating agent	2	Study has been completed
PS-341 and temozolomide	Proteosome inhibitor; alkylating agent	2	

Hsp—heat shock protein; MAPK—mitogen-activated protein kinase; MTD—maximum tolerated dose; mTOR—mammalian target of rapamycin; PDGFR—platelet-derived growth factor receptor; VEGFR—vascular endothelial growth factor receptor.