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ANTI-TUMOUR TREATMENT

Targeted therapy for uveal melanoma

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Summary Uveal melanoma is the most common primary intra-ocular malignancy in adults. Overall mortality rate remains high because of the development of metastatic disease, which is highly resistant to systemic therapy. Improved understanding of the molecular pathogenesis of cancers has led to a new generation of therapeutic agents that interfere with a specific pathway critical in tumor development or progression. Although no specific genes have been linked to the pathogenesis of uveal melanoma, which differs from that of cutaneous melanoma, progress has been made in identifying potential targets involved in uveal melanoma apoptosis, proliferation, invasion, metastasis, and angiogenesis. This review focuses on the prospects for improving the systemic therapy of uveal melanoma using molecularly targeted agents that are currently in clinical use as well as agents being tested in clinical trials. Preclinical studies suggest potential benefit of inhibitors of Bcl-2, ubiquitin-proteasome, histone deacetylase, mitogen-activated protein kinase and phosphatidylinositol-3-kinase-AKT pathways, and receptor tyrosine kinases. Modifiers of adhesion molecules, matrix metalloproteinase, and angiogenic factors also have demonstrated potential benefit. Clinical trials of some of these approaches have been initiated in patients with metastatic uveal melanoma as well as in the adjuvant setting after primary therapy.

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Introduction

The eye's uveal tract comprises the iris, ciliary body, and choroid. It contains a population of melanocytes from

which develops uveal melanoma, the most common primary intra-ocular malignancy in adults. Survival rates for uveal melanoma remain poor. Advances made in the treatment of the primary tumor have not resulted in any improvement in survival rates.¹ Up to 50% of patients will develop metastatic disease, even 10–15 years after diagnosis, which invariably leads to death. The median survival of patients with metastasis is less than 6 months. Drugs commonly used to treat cutaneous melanoma, such

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as dacarbazine, temozolomide, interferon- α , and interleukin-2, rarely produce durable responses in patients with uveal melanoma, and to what extent current treatments prolong survival as compared with no treatment at all is not known.² The resistance of uveal melanoma can be ascribed to its propensity to metastasize to the liver, which is recognized as a particularly refractory site for many cancers, but liver metastases from uveal melanoma may even be less responsive to chemotherapy than liver metastases from cutaneous melanoma.³ Systemic therapy may be more effective in the adjuvant setting treating micrometastatic rather than the macrometastatic disease, where multiple mechanisms of resistance may manifest. Clinical, histologic, and, more recently, cytogenetic factors can be used to identify patients with uveal melanoma harboring micrometastases.⁴ Administering dacarbazine or interferon- α -2b to high-risk patients after primary therapy has not been shown to improve outcome.^{5,6}

“Targeted therapy” refers to drugs designed to interfere with a specific molecular pathway that is believed to have a critical role in tumor development or progression. The identification of appropriate targets is based on an understanding of the molecular changes underlying the cancer. This approach contrasts with the conventional, more empirical approach used to develop cytotoxic chemotherapeutics and, for the most part, immunotherapeutics. This approach is not new. Hormonal therapies in breast and prostate cancer are examples of targeted therapies. Recent advances in understanding the molecular pathogenesis of cancers, however, have led to a new generation of therapeutic agents. These include drugs that can modify pathways that regulate the cell cycle and whether tumor cells undergo apoptosis or proliferation and drugs that can inhibit molecules involved in invasion and metastasis. These also include drugs that can inhibit tumor angiogenesis, a rate-limiting step in tumor growth and the formation and progression of metastases. Several targeted therapeutics have recently been approved to treat a variety of what had been refractory cancers.

Several targeted therapies are being tested in cutaneous melanoma.^{7,8} Whether the drugs under investigation will also be applicable to uveal melanoma is not known. Although there is a common embryologic origin of the melanocytes, there are many differences in clinical and biological features. Whereas the development of most cutaneous melanoma is strongly linked to exposure to ultraviolet irradiation, the etiology of uveal melanoma remains obscure. The eye lacks lymphatics, and uveal melanoma tends to spread by the hematogenous route, whereas cutaneous melanoma shows a greater predilection for lymphatic metastasis. Furthermore, the molecular pathways altered in development also appear to be quite different (Table 1). A number of molecular changes have been identified in uveal melanoma, and drugs that either directly or indirectly modify these pathways have been developed (Table 2). Clinical trials of some of these are being conducted in patients with uveal melanoma (Table 3). In this review, we have focused on the prospects for improving the systemic therapy of uveal melanoma using molecularly targeted agents that are currently in clinical use as well as agents being tested in clinical trials.

Table 1 Comparison of genetic alterations observed in uveal and cutaneous melanoma

Alteration	Uveal	Cutaneous
Monosomy 3	Common	Rare
Amplification 8q	Common	Rare
Deletions/translocations 1p	Infrequent	Common
<i>TP53</i> mutations ^a	Rare ^a	Common
<i>BRAF</i> mutations ^a	Rare ^a	Common
<i>CDKN2</i> mutations ^a	Rare ^a	Common
<i>PTEN</i> mutations ^a	Rare ^a	Infrequent

^a Somatic mutations. Other alterations in gene expression occur. See text.

Molecular pathogenesis

Establishing the molecular pathogenesis of uveal melanoma has been difficult. Uveal melanoma is rarely hereditary, which limits approaches such as linkage analysis to identify susceptibility genes.⁹ As uveal melanoma is now most often diagnosed clinically and treated with brachytherapy, tissue, particularly from smaller tumors, is not routinely obtained. Thus, it is difficult to develop natural history models for analysis of molecular pathways altered in progression. Furthermore, somatic mutations in major tumor suppressor genes have not been observed in uveal melanoma, and most of the alterations identified have been functional and not structural, which adds complexity to the analysis. Nonetheless, progress has been made, and the molecular basis for tumor development and progression is emerging (Fig. 1). Cytogenetic studies have established the presence of specific, nonrandom chromosomal aberrations in uveal melanoma that have provided clues, and gene array studies are providing new insights.

Melanocytes normally express the anti-apoptotic Bcl-2, one of a family of proteins that regulate the cell cycle, and melanocytes of the uveal tract are likely intrinsically resistant to apoptosis.¹⁰ Although somatic mutations of the genes encoding retinoblastoma (Rb) protein and p53 (*TP53*) are rare, there is evidence that alterations that inhibit the function of these tumor suppressor pathways occur that allow melanocytes of the uveal tract to enter the cell cycle and proliferate.¹¹ The earliest alterations appear to be in the Rb pathway. Overexpression of cyclin D1, which blocks the suppressor function of Rb, is frequent.¹² Mutations of the cyclin-dependent kinase inhibitor, p16INK4a (*CDKN2A*), which are implicated in familial cutaneous melanoma syndromes, are rare in uveal melanoma.^{13,14} Downregulation by promoter methylation of p16INK4a, an important mediator of the suppressor function of Rb, is, however, frequent.^{15,16} Upstream signaling to p53 in uveal melanoma appears to be functional, and thus the deregulation of p53 downstream effectors has been the focus of several studies. Overexpression of MDM2 (mouse double minute 2, also termed HDM2 for its human equivalent), a key negative regulator of p53, is frequent.¹⁷ Downregulation of the p53-induced, apoptosis effector, PERP, and overexpression of the cyclin-dependent kinase inhibitor, p21, which also inhibits p53, have also been observed.^{18,19}

Table 2 Molecular pathways implicated in uveal melanoma development and progression and pharmacologic modifiers

Pathway	Changes in uveal melanoma (approximate percent of tumors)	Pharmacologic modifiers
<i>Apoptosis/proliferation</i>		
Bcl-2	↑ Bcl-2 (100%)	Oblimersan
Rb	↑ Cyclin D1 (60%)	Vorinostat, depsipeptide
p53	↓ INK4A (30%)	Bortezomib, vorinostat, depsipeptide
	↑ HDM2 (100%)	
	↓ PERP (50%)	
MAPK	↑ p21 (20%)	Imatinib mesylate, sorafenib, sunitinib
	↑ pERK (70%)	
PI3K/AKT	↑ pAKT (50%)	Temsirolimus, everolimus, imatinib
	↓ PTEN (40%)	
RTK	↑ KIT (75%)	Mesylate, sorafenib, sunitinib, nepafenac
	↑ EGFR (30%)	
<i>Invasion and metastasis</i>		
Adhesion molecules	↑ $\alpha_5\beta_1$ (50%)	Vitaxin, volociximab
	↑ $\alpha_v\beta_3$ (20%)	
MMP	↑ MMP-2 (50%)	Marimastat
	↑ MMP-9 (50%)	
<i>Angiogenesis</i>		
bFGF	↑ FGF-2 (90%)	Lenalidomide
VEGF/VEGR	↑ VEGF (25%)	Bevacizumab, VEGF Trap, AZD2171, sorafenib, sunitinib, lenalidomide

Table 3 Targeted therapies in phase II clinical trials for patients with uveal melanoma

Drug	Mechanism(s) of action	Treatment (melanoma inclusion)	Primary endpoint
Bortezomib	Proteasome inhibitor Inhibits proliferation Inhibits adhesion and metastases	Combination with carboplatin and paclitaxel (uveal)	RR
Vorinostat	HDAC inhibitor Inhibits proliferation/induces apoptosis	Single agent (uveal and cutaneous)	RR
Imatinib mesylate	Inhibits KIT and PDGFR Inhibits proliferation Inhibits angiogenesis	Single agent (uveal)	PFS
Sorafenib	Inhibits VEGFR, RAF, KIT, and PDGFR Inhibits angiogenesis Inhibits proliferation	Combination with carboplatin and paclitaxel (uveal)	RR
Sunitinib	Inhibits VEGFR, KIT, PDGFR, and FLT-3	Combination with cyclophosphamide and lenalidomide (uveal)	RR
	Inhibits angiogenesis Inhibits proliferation	Combination with tamoxifen and cisplatin (uveal)	DFS, OS
VEGF Trap	Neutralizes VEGF Inhibits angiogenesis	Single agent (uveal and cutaneous)	RR, PFS
AZD2171	Inhibits VEGFR Inhibits angiogenesis	Single agent (uveal and cutaneous)	RR
Lenalidomide	Inhibits FGF-2 and VEGF	Single agent (uveal)	RR
	Inhibits angiogenesis	Combination with cyclophosphamide and sunitinib (uveal)	RR

Trial information was obtained from www.clinicaltrials.gov. RR, response rate; PFS, progression-free survival; DFS, disease-free survival; OS, overall survival.

Activation of mitogen-activated protein kinase (MAPK) signaling pathway, which involves the serine/threonine-spe-

cific protein kinases, RAS, RAF, MEK, and ERK, is an important mechanism through which many human cancers

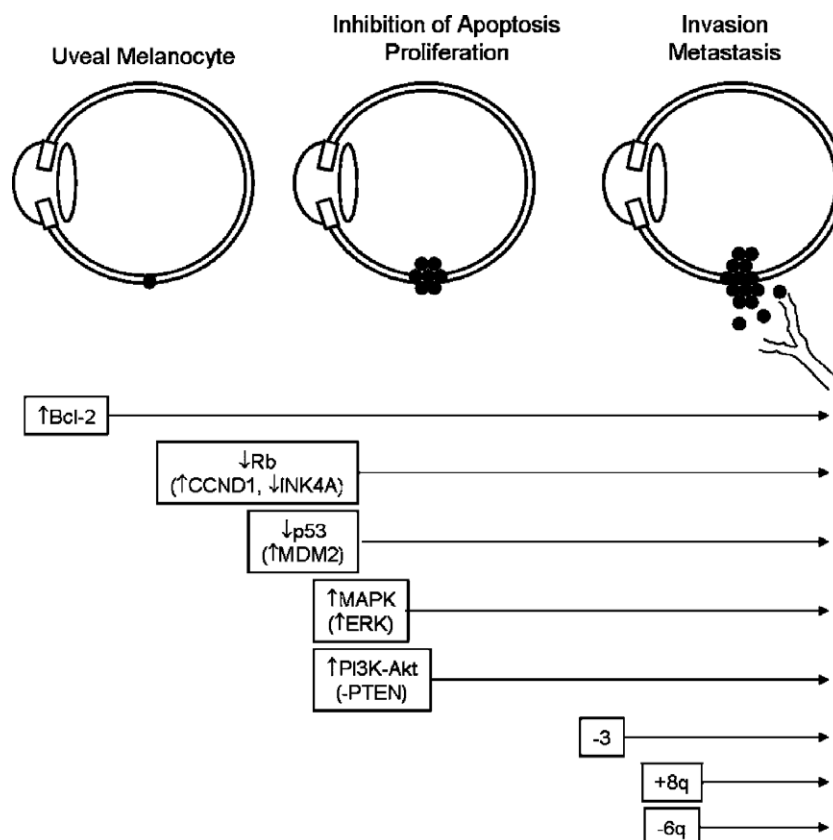


Figure 1 Molecular alterations implicated in the development and progression of uveal melanoma.

develop. The MAPK signal transduction pathway has been of particular interest since the discovery of frequent activating mutations of the B-RAF kinase in cutaneous melanoma.²⁰ Activation of the MAPK pathway, as manifested by activation of ERK, a downstream kinase in the pathway, also appears to be a common event in uveal melanoma, although it rarely occurs through mutation of *BRAF*.^{21,22} There is also evidence that the phosphatidylinositol-3-kinase (PI3K)/AKT pathway, one of the most commonly altered signaling pathways in human tumors, is altered in uveal melanoma.²³ Loss of the expression of PTEN, a dual-specificity phosphatase that blocks activation of the AKT pathway, is frequent.²⁴ In the majority of uveal melanomas, this loss of expression is secondary to hemizygous deletion of *PTEN*. However, the structure–function relationship may not be entirely straightforward, because it is only loss of cytoplasmic expression of PTEN that appears to correlate with shorter survival in uveal melanoma.²⁴ Alterations of the receptor tyrosine kinase (RTK), such as the KIT RTK, which initiate signaling through MAPK and PI3K/AKT, have also been demonstrated in uveal melanoma.²⁵

Uveal melanoma invasion and metastasis probably do not occur unless there is a major change in gene expression. Monosomy 3 has been closely associated with the development of metastasis and with mortality.^{26,27} In most series, the presence of monosomy 3, which has been observed in approximately 50% of tumors treated by enucleation, has been a better predictor than clinical and histologic factors.²⁸ Whether monosomy 3 plays a role in tumor progression through the deletion of a specific gene or genes or

whether it is simply a marker of genomic instability that accompanies tumor progression is not clearly established. Evidence to support the former comes from studies of metastasizing tumors that exhibit only partial deletions/loss of heterozygosity of chromosome 3, which suggest the involvement of tumor suppressor genes on 3p and 3q.^{29,30} There are several candidates on 3p, including the genes encoding the VHL protein (Von Hippel–Lindau disease) and Xeroderma Pigmentosum C. The type 2 transforming growth factor (TGF) β receptor is located at 3p22. TGF β 2 and also TGF β 1 have been shown to inhibit invasion and migration of uveal melanoma cells.³¹

Chromosome 8 gain resulting from trisomy 8, duplication of 8q, or isochromosome 8 has been observed in approximately 40% of uveal melanomas and has been associated with the development of metastasis.^{32–34} The 8q region most constantly amplified spans 8q24, the locus of the *MYC* proto-oncogene. *Myc* overexpression, however, has not been associated with the development of metastasis.³⁵ Loss of 6q occurs in approximately 25%, more commonly in metastasizing tumors.³⁶ Loss of 6q, however, may not be as tightly linked to metastasis as are the changes on chromosomes 3 and 8, and although one of the most common aberrations in tumors, the gene(s) involved on 6q has not been identified. Analysis of karyotype patterns has suggested that monosomy 3 probably occurs as an early event, and gain of 8q and loss of 6p as secondary events.³⁷ Amplification of 6p is found in approximately 25% of tumors. It is rarely observed with monosomy 3 and may correlate with better prognosis.³⁴ This observation and others suggest the

possibility of a bifurcation early in the development of uveal melanoma. Gene expression profiling has also suggested that metastasizing and non-metastasizing uveal melanomas represent two distinct entities.^{38,39}

The uvea is one of the most capillary-rich tissues of the body, and uveal melanoma is inherently a highly vascular tumor. Still, the presence of a high microvessel density is associated with worse survival, as is invasion of tumor cells into the lumen of tumor blood vessels and into the sclera.^{40,41} Uveal melanoma is also characterized by "vascular mimicry", intratumoral channels or loops composed of PAS-positive basement membrane in the absence of endothelial cells. Although function is still debated, the presence of these structures is also an adverse prognostic factor.⁴² Uveal melanoma cells express and produce several factors that promote invasion and metastasis, such as adhesion molecules and matrix metalloproteinases (MMPs).^{43–45} Several factors that promote angiogenesis in combination with factors that promote invasion and metastasis, such as basic fibroblast growth factor (FGF-2) and vascular endothelial growth factor (VEGF), are also frequently produced.⁴⁶

Targeting apoptosis/proliferation

Bcl-2

Bcl-2 blocks the mitochondrial release of cytochrome C and prevents the activation of pro-apoptotic caspase proteins. It is influenced by a variety of tumor suppressor pathways, particularly the p53 pathway. Virtually all uveal melanomas have been shown to overexpress Bcl-2. Although tumor Bcl-2 overexpression has been reported to be associated with an unfavorable outcome in cutaneous melanomas,⁴⁷ tumor Bcl-2 levels have not correlated with other clinical or histologic prognostic parameters or survival in uveal melanoma.^{35,48–50} The high levels of Bcl-2 expressed could, however, explain resistance of uveal melanoma cells to cytotoxic chemotherapy, which triggers cancer cell death by activating an apoptotic cascade that is initiated by cytochrome C release and caspase activation. Pharmacologic reduction or targeted inactivation of Bcl-2 has been shown to induce apoptosis of several tumor cells, including uveal melanoma cells, and has also been shown to enhance the sensitivity of uveal melanoma cells to chemotherapy.^{51,52} Oblimersen is an antisense oligonucleotide that binds *BCL2* mRNA and mediates its cleavage by RNase H. Oblimersen has been shown to reduce Bcl-2 and increase chemotherapy-induced apoptosis in melanoma models and is under investigation in patients with cutaneous melanoma.⁵³ Whether oblimersen is active in patients with uveal melanoma is not known. Patients with uveal melanoma were excluded in a randomized trial in which the addition of oblimersen to dacarbazine significantly improved multiple clinical outcomes in patients with advanced cutaneous melanoma and increased overall survival in patients with normal baseline serum lactate dehydrogenase.⁵⁴

Ubiquitin-proteasome

The ubiquitin-proteasome system is important in regulating the cell cycle. A number of regulatory proteins are degraded

during the cell cycle by the ubiquitin-proteasome pathway, and the ordered degradation of these proteins is required for the cell to undergo apoptosis or to proliferate. One of the targets for degradation is the tumor suppressor p53. Another is nuclear factor κ B (NF κ B), a transcription factor which regulates genes involved in apoptosis and proliferation as well as invasion, metastasis, and angiogenesis. NF κ B is activated by phosphorylation and deactivated by the inhibitor of nuclear factor kappaB (I κ B), which is degraded by the ubiquitin-proteasome pathway. Activation NF κ B has been directly implicated in tumorigenesis of various cancer types, including cutaneous melanoma.⁵⁵ The role of NF κ B activation in uveal melanoma, however, is not known. Bortezomib is a proteasome inhibitor that is used to treat multiple myeloma. Although single agent bortezomib did not manifest activity in a phase II trial in metastatic melanoma, preclinical studies suggest that it can enhance the sensitivity of melanoma cells to a variety of chemotherapeutics, an effect attributed to its ability to modulate cell regulatory proteins in the p53 pathway.^{56,57} Activity against uveal melanoma in particular has not been established. The combination of bortezomib with carboplatin and paclitaxel is being tested in a phase II clinical trial in metastatic melanoma that is including patients with metastatic uveal melanoma.

Histone deacetylase

Acetylation and deacetylation of chromatin histone protein by histone deacetylase (HDAC) alters chromatin structure and dynamically affects transcriptional regulation. Several lines of evidence indicate that histone hypo-acetylation induces repression of tumor suppressor gene expression. Inhibitors of HDAC are highly effective in up-regulating tumor suppressor genes, including those in the p53 and Rb pathways, and promoting apoptosis.^{58,59} HDAC inhibitors have also been shown to modulate the expression of genes involved in regulating tumor invasion and metastasis. Several small molecule HDAC inhibitors have been developed. These include depsipeptide, which has been demonstrated to be a potent inducer of apoptosis and inhibitor of cell proliferation of primary and metastatic uveal melanoma cell lines.⁶⁰ Vorinostat is a HDAC inhibitor that is used to treat patients with cutaneous T-cell lymphoma, the result of an apparent inherent sensitivity of this type of lymphoma to alterations in acetylation patterns that results in the suppression of apoptotic pathways. Vorinostat modulates a variety of genes in the p53 pathway, such as p21, and has been shown to induce apoptosis of melanoma cells.⁶¹ Activity against uveal melanoma in particular has not been established. Vorinostat is being studied in a phase II clinical trial in metastatic melanoma that is including patients with metastatic uveal melanoma.

MAPK

MAPK signaling is normally initiated at the cell membrane by growth factors interacting with RTK. As noted, in contrast to cutaneous melanoma, mutations in *BRAF* and also in *RAS*, are rare in uveal melanomas. Mutations in *BRAF* are also rare in mucosal melanoma suggesting that sun-related, ultraviolet-mediated effects may play a role in their development.⁶² Wild type B-Raf, however, plays a key role in the

proliferation of uveal melanoma cells in the absence of *BRAF* mutations by activating ERK. Active, namely phosphorylated, ERK (pERK), has been observed using immunohistochemical techniques in uveal melanomas of different cellular types with a nearly homogeneous intratumoral expression.^{21,22} The prognostic significance of this expression is not established. Numerous inhibitors of MAPK pathway mediators have been developed. These include sorafenib, which is used to treat patients with advanced renal cell carcinoma. Sorafenib has been shown to inhibit ERK-mediated, uveal melanoma cell proliferation.⁶³ Although the initial interest in sorafenib was based on its ability to inhibit protein kinases in the MAPK pathway, RAF in particular, sorafenib was also found to block several RTK effectively, including those of VEGF receptors (VEGFR) and platelet derived growth factor receptors (PDGFR), major regulators of tumor vascularity.⁶⁴ Animal models and correlative human studies suggest that most of the antitumor effects *in vivo* of sorafenib are the consequence of antiangiogenic effects mediated by inhibiting these RTKs.⁶⁵ A clinical trial exploiting the antivasular effects of sorafenib is in progress (see below).

PI3K-Akt

As with MAPK, PI3K-Akt signaling is initiated at the cell membrane by growth factors interacting with RTK as well as G-protein-coupled receptors. Phosphorylated Akt (pAkt), the major effector of the pathway, blocks apoptosis. The expression of tumor pAkt, as determined by immunohistochemistry, has been associated with negative prognostic indicators in patients with uveal melanoma.²³ PTEN is a major inhibitor of PI3K-Akt signaling. Loss of tumor cytoplasmic PTEN expression, as determined by immunohistochemistry, has been shown to be associated with shortened disease-free survival in patients with uveal melanoma.²⁴ Drugs that modify the PI3K-Akt pathway are under investigation. Inhibitors of mammalian target of rapamycin (mTOR) have been the best studied clinically. mTOR is a serine-threonine kinase downstream target of Akt that modulates cell cycle progression as well as angiogenesis.⁶⁶ The mTOR inhibitor, temsirolimus, is used to treat renal cell carcinoma. Clinical trials of temsirolimus and another mTOR inhibitor, everolimus, have been conducted in patients with metastatic melanoma.^{67,68} Responses have been infrequent, but stable disease has been observed. Whether mTOR inhibitors are active in uveal melanoma has not been reported. Several drugs can indirectly target the PI3K-Akt pathway and cause inhibition of Akt phosphorylation and induction of apoptosis. These include RTK inhibitors discussed below. They also include cyclooxygenase (COX) inhibitors. COX-2 expression has been found in approximately 60% of uveal melanomas, and expression has correlated with markers of poor prognosis.⁶⁹ Nepafenac, a COX-2 inhibitor used for the treatment of ocular inflammatory processes such as uveitis, has been shown to delay the progression of uveal melanoma in an animal model.⁷⁰ Drugs that more directly modify the PI3K-Akt pathway are being developed.⁷¹

KIT

The KIT receptor, which signals through MAPK and PI3K-Akt pathways, is expressed in approximately 75% of primary

uveal melanomas. Activation-related mutations of *KIT*, however, have not been identified.^{25,72,73} There is evidence that KIT is activated by its ligand, stem cell factor (SCF), during normal melanocyte proliferation.⁷⁴ There is also evidence that SCF/c-KIT autocrine loop interactions are involved in the transformation and proliferation of uveal melanoma cells.⁷⁵ The prognostic significance of KIT expression in uveal melanoma is, however, not known. Imatinib mesylate is an inhibitor of the RTK of KIT, bcr-abl, and PDGFR. It is used to treat patients with chronic myelogenous leukemia and gastrointestinal stromal tumors, where it acts by inhibition of bcr-abl and mutated KIT, respectively.⁷⁶ Treatment of uveal melanoma cell lines *in vitro* with imatinib mesylate inhibits proliferation.^{75,77,78} It has been noted, however, that the concentrations necessary for the inhibition of proliferation may not be clinically achievable.⁷⁸ As PDGF is a major regulator of tumor vascularity imatinib mesylate may also mediate an antivasular effect.⁷⁹ The clinical activity of imatinib mesylate in patients with uveal melanoma is under investigation. Fiorentini et al. treated three patients whose tumors expressed KIT with imatinib mesylate.⁸⁰ A reduction of malignant ascites in one, a partial reduction in lymph node metastasis in another, and progression of liver metastases in the third patient were observed. Progression was observed in two patients whose tumor did not express KIT who were also treated with imatinib mesylate. A phase II clinical trial of imatinib mesylate in patients with metastatic uveal melanoma that will assess the effect on progression-free survival is in progress. Imatinib mesylate has been evaluated in patients with cutaneous melanoma.^{81–83} Response rates have been disappointing, and therapy in some studies was unexpectedly toxic. Furthermore, the expression of KIT was not associated with response.

Epidermal growth factor receptor (EGFR)

The ErbB receptors and their ligands are involved in the pathogenesis of different types of cancers. Normal human melanocytes in culture express the four known ErbB receptors.^{84–86} ErbB receptors, which signal through MAPK and PI3K-Akt pathways, have been shown to mediate both the proliferative and migratory activities of melanocytes and melanoma cells.⁸⁷ ErbB1, also referred to as EGFR, was correlated with the metastatic potential of uveal melanoma cell lines and an increased capacity for these tumor cells to localize in the liver in a mouse model.⁸⁸ Tumor EGFR expression, as assessed by immunohistochemistry, has been associated with death due to liver metastasis in patients with uveal melanoma.^{89,90} In one study, however, the expression of EGFR was attributed to infiltrating macrophages and not the tumor.⁹¹ There are no clinical data regarding ErbB2 expression in uveal melanoma. Only a few cases of cutaneous melanoma express ErbB2, most of these being primary lesions rather than metastatic lesions.⁹² Two classes of anti-EGFR agents are currently used clinically: the monoclonal antibody, cetuximab, which is used to treat patients with colorectal and head and neck cancer, and low-molecular-weight ATP-competitive inhibitors of the RTK, such as erlotinib, which is used to treat patients with lung and pancreatic cancers, and gefitinib, which is used to treat patients with lung cancer. Gefitinib has been tested *in vitro* against uveal melanoma but only inhibited one of 12 lines tested.⁹³ This line did not overexpress EGFR. More recent clin-

ical experience has shown that responses to EGFR RTK inhibitors in lung cancer are not related to the surface level of EGFR expression but instead to the presence of activating mutations in the RTK.⁹⁴ Such mutations have yet to be identified in uveal or in cutaneous melanoma. Furthermore, EGFR RTK inhibitors have been shown to be ineffective when PTEN inactivation occurs in the presence of somatic *EGFR* activating mutations.⁹⁵ Given that PTEN inactivation is quite common in uveal melanoma, monotherapy with the EGFR RTK inhibitors currently used clinically is unlikely to be effective.

Targeting invasion and metastasis

Adhesion molecules

Cellular adhesion molecules play a central role in metastasis by mediating detachment of cells of the primary tumor and then attachment to components of the extracellular matrix or the vascular endothelium to be invaded. Invasive and noninvasive uveal melanomas have different adhesive properties.⁹⁶ Uveal melanomas have been characterized by increased expression of a variety of integrins, a family of heterodimeric adhesion molecules that bind ligands by recognizing short amino acid stretches on exposed loops, particularly the arginine-glycine-aspartic acid (RGD) sequence. These include $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_5\beta_1$, and $\alpha_v\beta_3$ integrins.^{96,97} Differences in the expression of integrins have been noted between uveal and cutaneous melanoma.⁹⁷ $\alpha_v\beta_3$ is an integrin, whose ligand is vitronectin, which is expressed by a large percentage of cancers, including melanoma, but not by normal melanocytes. Its expression in cutaneous melanoma parallels invasion and metastasis.⁹⁸ Although uveal melanoma cells do express $\alpha_v\beta_3$, expression is not common, and comparable levels of $\alpha_v\beta_3$ have been observed in invasive and noninvasive uveal melanoma cells.^{96,99} Thus, the acquisition of $\alpha_v\beta_3$, in contrast to cutaneous melanoma, may not be required for development of metastases. Uveal melanomas also express adhesion molecules in the immunoglobulin superfamily, including intercellular cellular molecule-1 (ICAM-1), neural cell adhesion molecule, and vascular cell adhesion molecule-1.¹⁰⁰ Of the adhesion molecules tested to date, only the expression of ICAM-1 has been shown to have prognostic significance. The loss of ICAM-1 expression has been associated with an increased risk of metastasis.¹⁰¹ Monoclonal antibodies targeting integrins and also peptide and non-peptide molecules mimicking the RGD sequence are being developed as therapeutics.¹⁰² Vitaxin, a humanized monoclonal antibody, binds $\alpha_v\beta_3$ triggering antibody-dependent cellular cytotoxicity. It also has been shown to block tumor growth in a mouse model by directly causing tumor cell apoptosis and inhibiting angiogenesis.⁹⁸ In a randomized study in patients with metastatic melanoma patients administered vitaxin alone had a median survival longer than patients administered vitaxin and dacarbazine.¹⁰³ Whether this reflects patient selection bias or a delayed effect of the therapy remains to be determined. Volociximab, an $\alpha_5\beta_1$ anti-integrin monoclonal antibody, is also being evaluated in patients with metastatic melanoma.¹⁰⁴ Whether either of these monoclonal antibodies has activity in uveal melanoma is not known. HDAC inhibitors have been shown to increase the expression

of ICAM-1.¹⁰⁵ The role of this effect in their antitumor activity is not known.

Matrix metalloproteinase (MMP)

MMPs are zinc-dependent endopeptidases that degrade the extracellular matrix during the processes of invasion and metastasis. They work synergistically with adhesion molecules in the invasion process. Uveal melanoma cells have been shown to express MMP-2 and -9, the MMP most commonly associated with tumor progression. Uveal melanoma cells also express MMP inhibitors, including tissue inhibitor of metalloproteinases (TIMP)-2.¹⁰⁶ There appears to be an association between tumor MMP-2 expression with metastasis and reduced survival in uveal melanoma.⁴⁵ Inhibitors of MMPs have been developed and tested clinically, but the results to date have been disappointing.¹⁰⁷ The MMP inhibitor, marimastat, which has been shown to inhibit MMP-2 and MMP-9, demonstrated only limited activity in patients with metastatic malignant melanoma.¹⁰⁸ HDAC inhibitors have also been shown to reduce the invasive properties of primary and metastatic uveal melanoma cell lines *in vitro*.¹⁰⁹ The HDAC inhibitor, depsipeptide, has been shown to decrease the migration of primary and metastatic uveal melanoma cell lines and the levels and activity of MMP-2 and MMP-9, while increasing the levels of TIMP-1 and TIMP-2. As noted, the HDAC inhibitor, vorinostat, is being evaluated in a clinical trial that includes patients with metastatic uveal melanoma.

Targeting angiogenesis

FGF-2

Fibroblast growth factors are a family of heparin-binding growth factors that exert their pro-angiogenic activity by interacting with various endothelial cell surface receptors, including RTK. They also interact with heparan-sulfate proteoglycans and integrins. Uveal melanoma cell lines have been shown to produce FGF-2.^{46,110} Immunohistochemistry studies have indicated that FGF-2 is expressed by most uveal melanomas.¹¹¹ FGF-2 occurs diffusely throughout the tumor and also to be associated with the microvasculature. The prognostic significance of FGF-2 expression in uveal melanoma is not known. Lenalidomide, an immunomodulatory derivative or "IMiD" of thalidomide, mediates a variety of effects, including antiangiogenic effects. These appear to be the result its ability to inhibit the secretion of angiogenic cytokines, including FGF-2, from tumor and from stromal cells.¹¹² Lenalidomide is used to treat patients with multiple myeloma and myelodysplastic syndrome. The single agent activity of lenalidomide in patients with metastatic uveal melanoma is being tested in a phase II clinical trial. Lenalidomide is also being evaluated in a phase II clinical trial in patients with metastatic uveal melanoma in combination with sunitinib and cyclophosphamide in a program designed to promote antiangiogenic effects.

VEGF

Most uveal melanoma cell lines produce several isoforms of VEGF.^{46,110} Primary tumors have also been shown to express

VEGF. A large variation in the frequency of VEGF expression has been reported, from 0% to 94%.^{111,113,114} Because the VEGF gene resides on 6p21 and, as noted, amplification of 6p occurs in approximately 25% of uveal melanomas, it was tempting to speculate the direct structure–function relationship. However, it has been shown recently that there was no statistically significant difference in VEGF expression between tumors with and without gain of 6p21.¹¹⁵ Thus, VEGF over-expression rather than structural amplification is probably significant in the pathogenesis of uveal melanomas, but its mechanism remains to be determined. In contrast to FGF-2, which is expressed diffusely, VEGF staining is associated with areas of fibrosis and necrosis.¹¹¹ In two studies that have examined prognostic significance in uveal melanoma, VEGF expression did not correlate with the occurrence of metastasis.^{114,116} The anti-VEGF monoclonal antibody, bevacizumab, is used in combination with cytotoxic chemotherapy to treat patients with colorectal and lung cancers. Bevacizumab is being tested in patients with metastatic melanoma.¹¹⁷ Although intravitreal application has been successfully applied to treat neovascular ocular diseases such as age-related macular degeneration and proliferative diabetic retinopathy, whether bevacizumab has activity against uveal melanoma is not known. VEGF Trap is a soluble decoy receptor created from the VEGFR that selectively inhibits VEGF and that has demonstrated activity against melanoma in preclinical studies.¹¹⁸ A phase II clinical trial of VEGF Trap is underway in patients with metastatic melanoma that includes patients with metastatic uveal melanoma. This trial will evaluate response rate and also progression-free survival, which will be compared to historical controls.

As noted, sorafenib inhibits the RTK of VEGFR. Sorafenib, which also can inhibit KIT and PDGFR, has demonstrated little or no antitumor activity in advanced melanoma patients as a single agent.¹¹⁹ Ongoing trials in advanced melanoma are evaluating sorafenib combination therapies. Promising results have been observed in a clinical trial of the combination of sorafenib with carboplatin and paclitaxel in patients with metastatic cutaneous melanoma, which may be an example of an antivascular agent enhancing chemotherapy effects, similar to what has been observed in other cancers with bevacizumab.¹²⁰ The combination of sorafenib with carboplatin and paclitaxel is currently being tested in a phase II clinical trial in patients with metastatic uveal melanoma. Sunitinib also inhibits the RTK of VEGFR as well as the RTK of KIT, PDGFR, and FLT-3.¹²¹ It too is used to treat patients with advanced renal cell carcinoma. Preclinical studies and phase I studies have suggested activity in melanoma.¹²¹ As noted, sunitinib is being tested in patients with metastatic uveal melanoma in combination with lenalidomide, which also can inhibit the secretion of VEGF, and cyclophosphamide. Sunitinib is also being tested in the adjuvant setting in patients with uveal melanoma in combination with a tamoxifen and cisplatin regimen, which is a previous study demonstrated promise in the adjuvant setting in patients with cutaneous melanoma.¹²² This study will determine the effect of this combination on disease-free survival and overall survival of patients with high-risk uveal melanoma, based on clinical and histologic factors, who have undergone primary therapy. AZD2171, an orally bioavailable inhibitor of the VEGFR RTK, has also demonstrated

promise.¹²³ A phase II clinical trial of AZD2171 is underway in patients with metastatic melanoma that is including patients with metastatic uveal melanoma.

Summary

Uveal melanoma is a refractory cancer. Systemic treatment options are limited, and at present there are insufficient data to recommend any chemotherapy or immunotherapy program. Targeted therapeutics address molecular abnormalities that are associated with tumor development and progression. This approach has been effectively applied in a broad range of what had been refractory cancers. Although no specific cancer genes have been linked, progress has been made in identifying potential targets involved in uveal melanoma apoptosis/proliferation, invasion and metastasis, and angiogenesis, and clinical trials have been initiated. Several targeted therapies are being developed for cutaneous melanoma. The molecular pathogenesis of uveal melanoma, however, differs from that of cutaneous, and specific approaches active/inactive in cutaneous melanoma may not be active/inactive in uveal. Targeted therapies may also be best applied in the adjuvant setting after initial treatment of the primary tumor. The increasing ability to identify a group of high-risk patients with primary uveal melanoma using molecular prognostication should allow the more rationale use of adjuvant systemic therapy and should facilitate testing of these approaches.

There are several challenges in developing targeted therapies for uveal melanoma. Although it is the most common ocular malignancy in adults, uveal melanoma is still a rare malignancy. The mean age-adjusted incidence of uveal melanoma is 4.3 per million in the United States, similar to that reported from European countries, making it at least 15 times less common than cutaneous melanoma.¹²⁴ Targeted therapies can be beneficial in terms of survival/progression parameters in the absence of an objective tumor response. Stable disease is more likely to be achieved than tumor response, and stable disease may be associated with improved survival. This has not, however, been established for uveal melanoma. Furthermore, there are little progression-free-survival or time-to-progression data available in this population, and studies with these types of endpoints usually involve larger numbers of patients.

Nonetheless, a new era of targeted therapies for uveal melanoma based on molecular/genetic profiles should be forthcoming. Novel technologies, such as single nucleotide polymorphisms typing platforms, should allow for association analyses for susceptibility loci. Recent gene expression profiling has demonstrated profound molecular difference between non-metastasizing tumors and metastasizing uveal melanoma.^{38,39,125} The distinctive gene signatures should provide a valuable new tool for understanding the biological processes underlying uveal melanoma development and progression. How these changes in gene expression lead to metastasis is now the subject of intense investigation. Proteomic studies have also recently identified proteins involved in tumor progression, and proteins specifically expressed in the metastases, which should also improve the understanding of uveal melanoma development as well

as have the potential of becoming clinically useful biomarkers.¹²⁶

Conflict of interest statement

None of the authors have any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work.

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