Therapeutic Implications of the Emerging Molecular Biology of Uveal Melanoma

Mininali Patel1, Elizabeth Smyth1, Paul B. Chapman1, Jedd D. Wolchok1, Gary K. Schwartz1, David H. Abramson2, and Richard D. Carvajal1

Abstract

Uveal melanoma represents the most common primary intraocular malignancy in adults. Although uveal and cutaneous melanomas both arise from melanocytes, uveal melanoma is clinically and biologically distinct from its more common cutaneous counterpart. Metastasis occurs frequently in this disease, and once distant spread occurs, outcomes are poor. No effective systemic therapies are currently available; however, recent advances in our understanding of the biology of this rare and devastating disease, combined with the growing availability of targeted agents, which can be used to rationally exploit these findings, hold the promise for novel and effective therapies in the foreseeable future. Herein, we review our rapidly growing understanding of the molecular biology of uveal melanoma, including the pathogenic roles of GNAQ (guanine nucleotide binding protein q polypeptide)/11, PTEN (phosphatase and tensin homolog), IGF (insulin-like growth factor)/IGF-1 receptor, MET (hepatocyte growth factor), BAP1 [breast cancer 1, early onset (BRCA1)-associated protein-1], and other key molecules, potential therapeutic strategies derived from this emerging biology, and the next generation of recently initiated clinical trials for the treatment of advanced uveal melanoma.

Introduction

Although uveal melanoma represents only 5% of all melanomas, this disease is the most common primary intraocular malignancy of the adult eye, affecting 6 individuals per million per year (1, 2). These tumors arise from melanocytes within the uveal tract, which consists of the iris, ciliary body, and choroid of the eye. Several features of the primary tumor have been associated with poor prognosis, including location in the ciliary body or choroid (as opposed to the iris), diffuse configuration, and larger size (3–6). Histologically, uveal melanomas with epithelioid morphology fare worse than those with spindle cells (7), as do those with higher mitotic activity, extracranial invasion, or the presence of microvascular networks (3, 8, 9). Genetic features, including monosomy of chromosome 3 and amplification of chromosome 8q, have also been identified as poor prognostic indicators (10, 11). Two independent groups have identified microarray gene expression profiles which accurately segregate uveal melanomas into 2 tumor classes by risk of metastasis (12–15). Class I tumors appear to have a low risk of metastasis, whereas class II tumors are more aggressive, correspond with monosomy in chromosome 3, and are associated with a higher rate of metastatic death.

The natural history of uveal melanoma is characterized by the frequent development of metastases, with over 50% of patients developing metastatic disease at any time from the initial diagnosis of the primary to several decades later (2, 16–21). A broad spectrum of therapies, including systemic therapies (22), hepatic artery infusion of chemotherapy, hepatic embolization, and metastastectomy, have been used to treat patients with metastatic uveal melanoma. However, a recent meta-analysis showed no compelling evidence that these interventions confer any survival benefit (23). Although a recently completed phase III trial that randomized 93 patients with hepatic metastases from uveal (n = 82) or cutaneous (n = 11) melanoma to percutaneous hepatic perfusion with melphalan to standard of care met its primary end point of hepatic progression-free survival (PFS), no survival advantage was observed (24).

Due to the lack of effective therapies for this disease, prognosis after the development of metastasis is poor. In the largest published series of patients with uveal melanoma, the median survival after diagnosis of metastatic disease was 3.6 months, with a 5-year cumulative survival of less than 1% (16). In this series, only 39% of patients received treatment for metastatic disease. In contrast, a smaller single institutional series of 119 cases treated at Memorial Sloan-Kettering Cancer Center (New York, NY) showed a 22% 5-year survival for patients with metastatic uveal melanoma (13, 18). In this study, 81% of patients received treatment for stage IV disease, including 20% who...
MAPK (extracellular signal-regulated kinase) pathway (25). In this signaling pathway, ligand exhibits activation of the mitogen-activated protein kinase (MEK). MEK phosphorylates and activates ERK, which dimerizes and translocates to the nucleus, where it mediates cell proliferation, survival, differentiation, and apoptosis. Preclinical studies show that inhibition of the MAPK pathway in uveal melanoma cell lines results in decreased cell proliferation (26, 27), suggesting that several key molecules in this pathway, including BRAF (v-raf murine sarcoma viral oncogene homolog B1) GNAQ (guanine nucleotide binding protein q polypeptide)/11, and MEK, may serve as potential therapeutic targets (Fig. 1).

**BRAF as a therapeutic target.** Cutaneous and uveal melanomas differ in many ways, including pattern of spread and responsiveness to chemotherapy; however, given their common melanocytic origin and the significantly larger body of knowledge about cutaneous melanoma, observations made in cutaneous melanoma have served to guide investigation into the molecular biology of uveal melanoma. BRAF has been shown to be of great significance in cutaneous melanoma, with up to 62% of cases harboring activating mutations in BRAF (28). Ninety-five percent of such cases result in a V600E mutation which involves a valine to glutamic acid substitution at position 600.

Based upon these findings in cutaneous melanoma, several groups have investigated the mutational status of BRAF in primary uveal melanomas (29–32), as well as in liver metastases of uveal melanoma (25). These studies have been overwhelmingly negative, with only 1 case harboring a BRAF V600E mutation (30). Several groups, however, have posited that uveal melanoma exhibits significant intratumoral heterogeneity and that conventional PCR techniques are insufficiently sensitive to identify BRAF mutations that may be present in a small subset of cells within a tumor. Using nested PCR and pyrophosphorylisis-activated polymerization techniques, these groups showed that subsets of tumor tissue, but not the entire tumor, harbor a BRAF mutation (33, 34). This observation, in part, may explain the identification of several uveal melanoma cell lines that harbor a BRAF mutation (29, 33, 35–37). Interestingly, Calipel and colleagues showed that uveal melanoma cell lines exhibit similar MAPK pathway activation and proliferation regardless of BRAF mutational status, indicating that other mechanisms of MAPK pathway activation are present in uveal melanoma (35).

Preclinical studies have shown that sorafenib, a small molecule inhibitor of the RAF family of kinases, PDGFR-β (platelet-derived growth factor receptor, beta polypeptide), VEGF receptor (VEGFR)-1 and VEGFR-3, and KIT, inhibits MAPK signaling and decreases proliferation, even in BRAF wild-type cell lines. Interestingly, studies of PLX4270, an inhibitor with relative selectivity for V600E BRAF, in uveal melanoma cell lines showed that only the BRAF mutant lines exhibited decreased cell viability with therapy, whereas no effect was observed in the wild-type cells (unpublished data). This is consistent with recent data indicating that effective RAF inhibition in BRAF wild-type cells may activate rather than inhibit MAPK pathway activation.

Eighty-six percent of primary uveal melanoma tissue specimens exhibit activation of the mitogen-activated protein kinase (MAPK) pathway (25). In this signaling pathway, ligand binding to cell surface tyrosine kinase receptors leads to exchange of GDP for GTP on Ras. Activated in its GTP-bound state, Ras activates Raf which, subsequently activates MAPK/ERK (extracellular signal-regulated kinase) kinase (MEK). MEK phosphorylates and activates ERK, which dimerizes and translocates to the nucleus, where it mediates cell proliferation, survival, differentiation, and apoptosis.
signaling (38–40), and that, although both BRAF mutant and wild-type uveal melanomas may require MAPK signaling for growth, inhibition of this pathway likely has different consequences depending upon the genetic background of the cell.

To date, there are a number of clinical trials evaluating various BRAF inhibitors such as PLX4032 (RO5185426, also known as RG7204), XL281, and GSK2118436 in melanoma (Table 1). Several of these have enrolled patients with uveal melanoma, and 1 trial of the combination of carboplatin, paclitaxel, and sorafenib is specifically enrolling patients with ocular melanoma. The ongoing phase I study of XL281 in patients with advanced solid tumors included 1 patient with uveal melanoma who achieved a confirmed partial response lasting for 4 months (41). It will be critical to carefully assess any clinical benefit observed in patients treated on these trials in relationship to tumor mutational status to optimally assess the role and future of BRAF inhibition as a therapeutic strategy for the treatment of uveal melanoma.

GNAQ/11 as therapeutic targets. Despite the absence of BRAF mutations, 86% of primary uveal melanomas exhibit
<table>
<thead>
<tr>
<th>Signaling pathway</th>
<th>Target</th>
<th>Agent</th>
<th>Development stage</th>
<th>Trial status</th>
<th>ClinicalTrials.gov identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP Kinase</td>
<td>BRAF</td>
<td>PLX4032 (RO5185426)</td>
<td>Phase III trial in cutaneous melanoma</td>
<td>Accrual completed</td>
<td>NCT01006980</td>
</tr>
<tr>
<td></td>
<td></td>
<td>XL281</td>
<td>Phase I trial in solid tumors</td>
<td>Ongoing</td>
<td>NCT00451880</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sorafenib (BAY 43-9006)</td>
<td>FDA approved for renal cell and hepatocellular carcinoma</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>MEK</td>
<td>AZD6244 (ARRY-142886)</td>
<td>Phase II trial in uveal melanoma</td>
<td>Ongoing</td>
<td>NCT01143402</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GSK1120212</td>
<td>Phase III trial in cutaneous melanoma</td>
<td>Ongoing</td>
<td>NCT01245062</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AS703026</td>
<td>Phase I trial in hematologic malignancies</td>
<td>Ongoing</td>
<td>NCT00957580</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MSC1936369B</td>
<td>Phase I trial in solid tumors</td>
<td>Ongoing</td>
<td>NCT00982865</td>
</tr>
<tr>
<td>PI3K/AKT</td>
<td>mTOR</td>
<td>Rapamycin</td>
<td>Approved for post-renal transplant immunosuppression</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tensrilmus</td>
<td>FDA approved for renal cell carcinoma</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Everolimus (RAD001)</td>
<td>Phase II with paclitaxel/carboplatin in stage IV melanoma</td>
<td>Ongoing</td>
<td>NCT01014351</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ridaforolimus (AP23573)</td>
<td>Phase VII in advanced/refractory malignancies</td>
<td>Accrual completed</td>
<td>NCT00112372</td>
</tr>
<tr>
<td></td>
<td>TORC1/2</td>
<td>AZD8055</td>
<td>Phase I in advanced solid malignancies</td>
<td>Ongoing</td>
<td>NCT00973076</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OSI027</td>
<td>Phase I in advanced solid/malignancies/lymphoma</td>
<td>Ongoing</td>
<td>NCT00698243</td>
</tr>
<tr>
<td></td>
<td></td>
<td>INK128</td>
<td>Phase I in advanced solid malignancies</td>
<td>Ongoing</td>
<td>NCT01058707</td>
</tr>
<tr>
<td></td>
<td>PI3K</td>
<td>XL147</td>
<td>Phase I in advanced solid tumors/lymphoma</td>
<td>Ongoing</td>
<td>NCT00486135</td>
</tr>
<tr>
<td></td>
<td></td>
<td>XL785</td>
<td>Phase I in patients with solid tumors</td>
<td>Ongoing</td>
<td>NCT00485719</td>
</tr>
<tr>
<td></td>
<td>AKT</td>
<td>Perifosine</td>
<td>Phase I in solid tumors/lymphoma</td>
<td>Accrual completed</td>
<td>NCT00389077</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GSK2141795</td>
<td>Phase I in solid tumors/lymphoma</td>
<td>Ongoing</td>
<td>NCT00920257</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GSK690693</td>
<td>Phase I in solid tumors/lymphoma</td>
<td>Ongoing</td>
<td>NCT00433818</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MK2206</td>
<td>Phase I in locally advanced or metastatic solid tumors</td>
<td>Ongoing</td>
<td>NCT01071018</td>
</tr>
<tr>
<td>Receptor Tyrosine Kinases</td>
<td>IGF-1R</td>
<td>IMC-A12</td>
<td>Phase I with temsirolimus in advanced cancers</td>
<td>Ongoing</td>
<td>NCT00678769</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R1507</td>
<td>Phase I in advanced solid tumors</td>
<td>Ongoing</td>
<td>NCT00400361</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MK0646</td>
<td>Phase I in advanced solid tumors</td>
<td>Accrual completed</td>
<td>NCT00635778</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OSI-906</td>
<td>Phase I in advanced solid tumors</td>
<td>Ongoing</td>
<td>NCT00514007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BIIB022</td>
<td>Phase I in advanced solid tumors</td>
<td>Ongoing</td>
<td>NCT00555724</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CP-751,871</td>
<td>Phase I with sunitinib in advanced solid tumors</td>
<td>Ongoing</td>
<td>NCT00729833</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AXL1717</td>
<td>Phase I in advanced solid tumors</td>
<td>Ongoing</td>
<td>NCT01062620</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AMG479</td>
<td>Phase I with biologics/chemo in advanced solid tumors</td>
<td>Ongoing</td>
<td>NCT00974896</td>
</tr>
</tbody>
</table>

(Continued on the following page)
### Table 1. Receptor tyrosine kinase inhibitors and inhibitors of the MAP kinase and PI3K/AKT pathways of interest in uveal melanoma (Cont’d)

<table>
<thead>
<tr>
<th>Signaling pathway</th>
<th>Target</th>
<th>Agent</th>
<th>Development stage</th>
<th>Trial status</th>
<th>ClinicalTrials.gov identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-Kit</td>
<td>Imatinib</td>
<td>Phase II in metastatic uveal melanoma</td>
<td>Ongoing</td>
<td>NCT00421317</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sunitinib</td>
<td>Phase II with cisplatin and tamoxifen in uveal melanoma</td>
<td>Ongoing</td>
<td>NCT00489944</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sorafenib</td>
<td>Phase II with carboplatin/paclitaxel in uveal melanoma</td>
<td>Ongoing</td>
<td>NCT00329641</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dasatinib</td>
<td>Phase I with bevacizumab in metastatic solid tumors</td>
<td>Ongoing</td>
<td>NCT00792545</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nilotinib</td>
<td>Phase II in c-kit mutated or amplified melanoma</td>
<td>Ongoing</td>
<td>NCT01168050</td>
<td></td>
</tr>
<tr>
<td>c-Met</td>
<td>PF-02341066</td>
<td>Phase I in solid tumors other than NSCLC</td>
<td>Not yet open</td>
<td>NCT01121588</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GSK1363089</td>
<td>Phase I in solid tumors</td>
<td>Accrual completed</td>
<td>NCT00742261</td>
<td></td>
</tr>
<tr>
<td></td>
<td>XL-184</td>
<td>Randomised discontinuation in advanced solid tumors</td>
<td>Ongoing</td>
<td>NCT00940225</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ARQ 197</td>
<td>Phase I in refractory/advanced solid tumors</td>
<td>Ongoing</td>
<td>NCT00609921</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EMD 1204831</td>
<td>Phase I in advanced solid tumors</td>
<td>Ongoing</td>
<td>NCT01110083</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PRO143966</td>
<td>Phase I in advanced solid tumors</td>
<td>Accrual completed</td>
<td>NCT01068977</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: n/a, not applicable.
activation of the MAPK pathway as evidenced by activation of phospho-ERK (26–32). In cutaneous melanoma, MAPK pathway activation has also been shown to be mediated by mutations in NRAS (42); however, these mutations have not been found in uveal melanoma (29). Thus, whereas uveal melanoma, such as cutaneous melanoma, is characterized by MAPK activation, the mechanism of MAPK activation differs between these 2 unique subtypes of melanoma.

Recent studies have identified G-proteins as potential drivers of MAPK activation in uveal melanoma. Genetic screens have shown that 46% to 53% of uveal melanoma exhibit mutations in GNAQ (26, 43–45). These mutations are not associated with clinical, pathologic, immunohistochemical, or genetic factors associated with advanced uveal melanoma, indicating that this alteration may represent an early event in disease pathogenesis (43). Recent data suggest that over half of uveal melanomas lacking a mutation in GNAQ exhibit a mutation in GNA11 (44). GNAQ is a G-protein α-subunit. G proteins are a family of heterotrimeric proteins (Gαβγ) coupled to cell surface, 7-transmembrane spanning receptors. Upon ligand binding to these receptors, the GDP bound to the Gα subunit of Gαβγ is exchanged for GTP, resulting in a conformational change and the subsequent dissociation of the Gα from the Gβγ subunits. These 2 subunits are then able to regulate various second messengers. Gαt activation is terminated by a GTPase intrinsic to the Gα subunit. The Gα class Gαt (Gtαt) mediates its activity through stimulation of phospholipase C-β (PLCβ), which cleaves PIP2 to IP3 and DAG. DAG goes on to activate protein kinase C (PKC), which ultimately activates downstream pathways including the MAPK signaling pathway.

Van Raamsdonk and colleagues showed that transfection of GNAQ Q209L in human melanocytes results in anchorage-independent growth, with cells able to grow in the absence of the DAG analogue 12-O-tetradecanoylphorbol-13-acetate, presumably due to high levels of DAG production by constitutively activated PLCβ. GNAQ Q209L transfected melanocytes have increased ERK activation as compared to melanocytes with wild-type GNAQ (26). Interestingly, small interfering RNA (siRNA) targeting GNAQ normalizes phospho-ERK levels, increases the number of resting cells, decreases cell number, and decreases anchorage-independent growth. Injection of nude mice with melanocytes harboring GNAQ Q209L, but not wild-type GNAQ melanocytes, leads to the development of pigmented tumors at the injection site (26). GNA11 has similarly been validated as an oncogene that results in MAPK activation, comparable to that achieved with GNAQ (44).

The somatic GNAQ exon 5 Q209L and Q209P mutations most commonly identified lead to a glutamine-to-lysine and glutamine-to-proline substitution, respectively, at position 209, which lies in the Ras-like domain of GNAQ. The GNA11 exon 5 mutation most commonly observed results in a Q209L substitution that is analogous to the Q209L substitution observed in GNAQ. Mutations at this site cause loss of the intrinsic GTPase activity, similar to that seen in Ras family members (46). Because Gαt inactivation is mediated by this intrinsic GTPase, such mutations lead to constitutive Gαt activation and downstream signaling. Exon 4 mutations in GNAQ and GNA11 have also been identified in 4.8% of uveal melanomas that lead to alterations at arginine 183 (R183; ref. 44). In all but one of the tumors tested, exon 5 Q209L and exon 4 R183 mutations were mutually exclusive. Interestingly, tumors characterized by these mutations display distinct biological activity. Injection of GNA11 Q209L transfect melanoma cells in immunocompromised mice produced rapidly growing tumors at all injection sites, whereas injection of R183C-transfected cells produced tumor growth at only one half of injection sites with a longer latent period. Furthermore, whereas all mice injected with the GNA11 Q209L variant developed visceral metastases, this was not observed with melan-a cells transfected with GNAQ Q209L, supporting the hypothesis that GNA11 Q209 mutations are more oncogenic than the GNAQ Q209 variant. Indeed, a recent study showed an inverse relationship of the frequency of GNAQ mutations and GNA11 mutations when comparing blue nevi, uveal melanoma, and uveal melanoma metastases. Whereas GNA11 mutations were observed in 7% of blue nevi, 32% of uveal melanoma, and 57% of uveal melanoma metastases, GNAQ mutations were present in 55% of blue nevi, 45% of uveal melanoma, and 22% of uveal melanoma metastases. This also suggests that mutations in GNA11 confer a greater risk of distant metastasis in uveal melanoma than GNAQ mutations.

Thus, in vitro and in vivo studies support the hypothesis that GNAQ/11 mutations result in MAPK activation and play an essential role in the development of uveal melanomas. There is currently a significant interest in investigating inhibition of the GNAQ/11 pathway for the treatment of uveal melanoma; however, whether inhibition of this pathway will be an effective strategy is yet to be determined. Importantly, it is also not known whether pathway inhibition at the level of GNAQ/11 or further downstream will be optimal. Currently, there are no clinically available specific inhibitors of GNAQ/11, PLCβ, or the various PKC isoforms with which to investigate these critical questions.

**MEK as a therapeutic target.** An alternative therapeutic strategy for these patients is the targeting, not of GNAQ/11, but rather of the downstream effector MEK. Treatment of uveal melanoma cell lines bearing GNAQ mutations with U0126, a small molecule inhibitor of MEK, leads to a decrease in phospho-ERK and cell number, a loss of anchorage-independent growth, and an increase in the sub-G0/G1 subpopulation. Moreover, in these cell lines, U0126 results in lower cell numbers than siRNA-mediated knockdown of GNAQ, suggesting that targeting the downstream target MEK may be more effective than inhibiting GNAQ itself (26). Additional in vitro studies indicate that uveal melanoma cell lines bearing the GNAQ Q209L mutation are sensitive to MEK inhibition with AZD6244, another potent, selective, orally available, and non–ATP-competitive small molecule inhibitor of MEK1/2 (47). MEK inhibition in
these cells is associated with decreased signaling through both the MAPK and PI3K pathways, as shown by inhibition of phospho-ERK and phospho-AKT. Although these effects are not observed in wild-type cells for GNAQ or BRAF, transfection of GNAQ wild-type cell lines resistant to AZD6244 with GNAQ Q209L leads to the induction of sensitivity to AZD6244 in terms of both ERK inhibition and decreased proliferation.

We have observed clinical efficacy of MEK inhibition in subset analysis of patients with metastatic uveal melanoma treated with AZD6244 on 3 completed trials (48–50). On a randomized phase II study of AZD6244 versus temozolomide for patients with melanoma, 17 received AZD6244 during the study: 7 received AZD6244 upfront whereas 10 received AZD6244 following progression on temozolomide (49). PFS hazard ratio (HR) was 0.76 (80% CI, 0.38–1.53) in favor of AZD6244, with a median PFS of 50 days for those randomized to temozolomide [80% confidence interval (CI) = 43 days, 83 days; 12 events/13 patients] and 114 days for those randomized to AZD6244 (80% CI = 70 days, 202 days; 5 events/7 patients). Insufficient numbers of patients have been treated thus far with AZD6244 to conclude a benefit over chemotherapy and to assess whether GNAQ/11 mutational status (Table 2). This study is powered to test the hypothesis that AZD6244 will decrease the 4-month progression rate by 40% when compared with temozolomide in the GNAQ/11-mutant patient population that is temozolomide/dacarbazine (DTIC) naïve. This study will also assess the efficacy of AZD6244 in temozolomide/DTIC naïve patients regardless of genetic background, as well as patients with tumor characterized by a GNAQ/11 mutation who have previously been treated with temozolomide/DTIC.

PI3K/AKT pathway. PI3K signaling is also implicated in uveal melanoma. PI3K is activated by G-protein–coupled receptors and by receptor tyrosine kinases. Upon activation, PI3K catalyzes the conversion of PIP2 to PIP3. PIP3 mediates translocation of AKT (also known as protein kinase B) to the cell membrane, where it is activated. AKT mediates several key proliferation and cell survival pathways. PI3K signaling is antagonized by PTEN, a protein that stimulates conversion of PIP3 to PIP2, and thus, decreases AKT activation.

A relative decrease in PTEN expression in aggressive primary uveal melanomas compared with less aggressive tumors was previously reported, with either decreased or complete loss of PTEN expression as measured by immunohistochemistry observed in 58.7% of cases evaluated (51). Loss of PTEN was associated with a less favorable profile for patients presenting with primary uveal melanoma, where patients with a total loss of PTEN have a
median survival of 60 months compared with more than 120 months for patients with normal or nearly normal PTEN expression.

Pi3K and AKT as therapeutic targets. Several uveal melanoma cell lines exhibit Pi3K activation (51–53). A few of these cell lines exhibit submicroscopic chromosomal deletions leading to loss of expression of PTEN, representing one mechanism of pathway activation (51). The addition of IGF-1R, which LY294002 in uveal melanoma cell lines results in decreased proliferation that is observed even in cell lines harboring a BRAF mutation (53, 54). Although LY294002 and the related nonreversible IGF-1R inhibitor wortmannin have limited clinical utility due to their poor solubility and high toxicity, more tolerable IGF-1R inhibitors such as XL147 are currently undergoing clinical investigation. In addition, inhibition of the Pi3K/AKT pathway at the level of AKT is currently being investigated with agents such as perifosine, GSK2141795, GSK690693, and MK2206 now in clinical trials (Table 1).

mTOR as a therapeutic target. mTOR is a downstream effector of the Pi3K pathway that stimulates cell proliferation through translational control of cell-cycle progression regulators. There exist 2 structurally and functionally distinct mTOR complexes: mTORC1 (mTOR complex 1, rapamycin sensitive) and mTORC2 (mTOR complex 2 rapamycin insensitive; ref. 55). mTORC1 is activated mainly via the Pi3K pathway through AKT and the tuberous sclerosis complex (56). Activated AKT phosphorylates TSC2, which leads to dissociation of the TSC1/TSC2 complex, thus inhibiting the activity of TSC2 to act as a GTPase activating protein. This allows Rheb, a small G-protein, to remain in a GTP-bound state and activate mTORC1. AKT can also activate mTORC1 by phosphorylating p70S6k, thereby relieving the PHAS-1-mediated inhibition of mTORC1 (57, 58).

Several mTOR inhibitors, including everolimus and temsirolimus, are being evaluated in clinical trials for melanoma, and a new class of compounds targeting both TORC1 and TORC2 complexes is also under investigation for advanced cancers (Table 1). A significant single-agent activity has thus far been shown in melanoma (59). Of significant clinical relevance, treatment of uveal melanoma cell lines with the mTOR inhibitor rapamycin at levels that inhibit downstream mTOR signaling by 100% results in only 9% to 21% inhibition of cell proliferation (53). This phenomenon is explained, in part, by the finding that mTOR inhibition induces AKT activation through loss of the mTOR pathway-dependent inhibition of IGF-1R signaling (discussed in more detail below; refs. 60, 61). IGF-1R blockade abrogates mTOR inhibition–mediated AKT activation and confers sensitivity to mTOR inhibition in cancer cells (60). Thus, IGF-1R–mediated feedback activation of PI3K signaling appears to confer resistance to mTOR inhibitors, and mTOR inhibition alone is likely insufficient for the successful treatment of uveal melanoma. Interestingly, it has been shown that activation of AKT due to mTOR blockade can be inhibited in vitro with treatment of an IGF-1R antibody (61, 62), suggesting that combination therapy targeting both mTOR and the IGF-1R pathway may produce more favorable results than those of mTOR inhibition alone.

Therapeutic targets upstream of the MAPK and PI3K/AKT pathways. Preclinical data show that simultaneous inhibition of both the MAPK and PI3K/AKT pathways result in the synergistic inhibition of cell proliferation (53), suggesting that dual-pathway inhibition may be necessary for the optimal management of uveal melanoma. Such inhibition may be achieved by combining 2 or more inhibitors targeting components of both pathways. Alternatively, as several key cell-surface receptors activate both pathways simultaneously, effective inhibition of such receptors may serve as an alternative therapeutic strategy.

KIT as a therapeutic target. A member of the PDGFR family of kinases, KIT is a receptor tyrosine kinase that mediates growth differentiation, as well as attachment, migration, and proliferation of cells. Binding of the KIT ligand stem cell–derived factor (SCF) results in receptor dimerization and autophosphorylation. Docking sites for several Src homology-2 signaling proteins such as those mediating PI3K, MAPK, and JAK/STAT pathway activation are subsequently revealed.

KIT expression has been identified in up to 87% of primary uveal melanomas by immunohistochemistry; however, less than 40% exhibit strong staining (63–65). Uveal melanoma cell lines as well as normal uveal melanocytes produce SCF; however, only uveal melanoma cell lines secrete SCF, suggesting the presence of a relevant autocrine loop in the setting of malignancy (66). Stimulation of normal uveal melanocytes with SCF results in activation of both ERK1/2 and AKT; however, in a KIT expressing uveal melanoma cell line, stimulation led to MAPK pathway activation only (65). Inhibition of KIT, using both imatinib mesylate, a small molecule inhibitor of several receptor tyrosine kinases, including ABL (c-abl oncogene 1), KIT, and PDGFR, as well as siRNA techniques, leads to a reduction in proliferation of uveal melanoma cell lines expressing the target. This effect was not observed in KIT negative cell lines or in normal uveal melanocytes (63, 65, 66). Treatment with imatinib abrogates both the MAPK and PI3K/AKT pathways in normal uveal melanocytes. In KIT expressing uveal melanoma, treatment with imatinib decreased the SCF-induced MAPK activation and resulted in decreased invasion by uveal cell melanoma lines as determined by penetration through a Matrigel-coated membrane (65). Interestingly, inhibition of MEK in the uveal melanoma cell line using UO126 decreased SCF-induced cell proliferation by 92% to 98%, but AKT inhibition had no significant effect, suggesting that the proliferative effects of the SCF/KIT autocrine loop in uveal melanoma likely funnel primarily through the MAPK pathway (66).

There are currently several clinical trials investigating various KIT inhibitors, including imatinib, nilotinib, and dasatinib, in patients with advanced melanoma (Table 1). Despite promising preclinical data, results observed in patients with uveal melanoma treated on these studies
have been underwhelming (67). In a phase II study of imatinib in 13 patients with uveal melanoma metastatic to the liver, 1 patient achieved stable disease for 5 months; however, no objective responses were observed (68). In a phase II study of sunitinib, a tyrosine kinase inhibitor of c-kit, PDGFR, VEGF receptor, and fms-related tyrosine kinase 3 (FLT-3), of 18 evaluable patients with advanced uveal melanoma, 1 patient achieved a partial response and 12 achieved stable disease (69). The median overall and PFS durations were 8.2 months and 4.0 months, respectively. Because the lack of the response observed in these studies might reflect treatment of patients with absent or very low KIT expression, Hoffman and colleagues hypothesized that more consistent responses might be achieved in a patient population with high tumor expression levels; however, in another study of 12 patients with metastatic uveal melanoma characterized by high KIT expression by immunohistochemistry treated with imatinib, no significant responses were observed (70).

The results observed in these clinical trials are disappointing but consistent with what has been observed in trials of KIT inhibition in cutaneous melanoma. The “oncogene addiction” hypothesis is based upon the hypothesis that tumorigenesis is dependent upon dysregulation of a gene or gene product. Protein expression is not indicative of such dysregulation in all cases and cannot be reliably used to guide drug development. Indeed, no difference in survival is observed between patients with uveal melanoma characterized by high KIT expression and those with disease characterized by low KIT expression (63). Rather than simple expression, activation of KIT via a mutation or amplification may be required to connote sensitivity to KIT inhibition as has been observed in tumors such as gastrointestinal stromal tumors and, more recently, in melanoma (71). Although such alterations have been associated with melanomas arising from acral, mucosal, and chronically sun-damaged surfaces (72, 73), thus far, no activating mutations have been identified in primary uveal melanoma samples (63, 64, 70).

**IGF-1R as a therapeutic target.** The IGF signaling pathway is implicated in both MAPK and PI3K signaling and way is implicated in both MAPK and PI3K signaling and inhibition of IGF-1R in uveal melanoma cell lines results in decreased proliferation (77, 78), and the IGF-1 signaling axis is implicated, not only in proliferation of uveal melanoma cells, but also in their metastatic potential (79, 80).

In a study of 36 patients with uveal melanoma, 10 of 18 patients (56%) who died of advanced disease bore tumors with high IGF-1R expression. In contrast, only 5 of 18 patients (28%) who survived more than 15 years following primary surgical enucleation had tumors with high levels of IGF-1R expression (77). This association between IGF-1R expression and melanoma-specific mortality was also suggested in a subsequent study of 132 patients with uveal melanoma, in which 24 of 42 patients (57%) with high expression of IGF-1R died of metastatic disease, whereas only 31 of 90 patients (34%) succumbed to metastatic uveal melanoma (80).

Treatment of uveal melanoma cell lines with PPP, a specific inhibitor of IGF-1R, results in decreased IGF-1R expression, decreased IGF-1R phosphorylation, decreased downstream MAPK and PI3K signaling, and a 60% to 90% decrease in cell survival (81, 82). PPP has a lower IC50 in uveal melanoma cell lines when compared with cisplatin, 5-fluorouracil, and doxorubicin, and has variable synergistic effects when used with chemotherapy. In *in vivo* xenograft studies using a uveal melanoma cell line in severe combined immunodeficient (SCID) mice showed that intraperitoneal, intravitreal, and oral treatment with PPP leads to tumor regression, decreased liver micrometastases, decreased IGF-1R phosphorylation, decreased PI3K and MAPK signaling, decreased MMP-2 expression, and increased apoptosis in tumor cells (81, 83). Interestingly, xenografts lacking IGF-1R exhibit no such response to PPP (82).

To date, there are several monoclonal antibodies and small molecule agents targeting IGF-1R in clinical development for advanced solid cancers. Several of these agents are being combined with agents targeting mTOR in an effort to overcome the feedback disinhibition observed with mTOR blockade alone discussed above (Table 1). Whether such a therapeutic strategy will be effective in uveal melanoma will be addressed in an upcoming phase II study of IMC-A12, the anti-IGF-1R monoclonal antibody, in patients with this disease (Table 2).

**IGF-1 as a therapeutic target.** An alternative strategy to directly targeting IGF-1R for the inhibition of the IGF pathway is suppression of the ligand. IGF-1. Basal serum IGF-1 levels have been associated with locally advanced disease as well as the development of liver metastases (84).

Octreotide is a somatostatin analogue that binds primarily to somatostatin receptor subtype sst2 and has been shown to suppress IGF-1 plasma levels in patients with solid tumors (85, 86). Octreotide has been further shown to decrease tyrosine phosphorylation levels of p85, the PI3K regulatory subunit, leading to dephosphorylation of phosphoinositide-dependent kinase 1 (PDK1) and AKT, without affecting PTEN, total PDK1 levels, or total AKT levels (87–89). Pasireotide (SOM230) is a novel, multireceptor, somatostatin analogue that binds with nanomolar affinity to somatostatin receptor subtypes sst1, sst2, sst3, and sst5, and potently suppresses growth hormone (GH) IGF-1, and adrenocorticotropic secretion (90). Pasireotide has been shown to significantly suppress IGF-1 plasma levels to a greater extent than that achieved with octreotide. Administration of pasireotide in dosages of 1 and 10 μg/kg/h to male Lewis rats significantly decreased plasma IGF-1 levels by 68% and 98%, respectively, on day 2 of therapy (91–93). This suppression was achieved primarily via the reduction of pituitary GH secretion.
although peripheral inhibitory effects of pasireotide on IGF-1 action have been shown as well (94).

In addition to affecting IGF-1 plasma levels, both octreotide and pasireotide may have direct effects upon uveal melanoma cells, as somatostatin receptors are known to be expressed on melanoma cells. One study showed that 96% of cutaneous melanomas tested expressed the somatostatin receptor sst1, 83% expressed sst2, 61% expressed sst3, 57% expressed sst4, and 9% expressed sst5 (95). Of 25 clinical uveal melanoma samples tested, all showed expression of sst2, 7 (28%) expressed sst3, and 14 (56%) expressed sst5 (96). Interestingly, octreotide or vapreotide showed dose-dependent inhibitory effects on cell proliferation in 3 uveal melanoma cell lines (OMM2.3, OCM3, and Mel270) tested (96).

The combination of the mTOR inhibitor RAD001 with pasireotide is being tested in an ongoing phase I trial, and the recommended phase II dose has been identified. A phase II study of this combination testing the hypothesis that mTOR inhibition in combination with inhibition of the IGF-1R pathway is an effective therapy for uveal melanoma is ongoing (Table 2).

**c-MET as a therapeutic target.** The c-MET proto-oncogene encodes a tyrosine kinase receptor responsible for biological functions as diverse as cell motility, proliferation, and survival (97–100). Hepatocyte growth factor/scatter factor (HGF) is a plasmogen-like protein that acts as the endogenous ligand for this receptor, binding of which leads to autophosphorylation of tyrosine residues within the receptor’s activation loop, activation of kinase and phosphorylation of additional tyrosine residues adjacent to the carboxyl terminus, which form a docking site for intracellular adaptors of downstream signaling (97, 100, 101). Signaling in this pathway is primarily mediated by Grb2, PI3K Src, Gab1, STAT3, PLCγ, Shc, Shp2, and Shp1 (100).

HGF acts as a mitogen to melanocytes, and c-MET overexpression correlates with the invasive growth phase of melanoma (102). Melanoma cells, but not melanocytes, express HGF, leading to a potential autocrine positive feedback loop in the development of melanoma (102). Although uveal melanomas overexpress c-MET, activating mutations or genetic amplifications of c-MET do not appear to play a significant role in this disease (103). Hendrix and colleagues first reported in 1998 the expression of c-MET by the more invasive interconverted phenotype of uveal melanoma cell lines, and subsequently showed a mitogenic response to HGF by c-Met expressing cells, but not by those who failed to express c-Met (104). Cell migration capacity appears to be enhanced by HGF via activation of phospho-AKT and the downregulation of the cell adhesion molecules e-cadherin and beta-catenin in a dose-dependent fashion (105). Both c-MET inhibition and AKT inhibition independently inhibited the downregulation of adhesion molecules by HGF and completely abolished the migration of these 2 cell lines, suggesting that activation of p-AKT via the HGF/c-MET axis is involved in HGF-induced uveal melanoma cell migration (105, 106). c-MET blockade using the small molecule SU11274 significantly inhibited both cell proliferation and migration in all uveal melanoma cell lines tested (103).

HGF and its receptor tyrosine kinase c-Met play essential roles in the processes of liver embryogenesis and in hepatic regeneration following injury in the adult state, emphasizing their role as both morphogen and mitogen for this organ (98, 106). Because uveal melanoma metastases preferentially involve the liver, the question arises as to whether local factors within the hepatic environment such as HGF or IGF-1 are responsible for the dominant pattern of metastases seen at this site, and whether inhibition of this pathway could decrease the risk of developing metastatic disease (107). In a series of 60 patients with resected uveal melanoma, higher levels of c-MET expression were associated with a significantly higher risk of death from metastatic disease (79); however, another series of 132 patients with uveal melanoma showed that, whereas expression of both c-MET and IGF-1R was predictive of poor prognosis in univariate analysis, the presence of c-MET alone was not predictive for decreased overall survival (108).

Work further exploring the role of c-MET in the pathogenesis of uveal melanoma is ongoing. Although there is great interest in targeting the HGF/c-MET pathway for the treatment of this disease, with a number of relevant agents currently in clinical development (Table 1), the efficacy of this strategy remains to be determined.

**Emerging insights into the biology of advanced uveal melanoma.** As discussed in the Introduction section, prior studies indicate that uveal melanomas segregate into 2 tumor classes based upon microarray gene expression profiles. Class I tumors have low rates of metastasis, whereas class II tumors are more aggressive and associated with a higher rate of death from metastatic disease (12–15). Harbour and colleagues interrogated a sample of 31 class II uveal melanoma metastatic samples and identified an 84% incidence of somatic mutations in the ubiquitin carboxy-terminal hydrolase breast cancer 1, early onset (BRCA1)-associated protein-1 (BAP1; ref. 109), a component of the ubiquitin proteasome system that has been implicated in cancers such as lung, breast, and renal cell carcinoma (110–113). BAP1 is a deubiquitinating enzyme that interacts with the breast cancer susceptibility gene, BRCA1, via its RING finger domain, but does not appear to function as a deubiquitinase of BRCA1 (114, 115). Unlike other members of the ubiquitin carboxyl hydrolase family, BAP1 possesses a large C-terminal domain which is predicted to coordinate the selective association with potential substrates or regulatory components (116). BAP1 participates in the assembly of multiprotein complexes containing numerous transcription factors and cofactors, and activates transcription in an enzymatic-activity–dependent manner, thereby regulating the expression of a variety of genes involved in various cellular processes (117).

Mutations, deletions, and rearrangements of BAP1 on chromosome 3p21.3 have been detected in lung and lung cancer cell lines, and in sporadic breast tumors (114, 118). Depletion of BAP1 resulted in an altered expression of 249 genes, including key mediators of cell-cycle progression,
DNA replication and repair, cell metabolism, survival, and apoptosis (117). BAP1 has tumor suppressor activity both in vitro and in vivo (119, 120). Expression of wild-type BAP1 significantly abolished tumorigenicity of a human non–small cell lung cancer cell line in nude mice, whereas expression of mutant BAP1 that lacks either deubiquitinating activity or nuclear localization did not suppress tumorigenicity, implying that both deubiquitinating activity and nuclear localization are necessary for the tumor-suppressive activity (119). Suppression of BAP1 by RNA interference has also been shown to inhibit cellular proliferation (120–122).

Microarray analysis comparing 92.1 uveal melanoma cells characterized by a wild-type BAP1 transfect with either control or BAP1 siRNA indicated that the gene expression profile of the BAP1 siRNA–treated cells shifted toward a class II tumor profile versus the class I profile observed in the control-treated cells, indicating a dominant role for BAP1 in the regulation of expression of these genes (109). mRNA levels of CDH1 and c-Kit were increased, whereas those for ROBO1, a neural crest differentiation gene, and the melanocyte differentiation genes CTNNB1, EDNRB, and SOX10 were downregulated. More work is required to elucidate the molecular mechanisms governing the function of BAP1 in uveal melanoma and to assess potential treatment strategies derived from these studies.

Summary
Because there are no effective systemic therapies for uveal melanoma, prognosis after the development of metastatic disease remains dismal. Recent studies have brought to light several key signaling cascades that are implicated in the development and progression of uveal melanoma, some of which serve as potential therapeutic targets. It is becoming increasingly clear that, like many cancers, uveal melanomas comprise a heterogenous group of diseases, each with distinct molecular features. Each molecular subtype may have unique clinical characteristics and may respond best to a specific therapeutic strategy. The identification of effective therapies for uveal melanoma will depend upon our ability to develop clinical trials with this possibility in mind.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Received November 29, 2010; revised February 4, 2011; accepted February 7, 2011; published OnlineFirst March 28, 2011.

References


Published OnlineFirst March 28, 2011; DOI: 10.1158/1078-0432.CCR-10-3169

Downloaded from clincancerres.aacrjournals.org on July 9, 2017. © 2011 American Association for Cancer Research.
Molecular Biology and Therapeutics of Uveal Melanoma


Clinical Cancer Research

Therapeutic Implications of the Emerging Molecular Biology of Uveal Melanoma


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-10-3169

Cited articles
This article cites 119 articles, 43 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/17/8/2087.full#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/17/8/2087.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.