

Uveal Melanoma: an Updated Review

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Abstract

This article reviews the epidemiology, pathophysiology, and treatment of uveal melanoma. Emphasis is placed on differential diagnosis and the genetics associated with tumor development and metastasis. The role of the *BRCA1-associated protein 1 (BAP1)* gene, a suggested uveal melanoma tumor suppressor gene, *EIF1AX*, a gene that encodes a protein that binds mRNA, among other genes, as well as the associated loss of chromosome 3 are discussed. While the treatment of primary uveal melanoma is generally successful, up to approximately 50% of patients ultimately develop metastatic disease; there is currently no FDA approved systemic therapy for metastatic uveal melanoma. The promising role of a variety of treatments is discussed, with emphasis on the immune checkpoint inhibitors.

Keywords

Uveal Melanoma; *BAP1*; *EIF1AX*; Immunotherapy; Differential diagnosis

Epidemiology

Melanomas develop from melanocytes that malignantly transform as a result of environmental or genetically induced changes in their DNA. While such transformation occurs most commonly in the skin, uveal melanoma represents 3-5% of all melanomas occurring in the United States and is the most frequent form of primary intraocular tumor in adults [1,2]; worldwide, the primary intraocular tumor is retinoblastoma with an incidence of 1:15,000 to 1:20,000 live births [3]. Approximately 85-90% of uveal melanomas develop from melanocytes in the choroid, 5-8% in the ciliary body, and 3-5% in the iris [1,4]. Unlike the pathogenesis of uveal melanoma, the malignant conversion of conjunctival melanocytes more closely approximates the development of cutaneous melanoma and is strongly associated with increased sun exposure [4].

In contrast to the rising incidence of cutaneous melanoma, the incidence of uveal melanoma has remained relatively stable at approximately 5 per million since the 1970s [1]. The distribution of uveal melanoma varies depending on sex, race, and geographic location [2]. Males were reported to have a significantly higher age-adjusted incidence of 5.9 per million compared to females, who had an average age-adjusted incidence of 4.5 per million [2,5,6]. Similarly, analysis of data from the European Cancer Registry-based study on survival and care of cancer patients (EUROCARE) in Europe, including 6,673 patients with uveal melanoma diagnosed from 1983 to 1994, revealed standardized incidence rates of 1.3-8.6 cases per million per year [7].

The US data were collected by the Surveillance and Epidemiology and End Result (SEER) program of the NIH which collects and provides reliable US population-based incidence data for a variety of cancers, including uveal melanoma [8]. In the US, there is a higher incidence of uveal melanoma in non-Hispanic whites (~6 per million) compared to Hispanics, Asians and blacks (~1.7, 0.4 and 0.3 per million, respectively) [2]. In contrast, cutaneous melanoma rates are 16 fold higher in whites than blacks [5]. Relative to uveal melanomas, conjunctival and mucosal melanoma rates are only about 2-3 fold higher in whites than blacks [9]. Overall, the rate of uveal melanoma in the US is lower in southern states, though the rate of iris and ciliary body melanomas are higher in southern and coastal states [5]. Uveal melanoma incidence appears to peak around the seventh or eighth decade. While, as mentioned, the incidence of uveal melanoma has remained relatively stable over the last decades [1,10], conjunctival melanomas were reported to have increased in incidence, especially among older white men, and while the incidence tended to rise in individuals 40-59 years of age, this increase was not statistically significant [11].

Risk Factors

Well established risk factors for the development of uveal melanoma include but are not limited to increasing age, lightly pigmented eyes, fair skin, inability to tan, ocular/oculodermal melanocytosis, dysplastic nevus syndrome, and arc welding [1,2,4,12]. The median age of diagnosis is 62 years, with many diagnoses occurring between the ages of 70-79 [1,2]. The lifetime risk of developing a uveal melanoma from oculodermal melanocytosis is 1 in 400 [13] while malignant transformation of choroidal nevi, which can be prevalent

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in Caucasians, is low [14]. That said, giant choroidal nevi (≥ 10 mm in diameter) were estimated to transform into melanoma tumors in 18% of patients over 10 years [15]. Common and atypical cutaneous nevi, cutaneous freckles, as well as iris nevi were all reported to be associated with a higher risk of developing uveal melanoma [16].

As mentioned, the rate of uveal melanoma is many times higher in whites than non-whites. While it is an obvious factor to consider as a cause of cutaneous melanoma, the role of ultraviolet (UV) exposure as a risk factor in uveal melanoma is controversial. In an Australian study, sun exposure was found to be an independent risk factor for choroidal and ciliary body melanoma, but surprisingly, evidence for an association between sun exposure and iris or conjunctival melanoma was not found, though the sample size of the study was small (≤ 25) [17]. Logically, as the pupils constrict during illuminated conditions, it would be expected that the iris would be most vulnerable to the tumorigenic effects of UV radiation; however, uveal melanoma occurs more frequently in the ciliary body and choroid [18]. de Lange et. al., studied the mutational status of 123 tumors and found that the role of UV light exposure varied with the location of the uveal melanoma [19]. Accordingly, anterior or ciliochoroidal melanomas preferentially developed in non-illuminated areas in lightly-colored eyes and posterior choroidal melanomas developed in illuminated regions. As 80% of uveal melanomas appear to be associated with mutations in the Gαq-proteins GNAQ and GNA11 (see below) [20], de Lange et. al.'s study analyzed the substitution mutations specific to these proteins. Interestingly, anterior tumors displayed GNAQ Q209L adenine to thymine mutations ($p=0.002$) while posterior tumors exhibited GNAQ/GNA11 Q209P adenine to cytosine mutations ($p=0.028$). Li and colleagues [21] reported that melanomas tended to develop in the macular area, with fewer occurring closer to the ciliary body. This distribution pattern correlated positively with the dose distribution of solar light on the retinal sphere, supporting a role for solar exposure in the induction of uveal melanoma. In another study using geographic tumor mapping, data suggested that UV exposure was unlikely to be responsible for inducing the development of choroidal melanoma in a study of 92 uveal melanomas [22]. These conflicting results must await reconciliation by future studies. Whether a protective effect of UV-generated vitamin D might mask its genotoxic effects has been speculated [23]. Finally, in a small study of 12 uveal melanomas, a UV radiation DNA damage signature was not identified [24].

Occupational cooking has also been suggested as a risk factor for uveal melanoma in a meta-analysis (OR: 1.81, 95% CI 1.33-2.46, $p<0.001$) [25]. Use of a mobile phone and occupational pesticide exposure were not proven risk factors for tumor development [26,27].

Etiology, Pathophysiology, Genetics, and Prognosis

Melanocytes differentiate from pluripotent neural crest stem cells [20]. These cells provide pigment to the skin, iris, ciliary body, choroid, and mucosal membranes of the body. An unregulated proliferation of melanocytes in any of these locations results in melanoma. As mentioned above, current evidence suggests that most uveal melanomas occur de novo and are infrequently the result of a transformed suspicious nevus [4]. For example, even though iris nevi are relatively common, their rate of transformation into a melanoma is only about 5% at 10 years [28]. Earlier diagnosis likely plays an important contributing role in the relatively good prognosis of patients with an iris melanoma, which is often managed with close monitoring, although large or fast-growing tumors may require immediate treatment. Iris melanomas are inherently more indolent and less likely to metastasize than ciliary body or choroidal melanomas.

Fatalities from all forms of melanoma are the result of metastasis [20]. Uveal melanoma is an aggressive form of cancer leading to metastasis in about 50% of cases, preferentially to the liver. While tumor location, thickness, diameter, and histopathology independently influence the development of metastatic disease [29-31], as a single modality, its genetic and molecular makeup may be

a more reliable marker [32,33]. Adding size to genetic analysis was shown to provide additive power for estimating metastatic potential [34].

While the MAPK pathway is activated and up-regulated in all forms of melanoma, the responsible somatic mutations differ between cutaneous and uveal lesions. Cutaneous melanoma (and most forms of conjunctival melanoma) is most commonly characterized by mutations in *BRAF* (40-50%), *NRAS* (15-20%) and more rarely *KIT* and *NFI* [2,17]. As mentioned, uveal melanomas are often associated with a mutation in Gαq-proteins GNAQ and GNA11 (80% of cases) [20]; mutations in genes *GNAQ* and *GNA11* are mutually exclusive. It is thought that these mutations increase proliferation by upregulating the MAPK pathway, but do not necessarily induce malignancy. Studies have also implicated *CYSLTR2*, which encodes a Leu129Gln substitution and drives phorbol ester-independent growth, as a uveal melanoma oncogene that occurs in a mutually exclusive manner with *GNAQ* and *GNA11* [35,36]. These mutations act as an initiating event and signal a change in chromosomal composition. In addition to being characteristic of primary uveal tumors, *GNAQ* mutations are also seen in epidermal lesions such as blue nevi (Mongolian spot), conjunctival lesions (Nevi of Ota), and peri-orbital lesions [37]; about 1 in 400 patients with a blue nevus ultimately develops uveal melanoma. Since *GNAQ* or *GNA11* gene mutations are also found in nevi, their presence cannot be used to predict the development of metastases [38,39] or patient outcome [40].

The mechanism by which uveal melanoma cells metastasize remains poorly understood. The presumption is that micrometastases spread throughout the body at the time of diagnosis [41]. While cutaneous melanomas metastasize through the lymphatic system, uveal melanoma spreads by way of the blood [42]. Models for estimating metastatic potential include the Liverpool Uveal Melanoma Prognosticator Online (LUMPCO), which estimates all-cause and melanoma-specific mortality well [43], and Prediction of Risk of Metastasis in Uveal Melanoma (PRiMeUM), which predicts an individual's personal risk of metastasis based on their individual and tumor characteristics with accuracy over 80% [44].

Inactivation of the *BRCA1*-associated protein 1 (*BAP1*) gene, a suggested uveal melanoma tumor suppressor gene, was reported in about 80% of metastatic uveal melanomas; this gene encodes a deubiquitinating enzyme [45]. Diagnoses made at a younger age are more likely to be associated with the presence of a *BAP1* mutation [1]. *BAP1* mutations in uveal melanoma cells seem to independently predict metastatic death and are associated with larger tumor size and ciliary body involvement [29]. However, *BAP1* mutations are not more frequently represented among metastatic lesions [46]. *BAP1* mutations correlate with reduced expression of *BAP1* protein and several studies have reported a link between *BAP1* immuno-staining and genetic analysis as well as the identification of a subgroup of atypical poor-prognosis disomy 3 (D3 - see below) patients [47,48]. Interestingly, germline mutations in *BAP1* have been associated with *BAP1*-Tumor Predisposition Syndrome (*BAP1*-TPDS), a condition that also predisposes patients to renal cell carcinoma, malignant mesothelioma, and cutaneous melanoma [49,50]. However, while 36 of 59 metastasizing tumors in one study carried a *BAP1* mutation, only 7% carried germline mutations compared to 54% with somatic mutations [49].

Clinicians have relied on the confirmed absence of chromosome 3, or monosomy 3 (M3), within a tumor sample to predict a patient's relapse free time and overall survival [51]. Gene expression profiling (GEP), which utilizes an RNA sample from the tumor, led clinicians to assign tumors to one of four subclasses - Class 1A, 1B, 2A and 2B [52,53] using an assay licensed to Castle Biosciences, Inc. which provides it for clinical use under the trade name DecisionDx-UM. Class 1 and Class 2 signatures predict low or high metastatic risk within the first five years after diagnosis, respectively [30,52]. For genetic analysis, if an eye has been enucleated, the tissue sample is collected from the removed eye; if not, a tissue sample is collected through fine needle aspiration biopsy (FNAB). Recent data suggest there is no increased metastatic risk after intraocular tumor biopsy [54].

Class 1A tumors express minimal aneuploidy and tissues reveal normal differentiated melanocytes [42,54]. Class 1B tumors exhibit chromosome 6p gain, which is also seen in retinoblastoma tumors [55]. Generally, Class 1 tumors are composed of spindle shaped-cells, have a low vascular/inflammatory profile, and exhibit disomy 3 (D3), 6p gain and a mutation in *EIF1AX*; this latter gene encodes a protein that interacts with mRNA, being a component of the 43S pre-initiation complex, and plays a role in the initiation of translation [54]. Mutations in *EIF1AX* were shown to protect against metastasis [56], even after adjusting for the effect of other known risk factors [57]. Interestingly, Ewens et. al., found that the combination of M3 with the *EIF1AX*-WT (risk) allele was significantly associated with metastasis (OR 29.7) and remained significant after adjustment for other tumor variables (OR 31.4) [58].

Class 2A tumors express M3 and Class 2B tumors express M3 and exhibit an 8p loss. Class 2 tumors are typically composed of epithelioid or mixed cell types and incorporate tumor-associated macrophages. They also display an increased vascular density and a mutation in *BAP1* [54,59]. The metastatic propensity of Class 2 tumors is believed to develop as a two-step process beginning with a loss of chromosome 3, followed by a deletion of the tumor suppressor gene *LZTS1* on chromosome 8p [42]. On the other hand, the presence of a gain of 6p and loss of 6q is usually associated with better patient survival even when chromosome 3 and 8 abnormalities are also present [42,53,60]. While Class 2 tumors have been associated with older age and a thicker mean ultrasound measurement prior to treatment, the identification of a characterizing clinical marker has yet to be discovered [59]. *PRAME*, a gene that expresses a surface protein targeted by cytotoxic T cells was reported to be associated with a shorter time to metastasis and increased risk of melanoma-associated mortality, and to be independently predictive when added to Class 1 or Class 2 distinction [61]; this protein is not expressed in normal tissues, except the testis [Table 1].

Analysis of 120 uveal melanoma tumors for numerical changes in chromosomes 1, 3, 6, and 8 with cytogenetic analysis, fluorescent *in situ* hybridization, and/or comparative genomic hybridization demonstrated that concurrent loss of the short arm of chromosome 1 and all of chromosome 3 is an independent predictor of decreased disease-free survival [62]. Use of multiplex ligation-dependent probe

amplification (MLPA) to detect abnormalities in chromosomes 1p, 3, 6q, 8p, and 8q showed that 10 year disease-specific mortality was 0% in 133 tumors with no chromosome 3 loss, 55% in tumors with chromosome 3 loss but no chromosome 8q gain, and 71% in 168 tumors showing combined chromosome 3 loss and 8q gain. In tumors with both of these abnormalities, epithelioid melanoma cytomorphology, closed microvascular loops, and high mitotic rate correlated with poor survival as did lack of chromosome 6p gain. These data support the use of MLPA for routine clinical prognostication, especially if the genetic data are considered together with clinical and histologic risk factors [63].

Despite prognostic correlations with the expression of a small panel of marker genes, with M3, and with *BAP1* aberrancy, the molecular pathways involved in the development of metastatic disease have not been elucidated, as the authors of the elaborate Rare Tumor Project of The Cancer Genome Atlas (TCGA) study pointed out [64]. This group performed a global and integrated molecular characterization of 80 primary uveal melanomas in an attempt to uncover the distinctions in the biological processes that underlie tumors that vary in aggressiveness. Their study identified four molecularly distinct, clinically relevant subtypes: two associated with poor-prognosis M3 and two with better-prognosis D3. *BAP1* loss was shown to follow M3 occurrence and correlated with a global DNA methylation state that is distinct from D3-uveal melanoma. Poor-prognosis M3-uveal melanoma subsets were shown to have distinct genomic, signaling, and immune profiles, and *EIF1AX* and *SRSF2/SF3B1* mutant D3-uveal melanoma had different genomic/DNA methylation profiles. Mutations in *EIF1AX* were previously reported in non-metastasizing tumors [65] and a hotspot mutation in *SF3B1*, the splicing factor 3 subunit 1-gene was detected in late metastasizing tumors [66,67]. *SF3B1* mutations are associated with a small group of cancers and in uveal melanoma, they are almost always mutually exclusive of *BAP1* mutations [68]. Developing a clinically relevant classification system was suggested to require prospective evaluation of copy number and/or gene expression data in tumors with similar clinical-pathological features to identify patients with higher- versus lower-risk M3-uveal melanoma, and to validate the differential metastasis intervals observed in the TCGA. Targeted next-generation sequencing should facilitate the prediction of patients' metastatic risk and potentially assess eligibility for new therapies [69].

Differential Diagnosis

While choroidal melanomas can leak fluid beneath the retina, causing the retina to detach and result in symptoms of flashing lights and floating specks, most uveal melanoma patients are asymptomatic and have their tumors discovered during a routine eye examination. Differential diagnosis must rule out large uveal nevi, which are most similar in appearance to small uveal melanoma tumors [70], and tumors that have metastasized to the uvea, especially from breast and lung cancers, which can be the first indication of an occult primary tumor [71]. Diagnosis is based on the results of a fundoscopic examination followed by ultrasound; optical coherence tomography (OCT) may be useful in some cases to differentiate the lesion from a choroidal metastasis. Studies reported that PET-CT is not sensitive in the diagnosis of malignant uveal melanoma [72,73]. Common lesions which simulate a uveal melanoma are described below, which in addition to suspicious choroidal nevi include central and peripheral exudative hemorrhagic chorioretinopathy, congenital hypertrophy of the retinal pigment epithelium (CHRPE), choroidal hemangiomas [74] and choroidal osteomas (Figure 1).

A suspicious choroidal nevus (Figure 2) is the most common mass prompting referral to ocular oncology specialists. Complicating the diagnosis, choroidal nevi may range from hyper-melanotic to hypo- and amelanotic, and exist in approximately 6.5% of the white population [75]. In this population, one in 8,000 choroidal nevi transform into a melanoma [76]. Modeling the clinically successful ABCD mnemonic (*asymmetry*, irregular *border*, *color* variegation, and *diameter* larger than 6mm) which aided in the identification of

	Low Metastatic Potential	High Metastatic Potential
Uveal location	Iris	Ciliary Body Choroid
*GEP class	Class 1	Class 2
Histopathology	Spindle cell type Low vascular/ inflammatory density	Epithelioid/mixed cell type High vascular/ inflammatory density
Gene mutation	Chromosome 6p gain <i>EIF1AX</i>	Monosomy 3 <i>BAP1</i> Chromosome 8p/8q imbalance <i>PRAME</i>
Tumor characteristics: T1-T4 (risk increases with increased tumor category) [27]	T1,T2 tumor thickness: ≤ 5.2 mm T1, T2 base diameter: ≤ 12 mm Absence of: mushroom configuration subretinal fluid extraocular extension	T3,T4 tumor thickness: ≥ 8.9 mm T3, T4 base diameter: ≥15 mm Presence of: mushroom configuration subretinal fluid extraocular extension

*Gene Expression Profiling (GEP)

Table 1: Metastatic Risk Profile

cutaneous melanoma, ocular oncologists developed a system intended to be used by eye care practitioners lacking advanced diagnostic equipment [76]. Thus, clinicians are prompted to make referrals based on findings guided by the mnemonic “To find small ocular melanoma using helpful hints daily,” which describes a suspicious nevus observed ophthalmoscopically and ultrasonographically with a *thickness* greater than 2mm, *subretinal fluid*, present *symptoms*, *orange* pigment (lipofuscin), *margin* within 3mm of the optic disc, *hollowness* on ultrasound, absent *halo*, and absent *drusen*. Additional testing that assists in the differentiation between a benign, suspicious choroidal nevus and a small malignant melanoma includes fluorescein angiography when the nevus is confined to the outer retina or has not altered the overlying pigment epithelium [77], OCT, and auto-fluorescence. Uncomplicated choroidal nevi will commonly reveal an area of hypo-fluorescence within the area of hyper-pigmentation; if the nevus has caused changes within the inner retina, it may begin to mimic the angiography of a malignant melanoma and will require further testing [77]. OCT and auto-fluorescence are particularly helpful in detecting subdued subretinal fluid and lipofuscin [76]. All patients with an indeterminate, suspicious choroidal nevus should be monitored frequently for evidence of growth and transformation.

The second most common pseudomelanoma is central exudative hemorrhagic chorioretinopathy (CEHC), followed by peripheral

exudative hemorrhagic chorioretinopathy (PEHC), which, but for its extramacular location, is similar to CEHC. This pathology is usually comfortably distinguished from malignant melanoma using indirect ophthalmoscopy and fluorescein angiography [78]. Additionally, evidence of age-related degeneration is usually present in the fellow eye, aiding in the diagnosis. Gass [77], however, cautioned the clinician to be alert to a malignant choroidal lesion masked by overlying angiographic findings of exudative hemorrhagic chorioretinopathy.

CHRPE (Figure 3) is the fourth most common simulating lesion, usually differentiated from a malignant melanoma by its ophthalmoscopic appearance alone, characterized by well-delineated margins, possible lacunae and a marginal halo [74,79]. While this lesion is typically flat, it is comprised of relatively large retinal pigment epithelial cells with large pigment granules that may moderately elevate the mass [77].

The fifth most common pseudomelanoma is a localized hemangioma of the choroid, which may be mistaken for an amelanotic melanoma or metastatic tumor [74,77]. An appropriate diagnosis can be made based on the noticeable orange-red color of the hemangioma, ultrasonography, and fluorescein angiography [77,80]. The ^{32}P uptake test has historically been used to aid in the diagnosis, though its clinical application is controversial [81,82].

Less commonly, a melanocytoma of the iris, ciliary body (Figure 4), and choroid may be mistaken for uveal melanoma [74]. Melanocytomas can occur anywhere along the uveal tract and may even emerge through a scleral emissary canal, resulting in conjunctival involvement [83]. Most melanocytomas arise from the juxtapapillary choroid; 2% of optic nerve head melanocytomas (Figure 5) undergo malignant transformation, and a smaller percentage of ciliary body and iris melanocytomas transform [83-85]. Herald signs of transformation include decreased vision, vitreous seeding, changes in intraocular pressure, and growth [86-88]. Thus, such lesions should be monitored for signs of malignancy.

Distinguishing retinal and choroidal detachments from malignant melanoma is aided by the use of fluorescein angiography and ultrasonography [77,89]. Taking a thorough patient history commonly identifies metastatic tumors to the choroid, as the patient will usually have a previous diagnosis of systemic malignancy or will present with ocular symptoms consistent with a systemic etiology [90].

Following the diagnostic protocol developed and reported in the 1990 Collaborative Ocular Melanoma Study (COMS), a misdiagnosis rate of only 0.48% was found in enucleated eyes gathered from various clinical centers located throughout the US and eastern Canada [91]. The results of this particular arm of the COMS study were so positive that the authors concluded that the major hurdles to the correct diagnosis of posterior uveal melanoma were overcome. Notably, more recent studies have tempered this enthusiasm, reporting misdiagnosis rates of up to 9.4% [92]. However, the first

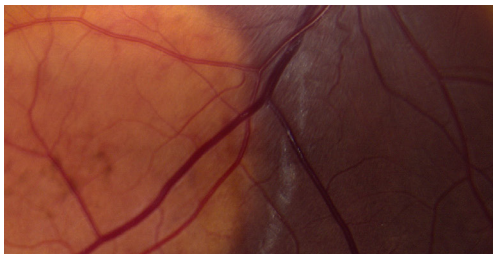


Figure 1: Choroidal Osteoma

Choroidal osteomas are similar in appearance to amelanotic choroidal melanomas. While osteomas are benign tumors composed of ossified choroid, they can experience growth. They can be distinguished from melanomas using ultrasonography, computerized tomography (CT), fluorescein angiography and optical coherence tomography (OCT).

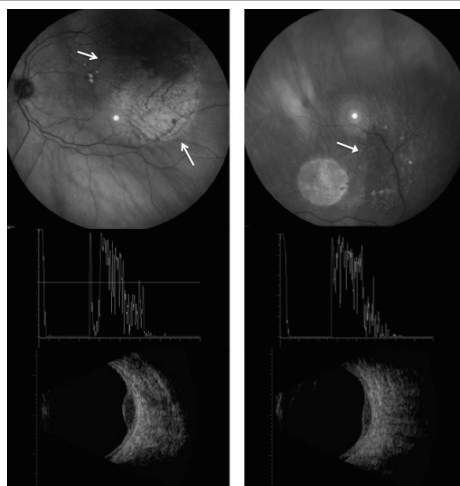


Figure 2: Choroidal Nevus

Image rows from top to bottom: fundus autofluorescence, A-scan, transverse B-scan. The images on the left and right show a dome-shaped choroidal melanoma and a similarly shaped choroidal nevus (indicated on the fundus images), respectively. An A-scan of the melanoma revealed low internal reflectivity and peaks of equal height, indicating a regularly structured lesion. In contrast, the A-scan of the nevus revealed medium to medium-high internal reflectivity and an irregular structure, consistent with a benign nevus.



Figure 3: CHRPE

This CHRPE was referred to an ocular oncology specialist due to its large size and presence of lacunae mimicking lipofuscin.

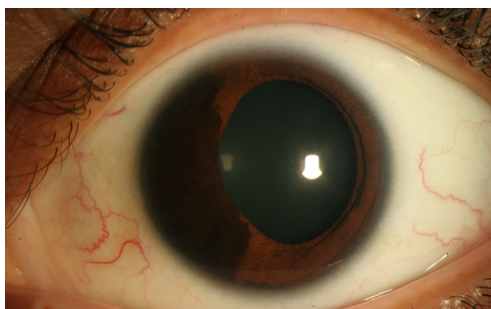


Figure 4: Ciliary Body Melanocytoma

Partial heterochromia in this eye is due to the presence of a ciliary body melanocytoma simulating a choroidal body melanoma. On gonioscopy, this lesion showed invasion of the iridocorneal angle.



Figure 5: Optic Nerve Head Melanocytoma

Note the substantial vitreal penetrance in this optic nerve head melanocytoma. A small percentage of these tumors do grow over several years but remain benign.

and only single-centered, population-based data on the rates of clinical misdiagnosis of posterior uveal melanoma in the era of modern diagnostic imaging reported that 2% of posterior uveal melanoma cases were clinically misdiagnosed [93].

Treatment

Primary uveal melanoma can be treated with plaque brachytherapy [94,95], charged particle radiation therapy [96,97] and photon stereotactic radiation therapy [98,99] though the latter treatments have been associated with ocular complications including dry eye, cataracts, uveitis, vitreous hemorrhage, and retinal detachment, among others [100]. High radiation doses are required as uveal melanoma cells are radio-resistant. In several studies, enucleation did not provide a survival advantage over radiotherapy [101-103]. Notably, in the COMS, 1,317 patients were randomly assigned to receive radiotherapy or enucleation and results showed no significant difference in survival between the two groups [104].

While the above treatments are generally successful, up to approximately 50% of patients ultimately develop metastatic disease; initial presentation with metastases is rare [105]. To date, there is no consensus regarding the optimal surveillance strategy following primary treatment and what role, if any, "liquid biopsy" might play in directed targeted therapies remains to be elucidated. A stepwise surveillance protocol based on serial hepatic ultrasonograms followed by confirmatory scans was reported to offer a high likelihood of detecting asymptomatic metastases in patients with primary uveal melanoma, while liver function tests did not [106,107]. While a chest X-ray is not generally effective in detecting metastatic disease, it did detect rare metastases to the heart in a recent study

[108]. On average, a quarter of patients die within five years of initial diagnosis, with nearly half dead within 15 years [11,109,110]. The most common initial metastatic sites, in descending order of frequency, are the liver, lung, skin/soft tissue, and bone [2], with the liver being the site of secondary growths in about 75% of patients. As mentioned above, cells that break away from primary uveal tumors disseminate through the blood. Accordingly, it is still unclear why metastatic growths do not develop at high rates in the capillary beds in the lungs, since those would be the first narrow vessels traversed by these cells. Once metastatic disease develops, median overall survival is approximately 13 months, with only 8% surviving two years [109,111,112], though 22% survived more than four years in another study [113].

There is no FDA approved systemic therapy for metastatic uveal melanoma as none have been shown to improve overall survival. Generally unsuccessful treatments have included bland embolization (BE), chemoembolization, radioembolization, immunoembolization, and hepatic arterial infusion of chemotherapy [114,115]. Accordingly, while percutaneous hepatic perfusion with melphalan, perhaps the approach most studied, showed an improvement in hepatic PFS, no improvement in overall survival was observed (10.6 vs 10.0 months; $p=0.77$) [116]. In a case series that combined data from two major centers, melphalan percutaneous hepatic perfusion (M-PHP) was reported to be safe in appropriately selected patients with primarily liver based disease, and outcomes compared favorably to currently available treatment modalities [117]. In a small randomized trial without clinical outcomes (phase II study), hepatic immunoembolization with granulocyte-macrophage colony-stimulating factor (gm-CSF) resulted in an overall response rate of 21.2% vs. 16.7% with bland embolization (BE). OS times were 21.5 months with immunoembolization and 17.2 months with BE [118].

Systemic chemotherapy with dacarbazine, an alkylating agent that, along with the other agents mentioned below was the standard of care for metastatic cutaneous melanoma prior to the development of immunotherapy, and adjuvant interferon offered no survival advantage compared to observation and matched historical controls following primary tumor treatment, respectively [119-121]. Other chemotherapeutic agents that failed to demonstrate efficacy include cisplatin, temozolomide, treosulfan, and fotemustine. Most prospective trials have been single-arm, phase II studies, which have generally demonstrated response rates under 10%, progression-free survival (PFS) less than five months, and overall survival (OS) less than one year [122]. The foregoing notwithstanding, the general recommendation is that patients enroll in a clinical trial when possible.

Data suggest that the response of tumor cells to hepatocyte growth factor (HGF) and insulin-like growth factor I (IGF-I), in addition to certain chemokines, may play a role in promoting liver metastatic growth [123]. Accordingly, some treatment strategies have concentrated on the inhibition of the receptor tyrosine kinases, MET and KIT, that are highly expressed in uveal melanoma. Crizotinib, an inhibitor of MET, significantly reduced metastatic growth in a murine model [124]. In a retrospective cohort study, the effectiveness of sunitinib, a multi-targeted receptor tyrosine kinase inhibitor first approved for the treatment of renal cell carcinoma, was compared to institutional controls [125]. Patients in the sunitinib group had worse cytogenetic/molecular features (M3 and 8q amplification or class 2; 87% vs. 57%), had smaller tumor sizes (T3-4 56% vs. 83%), and were younger. In the univariate analysis, the sunitinib group had longer OS (hazard ratio, 0.53). The following variables were statistically associated with prediction of OS: cytogenetic/molecular status, T-size category, gender, and adjuvant sunitinib in patients <60 years of age [125].

As mentioned, *BAP1* mutations in uveal melanoma cells seem to independently predict metastatic death and are associated with a larger tumor size and ciliary body involvement. Furthermore, germline mutations in *BAP1* were also reported to be more common in familial uveal melanoma. *BAP1* protein loss, as determined by immunohistochemistry was suggested as a rapid and cost-effective

means of identifying patients with aggressive disease; factors associated with improved survival were diffuse or heterogeneous *BAP1* expression [126]. More recently, non-genetic mechanisms were suggested to account for the loss of nuclear *BAP1* in some patients [127]. These authors also described a subset of nuclear *BAP1*-negative tumors in which *BAP1* was sequestered in peri-nuclear bodies, most likely within Golgi.

Histone deacetylase (HDAC) inhibitors induced morphologic differentiation, cell-cycle exit, and a shift to a differentiated, melanocytic gene expression profile in cultured uveal melanoma cells, and the anti-convulsive agent valproic acid (VPA) inhibited the growth of uveal melanoma tumors in vivo [128]. Based on these results, VPA and the HDAC inhibitor vorinostat are being evaluated in ongoing clinical trials (NCT03022565 and NCT02068586). While BRAF mutations are typically seen in cutaneous melanoma, they are not seen in uveal melanoma and as such, BRAF inhibitors are not a treatment option in these patients.

In light of the importance of Gαq-protein signaling in uveal melanoma tumors, therapies targeting downstream receptor pathways such as MAPK and PI3K/AKT have been explored. A recent review analyzed the results from six studies that examined the effects of selumetinib +/- dacarbazine (n = 3), trametinib +/- an AKT inhibitor (n = 2) and binimetinib plus sotrastaurin (n = 1) from three open-label phase II, two open-label phase I and one placebo-controlled phase III trials [129]. The overall response rate was available in five studies and ranged from 0 to 14% with an average of 2.5%. The median PFS ranged from 3.1 weeks to 16 weeks. Data on OS and one-year survival rates were not consistently reported. Patients displayed severe treatment-related adverse events, most commonly for the combination use of selumetinib plus dacarbazine (62%) and binimetinib plus sotrastaurin (75%). These authors concluded that uveal melanoma cells were not inhibited by these agents. Other agents that have failed to demonstrate effectiveness include those targeting EGF [130] and VEGF [130-133]. Recently, expression of ADAM10 (a disintegrin and metalloprotease-10) was reported to be associated with a more rapid metastatic progression; whether the targeting of this molecule might enhance survival remains to be determined [134].

To date, only limited activity has been observed in patients treated with immune checkpoint inhibitors, despite their demonstrated effectiveness for the treatment of cutaneous melanoma. However, a retrospective review of the Dana-Farber Cancer Institute, Massachusetts General Hospital, Memorial Sloan-Kettering Cancer Center, and University Hospital of Lausanne experience reported durable responses to the CTLA-4 inhibitor ipilimumab, with manageable toxicity [135]. Accordingly, in two studies of the effectiveness of ipilimumab, median OS ranged from approximately seven to ten months [136,137]; a study using tremelimumab, another anti-CTLA-4 antibody, was terminated for lack of efficacy [138]. In an early study in 10 patients of the effectiveness of pembrolizumab, a PD-1 blocking antibody, there was one complete response, two partial responses, and one patient with stable disease; four patients had rapidly progressive disease [139]. In a multicenter, retrospective series, 56 patients with stage IV uveal melanoma received PD-1 or PD-1 ligand (PD-L1) antibodies between 2009 and 2015 at nine academic centers; 48 patients (86%) had received prior therapy, and 35 (63%) had received treatment with ipilimumab [140]. Three patients had an objective response to ipilimumab, and eight had stable disease as their best response. Thirty-eight patients (68%) received pembrolizumab, 16 (29%) received nivolumab, and two (4%) received atezolizumab. Objective tumor responses were observed in two patients for an overall response rate of 3.6%; stable disease (≥6 months) was observed in five patients (9%). The median PFS was 2.6 months and the median OS was 7.6 months. There was no association between prior treatment with ipilimumab or liver-directed therapy and PFS or OS. More recently, the E1L3N clone of the anti-PD-L1 monoclonal antibody was reported to be a specific marker for PD-L1 in uveal melanoma and its expression correlated with a better outcome and decreased numbers of tumor-infiltrating lymphocytes

(TILs) [141]. The authors speculated that this antibody clone may have the potential to identify subgroups of uveal melanoma patients that might benefit from PD-1/PD-L1 pathway blockade.

Combination treatment with anti-PD-1/anti-CTLA-4 therapy showed a durable response in a 72-year-old Caucasian male despite possessing worse prognostic features (*GNA11* mutation, older age at presentation, male gender, short metastasis free interval and extraocular extension) [142]. The patient achieved a durable response to the combination therapy despite early progression from the original melanoma treatment and continued to do well 22 months after four cycles of ipilimumab/nivolumab followed by one dose of nivolumab, without any evidence of the recurrent disease. Clinical trials to assess the efficacy of combination therapy are ongoing.

In the TCGA study referenced above, mRNAs for the immune checkpoint inhibitors IDO1 and TIGIT were found to be among the most highly expressed in CD8-enriched M3 uveal melanomas. The authors speculated that this may account, in part, for the clinical observations suggesting that single-agent anti-CTLA-4 or anti-PD1 immune checkpoint inhibitors have low efficacy in patients with metastatic disease. They further suggested that agents targeting IDO1 and/or TIGIT, which are currently in clinical trials, may help overcome immune suppression in uveal melanomas.

In light of the responses reported to adoptive T-cell therapy in different refractory solid tumors, there has been an interest in examining the effects of such therapy in uveal melanoma. In the first of its kind study in metastatic uveal melanoma patients, individuals with histologically confirmed metastatic uveal melanoma were enrolled in an ongoing single-center, two-stage, phase 2, single-arm trial to determine whether adoptive transfer of autologous TILs could mediate regression of their metastatic growths [143]. Results showed that of 21 enrolled patients, 7 (35%) of 20 evaluable patients had objective tumor regression. Among the responders, six patients achieved a partial response, two of which are ongoing and have not reached maximum response. One patient achieved complete response of numerous hepatic metastases, for at least 21 months post therapy. Three of the responders were refractory to previous immune checkpoint blockade. Common grade 3 or worse toxic effects were related to the requisite lymphodepleting chemotherapy regimen and included lymphopenia, neutropenia, and thrombocytopenia (21 [100%] patients for each toxicity), anemia (14 [67%] patients), and infection (6 [29%] patients). There was one treatment-related death secondary to sepsis-induced multiorgan failure. The data appear to support the further investigation of immune-based therapies in metastatic uveal melanoma patients. Further supporting this approach is a report that adjuvant treatment with dendritic cell vaccination in high risk uveal melanoma patients correlates with favorable overall survival in those with a detectable tumor antigen-specific immune response after vaccination [144].

In a small study of the effects of IMCgp100, a bispecific molecule comprised of a targeting end that constitutes a soluble T cell receptor targeting glycoprotein, a uveal melanoma antigen, and an effector end targeting CD3, 20% of patients achieved a partial response and 47% had stable disease at eight weeks. The disease control rate was 53% at 16 weeks and 40% at 24 weeks [145]. A phase I trial of IMCgp100 using a dose escalation strategy reported objective responses in 11% of patients with 63% showing stable disease at around 170 days post treatment. The estimated OS at one year was 79.5% [146].

Conclusion and Future studies

The methods currently used for treating primary uveal tumors are generally highly successful in obliterating the tumor. However, vision often deteriorates and the treated eye is vulnerable to development of retinal vascular occlusions, vitreous hemorrhages, and retinal detachments, even after conservative treatment using laser photocoagulation. Thus, active research is being done to reduce subsequent retinal damage in order to preserve vision. One approach being investigated is the treatment of small primary choroidal melanomas with an intravitreal injection of a papillomavirus-like particle (VLP) [147]. The viral nanoparticle is conjugated to an

infrared activated photodynamic dye. Within the vitreous, the virus selectively binds to malignant cells due to their over expression and modification of heparin sulfate proteoglycans, leading to the destruction of the cell when exposed to an ophthalmic laser. As noted above, a majority of choroidal melanomas occur within the posterior pole and are therefore more likely to reduce central vision following treatment. Further exploration of vision-preserving treatment options will benefit the patient and are thus likely to be the focus of ongoing research.

Because of the important role mutations in genes such as *GNAQ*, *GNA11* and *BAP1* have in the development and prognosis of uveal melanoma, clinical trials that concentrate on altering components within these pathways are, and will, likely be an area of investigation over the coming years. As previously mentioned, there is as yet no FDA approved treatment for metastatic uveal melanoma. While the use of genetic biomarkers is currently the most reliable method for predicting patient outcome, how specifically these changes contribute to metastasis and mortality require further investigation. Furthermore, what role epigenetic changes may play in this process must also be explored, hopefully paving the way for new adjuvant therapy trials. Ways to predict responsiveness to immunotherapy with other targeted pharmaceuticals, and the potential use of immunotherapeutic antibodies to target both primary and secondary tumors, all need to be investigated.

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