Uveal Melanoma

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Preface

Traditionally, uveal melanomas have been afforded a single chapter in textbooks on its more populous cousin, cutaneous melanoma. However, advances in understanding its unique biology, coupled with the necessary multidisciplinary teamwork so different than what is required for cutaneous melanoma, makes a stand-alone volume for this disease necessary and imperative.

UM is a well described yet rare disease. The most common ocular malignancy, its care requires coordination between ophthalmologists, radiation therapists, molecular biologists, pathologists and medical oncologists. Again, unlike melanomas arising in the skin from UV damage, these tumors have a low tumor mutational burden, do not yet have driver mutations that can be targeted (like BRAF V600E mutations) and are poorly responsive to immunotherapeutic agents that have transformed the care of patients with advanced cutaneous melanoma.

Yet there is reason to be far less nihilistic regarding the care of this disease than in the past. Plaque brachytherapy and other radiation techniques have substantially improved the care of the eye. Gene expression profiling at the time of diagnosis allows better prognostication for chances of recurrence, allowing patients and physicians to better surveil patients. While immunotherapy has not had the responses that had been hoped for after seeing the results in cutaneous melanoma, there still are patients who have substantial benefit from these medications and novel immune approaches are entering clinical trials.

Optimal care of the patient with uveal melanoma highlights the need for coordination across a multidisciplinary team of many specialties. It is also hoped that, as we gain better understanding of strategies to improve responses in tumors with low mutational burdens, we will not only improve survival of these patients but perhaps provide a model that can be extended to other difficult-to-treat tumors.

We dedicate this book to our patients and their families.

Houston, TX, USA — Eric H. Bernicker

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Part I
Basic Science

Chapter 1
Molecular Basis of Uveal Melanoma and Emerging Therapeutic Targets

J. William Harbour and Zelia M. Correa

Introduction

Uveal melanoma (UM) is the most common primary cancer of the eye and can arise from the choroid, ciliary body, and iris (Fig. 1.1). Despite successful treatment of the primary tumor and an absence of systemic dissemination at initial presentation in most cases, up to half of patients will later develop deadly metastatic disease. The most common site of initial metastasis is the liver in over 90% of patients, followed by the lung, bone, and subcutaneous tissue [1]. Clinical and histopathologic features associated with increased risk of metastasis include advanced age, ciliary body location, larger basal diameter, epithelioid cells, vasculogenic mimicry extracellular matrix patterns, lymphocytic and monocytic infiltrate, and extrascleral tumor extension [2, 3]. In recent years, key genetic events and their relationship to prognosis in UM have been elucidated. Fundamentally, UM can be divided based on gene expression profile into class 1 (low metastatic risk) and class 2 (high metastatic risk) tumors [4]. This molecular classification has been found to be more accurate than clinical, histopathologic, and chromosomal prognostic factors, and it has been prospectively validated as a clinical test that is now widely used for routine patient prognostication [5–10]. Class 2 tumors are associated with mutational inactivation of the tumor suppressor BAP1 [11], the loss of which leads to the dedifferentiated "epithelioid" melanoma cells that have a penchant for liver metastasis [11–13]. In contrast, class 1 tumors usually have mutations in either EIF1AX or SF3B1, associated with low and intermediate metastatic risk, respectively [14–16]. Another important prognostic factor in UM is the cancer-testis-antigen PRAME, which is normally expressed only in testis but becomes aberrantly reexpressed in UM and

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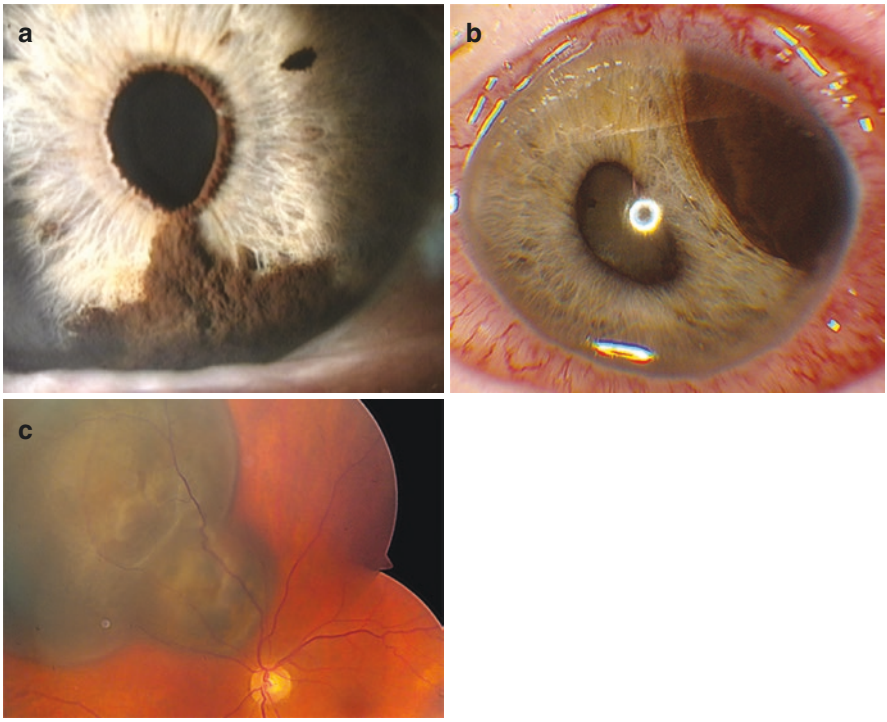

Fig. 1.1 Clinical presentations of uveal melanoma. (**a**) Iris melanoma. (**b**) Ciliary body melanoma. (**c**) Choroidal melanoma

many other cancer types, where it is usually associated with poor patient outcome [17–19]. PRAME expression worsens the prognosis of patients with both class 1 and class 2 UM [17, 18], such that PRAME expression has now been incorporated into routine clinical prognostic testing alongside the GEP class 1/class 2 assay for UM [20].

Recent Genomic Findings in Uveal Melanoma

Recent advances in technology have allowed these and other key genetic determinants of clinical outcome in UM to be understood in the context of tumor evolution and microenvironment. Bioinformatic methods have allowed bulk (whole tumor) next-generation DNA sequencing data to be deconvoluted in order to infer the evolutionary order in which genetic events accrue during UM progression [21]. Such models indicate that the Gαq family of mutually exclusive mutations in GNAQ, GNA11, CYSLTR2, and PCLB4 occur early and are most likely initiating events (Fig. 1.2) [22–26]. Most nascent uveal melanocytic tumors became arrested at this

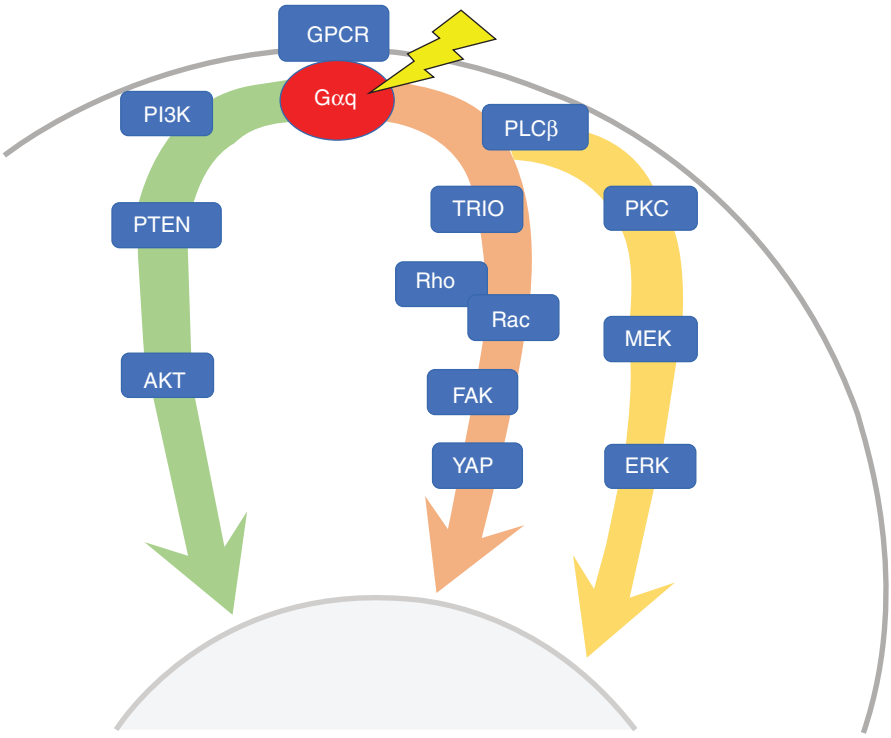

Fig. 1.2 Signaling pathways affected by mutant Gαq signaling in uveal melanoma. Mutations in GNAQ, GNA11, CYSLTR2, or PLCB4 (represented by red oval) result in constitutive signaling to multiple downstream mitogenic pathways, including the PI3K/AKT (green arrow), FAK, Hippo/Trio/FAK/YAP (orange arrow), and MAPK (yellow arrow) pathways. Transmission of these signals to the nucleus results in chromatin changes that drive uveal melanoma progression

stage by tumor suppressor checkpoints, resulting in benign uveal nevi that can be found in 4–6% of white individuals [27, 28]. For further tumor progression to occur, additional genomic aberrations are required, including certain chromosome copy number variations (CNVs) and secondary driver mutations. The most common CNVs include 1p loss, chromosome 3 loss, 6p gain, 6q loss, and 8p loss, and 8q gain. Chromosome 3 loss, sometimes referred to as monosomy 3, is more accurately referred to as loss of heterozygosity for chromosome 3 (LOH3), since the aberrant single chromosome is sometimes duplicated to form isodisomy 3 [29, 30]. LOH3 often arises in tumor evolution, whereas the other CNVs may occur later [21]. Secondary driver mutations occur in one of three main genes – BAP1, SF3B1, and EIF1AX (the so-called "BSE" mutations) – and occur in a largely mutually exclusive manner [16, 21, 31]. Unlike the Gαq mutations, the BSE mutations are highly prognostically relevant. Inactivating mutations in BAP1, usually accompanied by LOH3 which eliminates the second copy of the gene, are highly associated with class 2 UM and a 5-year metastatic rate of ~70% [11]. In contrast, hemizygous

change-of-function mutations in SF3B1 and EIF1AX are associated with class 1 UM with intermediate to low metastatic risk, respectively [15, 16, 32].

In contrast to some cancer types where mutations and other genomic aberrations may accumulate over long periods of time with many evolutionary subclones coexisting within the tumor [33], all of the canonical aberrations in UM seem to be present in most or all tumor cells at the time of diagnosis, suggesting that they may occur relatively close in evolutionary time as a punctuated burst or selective sweep [21]. Subsequent evolution occurs in a neutral or undirected manner in which rare, one-off mutations may be acquired during subsequent metastasis but are not sufficiently common to be the focus of targeted therapy [34].

Rational Therapeutic Targets in Uveal Melanoma

A clinically important implication of UM genetic evolution is that targeted therapy may be most effective when directed against early canonical genomic aberrations, especially the Gαq and BSE mutation clusters, which are present in most or all tumor cells in most cases. The two most common Gαq mutations occur in GNAQ and GNA11 [23, 24]. While these mutations confer a dominant effect at the cellular level, they are recessive at the biochemical level, leading to an inability to hydrolyze and inactivate the Gαq stimulatory subunit [35]. As such, these mutations have been difficult to target through direct pharmacologic inhibition. Rather, most strategies have attempted to inhibit downstream signaling pathways, including MAPK, PI3K/AKT, PLCβ/PKC, FAK, Hippo/FAK, and Trio/YAP [36–39]. Because of the pleiotropic effects of Gαq signaling, monotherapy targeting individual signaling pathways has been ineffective due to pathway switching [40]. Simultaneous targeting of multiple pathways has shown promise in preclinical models [41, 42] but is associated with significant toxicity and uncertain efficacy in patients.

Since the vast majority of metastasizing UM harbor BAP1 mutations [11], there have been efforts to target therapy against this aberration. However, since BAP1 mutations are loss-of-function events, therapeutic strategies have focused largely on exploiting vulnerabilities created by BAP1 loss or inhibiting downstream effectors of the BAP1-deficient phenotype. Inhibition of the DNA damage repair protein poly (ADP-ribose) polymerase (PARP) has been suggested to take advantage of defects in DNA damage repair in BAP1-mutant cancers such as UM [43]. Since UM genomes do not reveal a BRCA1-like DNA damage signature [21, 31], it remains unclear whether BAP1-mutant UM will be sensitive to PARP inhibitors. Nevertheless, a clinical trial is currently underway to test this hypothesis [44]. While all of the functions of BAP1 have yet to be elucidated and verified, BAP1 has clearly been shown to play a role in development and differentiation. Loss of BAP1 in uveal melanocytes causes a loss of differentiated cell identity [13]. Similarly, depletion of BAP1 causes defects during the switch from pluripotency to differentiation in *Xenopus* frog embryos [45]. This differentiation block in the melanocytic lineage

may be responsible for the metastatic phenotype in BAP1-mutant UM cells and appears to be mediated at least in part downstream activation of HDAC4, which represses expression of differentiation genes by acetylation of the histone H3K27 at the promoter and enhancers associated with these genes [45]. As such, inhibition of HDAC4 and perhaps other HDACs may reverse the differentiation block caused by BAP1 loss. Indeed, this appears to be the case in cultured UM cells [46] and in preclinical animal models of UM [47].

PRAME is another promising target of therapy in UM. Since PRAME is not normally expressed in somatic cells, its aberrant reexpression can lead to recognition by the immune system and activation of cytotoxic T cells [48, 49]. Consequently, various PRAME-directed immunotherapy approaches are being explored, including engineered T cells and bispecific T-cell redirection therapy. Further, since PRAME is associated with aneuploidy in UM and other cancer types, this may create a vulnerability to perturbation of cytosolic DNA sensing pathways such as with STING agonists [50, 51].

UM is an immunologically "cold" tumor that responds poorly to checkpoint inhibitor therapy directed against PD1 and CTLA4 [52]. However, there have been recent advances suggesting that UM can be rendered susceptible to immunotherapy. First, there have been promising early results using T-cell redirection therapy targeting the pan-melanocyte antigen gp100 in metastatic UM [53]. Second, some metastatic UMs respond to adoptive tumor-infiltrating lymphocyte (TIL) therapy [54]. Recently, new light has been shed on the lack of responsiveness to checkpoint inhibitor immunotherapy. In the first single-cell sequencing study in UM, it was that LAG3, rather than PD1 or CTLA4, is the predominant checkpoint molecule expressed on primary and metastatic UM [55]. This discovery has led to a new clinical trial (ClinicalTrials.gov NCT04552223) and expanding interest in checkpoint inhibitor immunotherapy for UM.

Summary

Given the lack of effective therapies for metastatic UM, there is an urgent need for clinical trials in both the adjuvant and metastatic settings. Yet, to maximize the value and likelihood of success for such trials, a deeper understanding is needed of the mechanisms driving the metastatic process. The two most important predictors of metastasis in UM are (1) the class 2 GEP caused by loss of BAP1 and (2) aberrant expression of the cancer-testis-antigen PRAME [16–18, 56–58] (Fig. 1.3). Appropriately, there has been a major emphasis on targeting these pathways through molecular and immunologic strategies [44, 47, 49, 59]. While not predictive of metastasis, Gαq pathway mutations are initiating events that are present in almost all UM [22–24, 60]. As such, these mutations have also received extensive attention in the development of targeted therapy through the inhibition of downstream signaling pathways. However, a major challenge with targeting these mutations has been

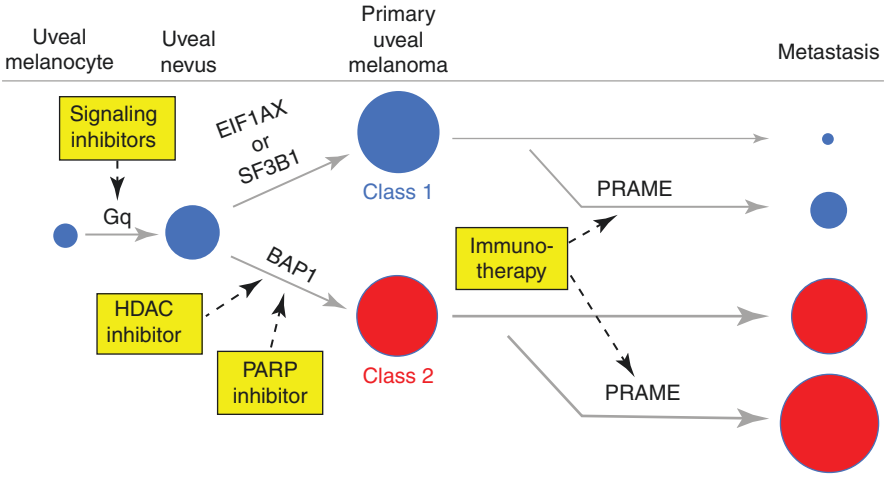

Fig. 1.3 Major molecular targets for therapeutic intervention in uveal melanoma. Mutations in the Gαq signaling pathway are initiating events in uveal melanoma that are difficult to inhibit direction, such that most efforts have focus on inhibiting kinases in downstream signaling pathways. BAP1 loss is the strongest predictor of metastasis in uveal melanoma, and efforts to direct therapy against this aberration have focused on inhibition of downstream targets that may render BAP1-mutant tumor cells vulnerable, such as HDAC and PARP inhibitors. Aberrant expression of the cancer-testis-antigen PRAME increases the risk of metastasis in both class 1 and class 2 uveal melanoma, and most therapeutic strategies have focused on immune-directed therapies such as engineered T cells and T-cell redirection therapy

the emergence of therapeutic resistance through pathway switching [61, 62]. While there are now a growing number of clinical trials for patients with high-risk and metastatic UM [44, 52, 63–65], it has been challenging in this rare cancer to obtain sufficient funding and numbers of subjects. Collaborative groups around the world are responding to this challenge. The Collaborative Ocular Oncology Group (COOG), which has undertaken large multicenter prospective clinical studies [7], is spearheading efforts to coordinate and seek funding for such trials in North America.

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Chapter 2
Clinical/Commercial Genetic Testing in UM

Thomas M. Aaberg Jr and Aditya Shah

Following the emotional and physical challenges of undergoing definitive local treatment for their primary uveal melanoma, a patient must then consider their risk for developing metastatic disease. If the risk is high, should the managing physician suggest more frequent surveillance for metastatic disease? Should adjuvant therapy be recommended? These are valid questions which can only be answered if one has a reasonably accurate measure of metastatic risk.

Historically, ocular oncologists have relied on clinical and histopathologic features of uveal melanoma to prognosticate metastatic risk. The most well-known controlled clinical trial for uveal melanoma, the Collaborative Ocular Oncology Study (COMS), categorized tumors by size: small, medium, and large. The COMS trials found a 5-year melanoma-specific mortality was approximately 40% for large melanomas, 20% for medium, and 5% for small tumors [1, 2], thus suggesting that treating a melanoma when small reduces the risk of metastatic disease. A study by Seddon et al. [3] further supported the COMS findings and reported worsening prognosis with every 2 mm increase in largest basal diameter. However, many experts have suggested that lead time bias was responsible for the perceived reduced mortality [4]. When patients were followed for more than 5 years, a greater number of treated medium- and small-tumor patients developed metastatic disease.

Histologic features have also been used to predict prognosis. First published by Callender in 1931, uveal melanoma cell type was described as either spindle cell A, spindle cell B, epithelioid, or mixed cell (spindle and epithelioid) [5]. Jensen

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correlated cell type with mortality rates, showing that a primarily epithelioid cellular composition portends a worse prognosis [6]. However, this too has limitations. There are no uniformly accepted histologic criteria to distinguish spindle A cell from nevi. There are no criteria for classification of a mixture of spindle A and B, and there are no criteria for the large number of patients with mixed spindle and epithelioid. This confusion led to counting the number of epithelioid cells per HPF, but this method is pathologist dependent—dictated by accuracy and reproducibility of epithelial cell identification. Other histologic features used to predict metastatic prognosis have included tumor microcirculation, tumor microvascular density, and tumor vascular loops. For example, contiguous closed vascular loops conferred a 10-year all-cause survival of 50.7% when present and 88.3% when absent [7]. However, the majority of these histologic features (particularly the vascular features) require an enucleated eye and therefore cannot be used for the majority of patients who have radiation therapy.

To better stratify metastatic risk and ultimately to stratify the uveal melanoma patient population appropriately for different metastatic screening protocols and for adjuvant treatment trials, investigators began exploring cytogenetic analysis of the primary tumor. Cytogenetics is a branch of genetics that is concerned with analysis of specific chromosomes and how they may affect cellular behavior. In uveal melanoma, studies have shown that the loss of chromosome 3 is associated with a worse prognosis, any abnormality in chromosome 6 is associated with a better prognosis, and a gain in chromosome 8 is associated with a worse prognosis [8, 9]. Going through each of these findings, it is important to note that there is no clear answer whether changes in these chromosomes cause deregulation of genes or if they are markers for tumor progression.

Monosomy 3 has the strongest association with uveal melanoma-specific death. Sandinha et al. [10] reported this association showing that a 5-year survival time was 100% for patients with disomy chromosome 3 tumor status as compared to 30% for monosomy 3. Investigators have also demonstrated that monosomy 3 has a stronger association with a worse prognosis when compared to the traditionally high risk clinical or histologic parameters discussed above [10, 11]. This makes monosomy chromosome 3 status an important area of interest in UM cytogenetics. Monosomy chromosome 3 prognostic value has been confirmed by several other studies. One study done by Prescher et al. [12] examined 30 patients whose eyes were primary enucleated and had monosomy 3. Out of the 30 patients, 17 (57%) of them developed metastasis with a 3-year relapse-free survival rate of 50%. This was compared to 24 patients whose tumors had disomy chromosome 3, all of whom did not develop metastasis. Scholes et al. [11] had a similar result when they were examining 60 patients with loss of heterozygosity in chromosome 3. They found that the rate of metastasis-related death associated with disomy 3 versus monosomy 3 increased from 0% to 51% at 2 years. This was stronger than any other association including cell type, ciliary body involvement, PAS-positive loops, or large basal tumor diameter.

Chromosome 6 is unique because any abnormality in the chromosome will have a positive effect on prognosis. Helgadottir and Hoiom [13] stated that the gain of

chromosome 6p and the loss of 6q have been detected in about one-third of the tumors they studied. These changes were usually detected in the same tumor. These chromosomal changes are rarely associated with monosomy of chromosome 3. White et al. [14] examined the tumors of 54 uveal melanoma patients of which 25 had abnormalities of chromosome 6. The abnormalities in chromosome 6 had a relative increase in 6p material from either an isochromosome of 6p or the deletion of 6p. It was found that the patients who had an abnormality in chromosome 6 did better than those without an abnormality. When relating this to other aberrations in chromosomes 3 and 8, it was most favorable to have a chromosome 6 abnormality without any abnormalities in 3 and 8. It was least favorable to have an abnormality in chromosomes 3 and 8 without having any in chromosome 6. White et al. [14] explained how the abnormality in chromosome 6 seems to have a protective effect, but the underlying mechanism needs to be studied further.

Chromosome 8 is the final major finding that is associated with a worsened prognosis in uveal melanoma. Harbour [15] stated that chromosome 8p loss occurs in about 25% of uveal melanomas and 8q gain in almost 40% of patients. The gain of 8q is significantly associated with metastasis. Prescher et al. [16] found a region with common gain on 8q, identified as 8q23-24→qter. This region contains many potential oncogenes, such as DDEF1, NBS1, and MYC, that when overexpressed in uveal melanoma result in a poor prognosis. Harbour [15] also theorized that 8p loss may be more of an important prognostic factor than the gain of 8p. Onken et al. [17] found that all tumors that exhibited 8p loss also showed 8q gain, which was consistent with Prescher et al. [16] suggesting the formation of an isochromosome of 8q. This finding is responsible for about 25% of uveal melanoma cases that have 8q gain and appears to occur exclusively with monosomy 3 tumors [15]. Sisley et al. [18] studied how the number of increased copies affected prognosis. Having tumors that contained one or two additional copies of 8q had a disease-free interval with a median of only 32 months. Patients with tumors who had three or more additional copies had a significantly lower disease-free interval (median of 14 months).

Chromosomal analysis has evolved from using light or electron microscopic evaluation to methods that analyze extended DNA fibers using DNA probes, digital fluorescence microscopy, and image analysis [19]. There are a number of genetic analysis techniques available, including karyotyping, fluorescence in situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA), array-based comparative genomic hybridization (aCGH), and single-nucleotide polymorphism (SNP). There are limitations and benefits for each technique which make some tests more appropriate for commercial use over others. For example, karyotyping, while providing information on all chromosomes in a single assay, is labor intensive and more importantly requires viable dividing cells, i.e., fresh tissue which can be cultured. FISH can be performed on fresh, aged, frozen, or paraffin-embedded specimens. In this technique, a specific fluorescein-colored probe is used to bind specific chromosome sites. FISH does not require an experienced cytogeneticist and circumvents the need for viable cells [20]. It has been a reliable technique for detecting chromosome 3 and 8 aberrations [21]; however, it cannot detect isodisomy of chromosome 3 or structural changes such as partial deletions. FISH is also prone to

sampling errors due to tumor heterogeneity [22–24]. Alternatively, MLPA is capable of detecting partial deletions of chromosome 3 [25, 26], can be performed on smaller samples (making it suitable for fine needle biopsy specimens), but optimally is performed on fresh or snap-frozen specimens. Like FISH, MLPA is subject to sampling errors due to tumor heterogeneity [25]. The aCGH technique uses DNA from the uveal melanoma and from a reference DNA which are labeled differently and hybridized with cloned DNA fragments. The DNA fragments have known chromosomal locations and consequently can provide genome-wide data on small aberrations and copy number variations [27]. But like the other techniques already mentioned, aCGH cannot identify isodisomy of chromosome 3. SNP array is more sensitive than the aforementioned techniques. Variations of single nucleotides are assayed. Therefore, a significant advantage of SNP is the ability to detect isodisomy of chromosome 3.

Uveal melanoma chromosomal analysis is now commercially available by Impact Genetics. The commercial laboratory will analyze chromosome copy number using multiplex ligation-dependent probe amplification (MLPA) on chromosomes 1, 3, 6, and 8 is performed to detect monosomy, disomy, or trisomy status. Microsatellite analysis (MSA) is performed on chromosome 3 to detect chromosome copy loss and/or isodisomy. Where indicated, sequencing of GNAQ, GNA11, SF3B1, and EIF1AX is performed to detect frequently occurring mutations in UM tumors. The specimen that is sent to Impact Genetics is a fine-needle aspirate biopsy (FNAB) from the patient's tumor that is expelled into a lysis buffer. The DNA is then extracted from the specimen. The laboratory requires 200 ng of DNA, but results can be possible with less than 100 ng of DNA. The specimen can be fresh or frozen and safely sent with up to 2 days in transit. Tumor and buccal samples for DNA analysis can be safely sent with up to 7 days in transit at room temperature. With the cytogenetic information, coupled with patient demographics such as age and gender, and tumor histology, the company generates a "Survivorship Prediction." The uveal melanoma survivorship prediction report estimates the 3-, 5-, and 10-year survival compared to age- and sex-matched general population controls. The report also includes the actual MLPA test results for each of the chromosomes tested and MLA analysis results.

In contrast to cytogenetic testing which specifically analyzes the tumor DNA, gene expression profiling (GEP) is a field in molecular biology that is concerned with measuring the activity of thousands of genes under a specific situation or in a specific cell to understand the large-scale picture of cellular function. Harbour [28] demonstrated how GEP can be used to stratify uveal melanoma patients into those with a low risk of developing metastatic disease versus those with a high risk. This method is performed by migrating the proteins expressed from genes in a high-density microarray platform to a 15-gene, qPCR-based assay. Twelve of the 15 genes (CDH1, ECM1, HTR2B, RAB31, EIF1B, FXR1, ID2, LMCD1, LTA4H, MTUS1, ROBO1, and SATB1) are discriminating genes, and 3 (MRPS21, RBM23, SAP130) are control genes. With this assay, Harbour [28] found, for example, that, when specific genes (CDH1, ECM1, HTR2B, and RAB31) were upregulated and other specific genes (EIF1B, FXR1, ID2, LMCD1, LTA4H, MTUS1, ROBO1, and

SATB1) were downregulated, patients had a significantly worse prognosis for survival. Uveal melanomas with this expression were termed class 2 uveal melanomas. In contrast, class 1 uveal melanomas were found to have a favorable prognosis for survival.

Further validating the power of the gene expression profiling test, GEP class 2 showed a significant association with other known prognostic factors, including increased patient age, greater tumor diameter and thickness, ciliary body involvement, and mixed/epithelioid cell type. However, statistical analysis concluded that GEP class 2 was more strongly associated with metastasis than any of the other clinical or pathological prognostic factors, including chromosome 3 status [29, 30]. The GEP assay has been independently validated by other investigators [31–33].

As investigation of GEP testing progressed, investigators noticed that there was a cluster of class 1 tumor patients who developed metastasis greater than 5 years from treatment, were younger patients, and had disomy chromosome 3. As a consequence, class 1 melanomas were subdivided into class 1A and class 1B [28]. The reported rate of detectable metastatic disease at 3 and 5 years is 98% and 98% for class 1A, 93% and 79% for class 1B, and 50% and 28% for class 2.

GEP assay has been translated into a College of American Pathologists-accredited Clinical Laboratory Improvement Amendments-certified laboratory test which can be used on a routine basis. GEP is now available commercially as the DecisionDx-UM test from Castle Biosciences, Friendswood, Texas, USA. So how does a surgeon obtain GEP testing? At the time of surgery (either enucleation, brachytherapy, clip placement, etc.), a fine-needle biopsy of the tumor is performed by the surgeon's preferred method. Typically, the use of a 27- or 25-gauge needle is sufficient. In the operating room, the specimen is expelled into an empty RNase-free tube provided by Castle Laboratories. The empty syringe is filled with 200 μL of extraction buffer (XB) also provided by Castle Laboratories and is used to rinse any remaining cells from the biopsy needle and syringe. The specimen tube is frozen in −80 C freezer until it can be shipped on dry ice by overnight courier to Castle Laboratories. On arrival in the laboratory, RNA is isolated. The RNA samples are converted to cDNA. Then, the cDNA is pre-amplified. RNA expression for each of the 15 genes is quantified. As previously mentioned, the assay includes 12 discriminating genes and three control genes. A gene is considered undetectable if its amplification product registers no Ct value after 40 cycles of qPCR. A sample is considered a technical failure if the three discriminating genes are undetectable. The report received by the physician will list the UM class type and the discriminant value (i.e., the confidence level; a discriminant value $\geq$0.100 is reported with normal confidence levels).

Another prognostic and potentially targetable antigen is preferentially expressed antigen in melanoma, or PRAME. PRAME is part of the cancer-testis antigen family present on cell surfaces. Normal tissue typically has little to no PRAME expression. However, in some solid tumors (such as melanoma), the gene becomes aberrantly expressed. PRAME acts as a repressor of retinoic acid signaling leading to a role in proliferation, differentiation, and apoptosis. Studies suggest that elevated expression of PRAME is a risk factor for metastasis in uveal melanoma patients.

This is currently under investigation and is also commercially available through Castle Laboratories.

Are there advantages or disadvantages of one commercially available test over the other? At the time this chapter was written, only the two commercial laboratories, Impact Genetics and Castle Laboratories, offered commercial testing. As mentioned, Impact Genetics performs primarily chromosomal analysis while Castle Laboratories performs gene expression profiling.

The first potential significant difference is the amount of specimen required. Initially, cytogenetic testing was primarily used for uveal melanomas that were treated by enucleation, which allowed a large amount of tumor tissue to be obtained. However, approximately 80–90% of uveal melanomas are treated by radiotherapy rather than enucleation, which means the sample must be obtained by needle biopsy. Chromosomal analysis by FISH or MLPA testing requires more tissue as compared to GEP testing which uses PCR amplification [28]. As mentioned, Impact Genetics prefers 200 ng of DNA. A human cell contains about 6 picograms of DNA [34]. Consequently, the Impact Genetic test optimally requires over 30,000 cells. The Castle Laboratory test requires as few as ten cells. The clinical consequence of this issue has been reported. Shields et al. [35] biopsied 140 melanomas at the time of plaque radiotherapy. Seventy-nine of the melanomas biopsied measured more than 3 mm in thickness with enough aspirate obtained in 71 cases (90%) to perform cytogenetic testing. Sixty-one tumors were 3 mm or less. Out of the 61 cases, 49 (80%) could have chromosomal analysis, but 12/61 (20%) did not have adequate specimen for analysis [35]. McCannel et al. [36] also found that biopsies from tumors with a height of 5 mm or more were sufficient to conduct FISH testing in 91% (58/64) of cases. However, only 53% (27/49) of tumors with a height of less than 3 mm had an adequate amount of tissue for analysis. These studies show the importance of how much tissue is needed to perform cytogenetic analysis under FISH. By comparison, there have been situations where there were issues with GEP testing. Afshar et al. [37] had 62 tests sent to Castle Biosciences with three of them being reported as a technical failure and five of them with GEP not being performed. Out of the five not being performed, one of them had a problem with transport, two of the patients declined the Castle testing, and the last two tumors did not have enough tissue. The samples that did not have enough tissue were from tumors that were <1 mm thick. The study by which we can best compare tissue requirements for chromosomal analysis vs GEP analysis was performed by Corrêa and Augsburger [38]. The authors specifically compared the sufficiency of FNAB aspirates to provide cytologic vs GEP results. FNAB aspirates were insufficient for cytopathologic classification in 34 of 159 cases (21.9%). In contrast, FNAB aspirates were insufficient for GEP classification in only 1 of 159 cases (0.6%).

There may be a difference in the strength of the two prognostic tests; however, to date there has not been a trial directly comparing the two. When GEP testing is compared to chromosome 3 status, Onken et al. demonstrated a 20% discordant rate; specifically 6% of class 2 melanomas had disomy 3 and 14% of class 1 melanomas had monosomy chromosome 3 [30]. In nearly all cases, the GEP status was

more accurately predictive of metastasis. Chromosome 3 status was collected from the first 260 cases and was identified with GEP/chromosome 3 status. There were 119 (45.8%) with class 1/disomy 3, 87 (33.5%) with class 2/monosomy 3, 38 (14.6%) with class 1/monosomy 3, and 16 (6.2%) with class 2/disomy 3. A significant association was found between class 1 and disomy 3 and between class 2 and monosomy 3. However, the GEP and chromosome 3 results were discordant in 54 (20.8%) of cases. From the 16 cases that were class 2/disomy 3, there were 7 (43.8%) of them that had metastasized. Among the 38 cases that were class 1/monosomy 3, only 1 (2.6%) of them had metastasized. This showed that GEP was more strongly associated with metastasis than chromosome 3 when looking at the discordant cases. When GEP was compared to clinical features typically used to predict metastasis using Cox proportional hazard statistical multivariable analysis, chromosome 3 status and other variables did not contribute prognostic information that was independent of GEP ($P = 0.2$) [30]. As mentioned, Impact Genetics uses a combination of cytogenetic information, demographics, and histologic features to create their Survivorship Prediction. Therefore, it is possible that the totality of the Impact Genetic data analysis equates to that of GEP analysis.

The future of tumor cytogenetic analysis may rest in next-generation sequencing. There is evidence that specific frequently mutated genes could be drivers in UM development, including GNA11, GNAQ, BAP1, EIF1AX, and SF3B1. These drivers of UM development have been correlated with chromosomal and GEP analysis. The gene drivers GNAQ and GNA11 are related G-alpha proteins with 90% amino acid sequence homology. Shoushtari and Carvajal [39] found the prevalence of GNAQ mutations in 33% of uveal melanomas and GNA11 mutations in 39%. These mutations are mutually exclusive with each other and are considered an early event in UM evolution. However, neither GNAQ nor GNA11 mutations were found to have an association with prognosis. BRCA1-associated protein 1 (BAP1), a tumor suppressor gene located on chromosome 3, was found to be one of the most significant mutated genes associated with poor prognosis [13]. It is almost mutually exclusive with SF3B1 and EIF1AX mutations. Johansson et al. [40] reported that BAP1 had a frequency of 30–40% in primary uveal melanomas. Also, over 80% of metastasizing uveal melanomas have been found to have a mutation in this gene. Using next-generation sequencing, Helgadottir and Höiom [13] reported that a majority of the class 2 tumors carried a mutation in the BAP1 gene, mapped to chromosome 3p21.1. Very few of the class 1 tumors had a mutation in the BAP1 gene. Gene driver EIF1AX is almost mutually exclusive with BAP1 and SF3B1 mutations and was found to have an association with disomy 3, a low prevalence in monosomy 3 tumors, and is associated with class 1A GEP. This gene driver is unique because it portends a good prognosis. The gene driver SF3B1 is again almost mutually exclusive with BAP1 and EIF1AX mutations. This mutation is associated with disomy 3, younger patient age, and poor prognosis with development of late metastasis. In a study by Martin et al. [41], they found that the prevalence of EIF1AX mutations occurred in 15 out of 31 tumors (48%), and SF3B1 mutations occurred in 9 out of 31 tumors (29%). Interestingly, these driver mutations appear to correspond to GEP class type [42]. Next-generation sequencing is currently being performed in a

prospective observational clinical trial by the Collaborative Ocular Oncology Group 2 (COOG2).

The ability to accurately assess a patient's risk for metastatic disease is playing a greater and greater role as medical advances continue. Risk assessment is, has been, and will continue to be critical to two important aspects of patient care: the appropriate intensity at which we surveil a patient for metastatic disease and identifying patients appropriate for adjuvant therapy [43–45]. At the time of this chapter's publication, adjuvant therapies have not been identified and treatments for metastatic UM have at best extended survival by months. However, also at the time of this chapter's publication, investigators are reporting potential targetable UM sites, and new drugs showing potential therapeutic promise are undergoing investigation.

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Chapter 3
BAP1 Tumor Predisposition Syndrome

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The BAP1 tumor predisposition syndrome (BAP1-TPDS) was identified by compiling the work of several researchers who were characterizing mutations responsible for cancer predisposition in various organ systems. In 2010, somatic mutations in *BAP1* were reported in 26 of 31 metastasizing uveal melanoma studied, with 1 of these mutations being germline [1]. The following year, three independent groups reported germline *BAP1* mutations in two families with mesothelioma [2], two families with atypical melanocytic cutaneous neoplasms and cutaneous melanomas [3], and a family with uveal melanoma, mesothelioma, lung adenocarcinoma, and meningiomas [4]. Some of the patients from the mesothelioma families were noted to have been diagnosed with uveal melanomas [2]. Two years later, germline *BAP1* mutations were identified in several patients with renal cell carcinoma and other cancers, which prompted additional characterization of families with *BAP1*-associated cancers [5]. As tumors, patients, and families with *BAP1* mutations have been further studied, the association of the syndrome with these four

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cancers (Fig. 3.1) has been confirmed, and screening guidelines for cancers associated with the syndrome have been suggested [6].

BRCA1-associated protein-1 (BAP1) was first identified as binding to BRCA1 and thus as likely having a role in tumor suppression [7], but later studies confirmed that it is an independent tumor suppresser gene. BAP1 is a deubiquitinating enzyme important in up- and downregulation of proteins important in cellular proliferation

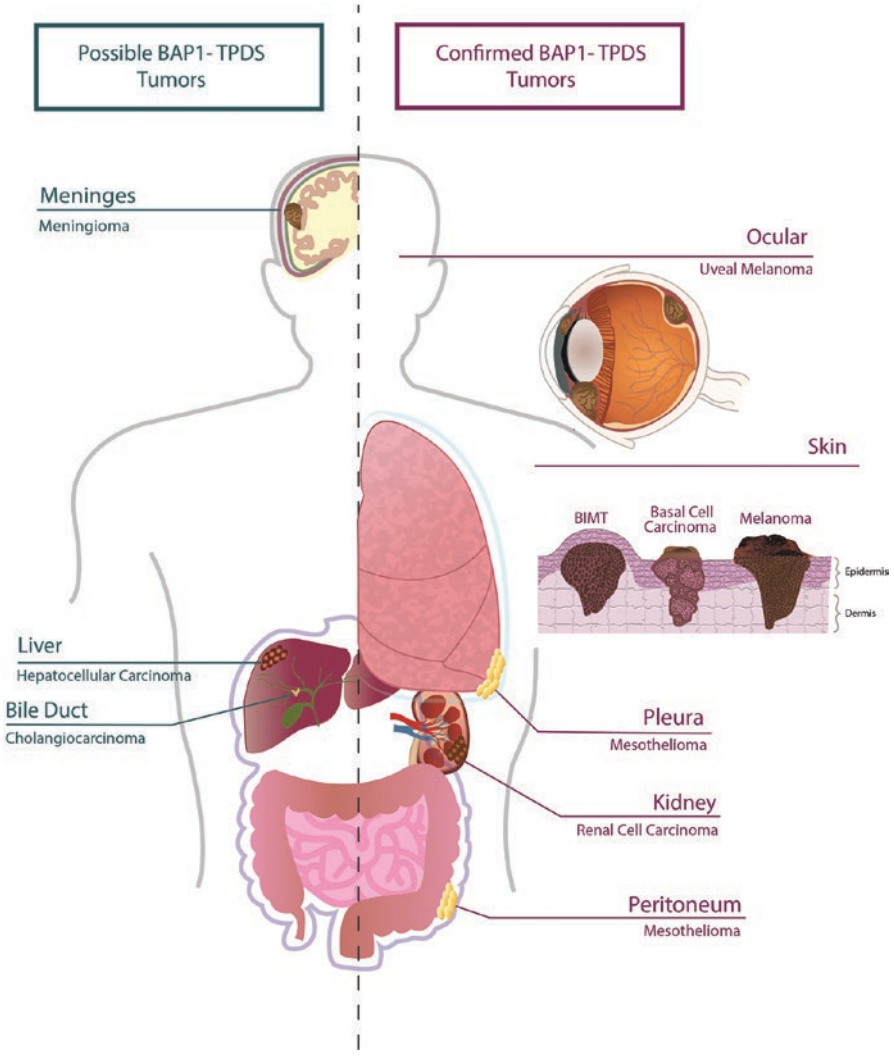

Fig. 3.1 Confirmed BAP1-TPDS-associated tumors (right side of figure) include uveal melanoma, BAP1-inactivated melanocytic tumors (BIMT), basal cell carcinoma (BCC), cutaneous melanoma, pleural and peritoneal mesothelioma, and renal cell carcinoma. Possible BAP1-TPDS-associated tumors (left side of figure) include meningioma, hepatocellular carcinoma, and cholangiocarcinoma

[8], and it has recently been found to have several additional functions (Fig. 3.2). In the nucleus, BAP1 has roles in chromatin modification, genome stability, transcription factor regulation, direct regulation of gene transcription, and double-strand break repair [9]. In the cytoplasm, BAP1 is involved in oxidative phosphorylation, apoptosis, and ferroptosis [9]. Several *BAP1* variants have been identified, and most of those associated with BAP1-TPDS are loss-of-function mutations or deletions. It has been suggested that some of the missense variants could also contribute to the BAP1-TPDS phenotype [10]. Studies of tumors from patients with germline *BAP-1* mutation have shown somatic mutations in or deletion of the second copy of the gene in several tumors including uveal melanoma, mesothelioma, renal cell carcinoma, cutaneous melanoma, and *BAP1*-inactivated melanocytic tumor (BIMT) [10, 11]. Therefore, it appears that germline mutation of *BAP-1* results in a haploinsufficiency, where a second somatic mutation or deletion results in loss of BAP1 function and increased chance of tumor development following the Knudson's two-hit model for biallelic inactivation.

Considering the inheritance pattern observed in familial studies and haploinsufficiency as described above, the BAP1 tumor predisposition syndrome is characterized as autosomal dominant. Most patients with germline *BAP1* mutations have an affected parent. Penetrance is likely high with approximately 82.5–88% of individuals with *BAP1* mutation diagnosed with at least one cancer [10]. However, this could be due to ascertainment bias of studying high-risk families. There has only been one patient identified thus far whose parents were both confirmed as not harboring the proband's *BAP1* mutation, suggesting de novo origin of the

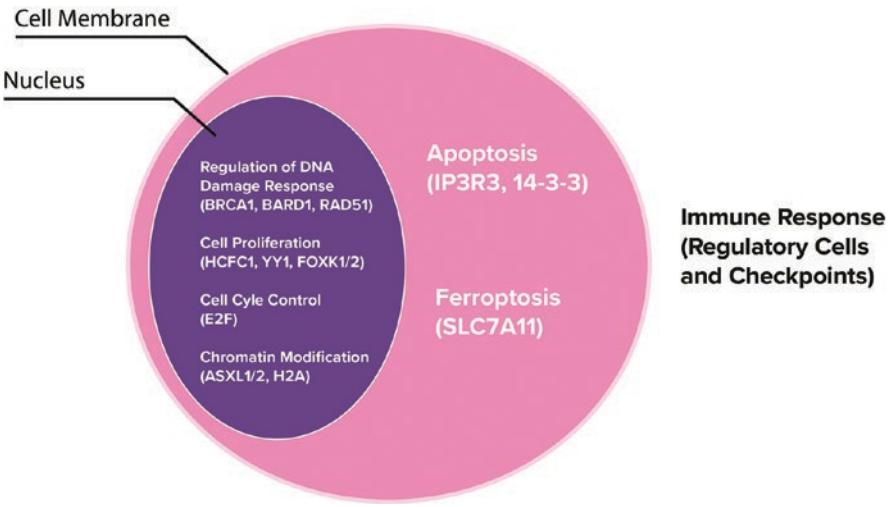

Fig. 3.2 Intranuclear, intracytoplasmic, and extracellular functions of BAP1. Intranuclear functions include regulation of the DNA damage response [85–87], cell proliferation, cell cycle control [88–91], and chromatin modification [92–94]. Intracytoplasmic functions include roles in apoptosis and ferroptosis [95–97]. Extracellularly, BAP1 is involved in the immune response [81, 83, 98]

mutation [10]. Therefore, the rate of de novo *BAP1* germline mutation is believed to be low. Overall prevalence of germline *BAP1* mutation is unknown. Carrier frequency in the general population is 1:26,837 based on the Genome Aggregation Database (gnomAD). The frequency of germline *BAP1* mutations was 1:1299 in an unselected cancer cohort of patients included in The Cancer Genome Atlas (TCGA) project [12].

It has been suggested that the most common tumor associated with BAP1 predisposition syndrome is the BIMT. In one study of patients with germline *BAP1* mutations, up to 75% had BIMT lesions, and most had multiple [13]. Median age of onset was younger than with other *BAP1*-associated lesions in this study, which suggests that identification of these lesions could aid in early diagnosis. These tumors were formerly called atypical Spitz tumors (AST), nevoid melanoma-like melanocytic proliferations (NEMMP) [14], and melanocytic *BAP1*-mutated atypical intradermal tumors (MBAITS) [15]. BIMT lesions vary from skin-toned to reddish brown. They may be minimally or significantly elevated and average 5 mm in diameter. The clinical and histological diagnosis can be difficult, with findings that are somewhere between the benign Spitz nevus and a melanoma. BIMT lesions are not themselves malignant, but they can undergo malignant transformation. In cases of uncertainty, immunostaining of biopsied lesions will show lack of BAP1 protein because both copies are inactivated. BIMT also typically has the *BRAF* pathogenic variant.

Uveal melanoma is the malignancy most associated with BAP1 tumor predisposition syndrome. Among all individuals with uveal melanoma, only approximately 1–2% harbor germline *BAP1* mutations [16–18]. However, in uveal melanoma patients with positive family history of uveal melanoma, 20% have germline *BAP1* mutations [19, 20]. Among individuals diagnosed with *BAP1* mutation, 36% of probands and 16% of relatives with BAP1-TPDS have uveal melanoma [10]. Uveal melanoma is the *BAP1*-associated cancer with the earliest age of diagnosis (16 years), and it is often diagnosed much younger than are uveal melanomas in the general population, with a median age at diagnosis of 53 versus the general population median of 62. The uveal melanomas of patients harboring germline *BAP1* mutations are more aggressive than comparably sized melanomas with higher metastatic risk and reduced survival [14, 21]. Moreover, somatic mutation of *BAP1* in uveal melanoma is a poor prognostic feature and is present in most metastasizing uveal melanomas [1, 22, 23].

The second most frequent cancer associated with BAP1-TPDS is malignant mesothelioma. 1–3% of patients with malignant mesothelioma have *BAP1* germline mutations, though 6–7.7% with positive family history have BAP1-TPDS [24–26]. Malignant mesothelioma is present in approximately 25% of probands and 19% of relatives with BAP1-TPDS [10]. As with uveal melanoma, malignant mesothelioma in BAP1-TPDS tends to occur at a younger than average age than that of sporadic malignant mesothelioma [10, 25, 27]. In BAP1-TPDS, patients are much more likely to have peritoneal malignant mesothelioma than in the general population, where the ratio of peritoneal to pleural malignant mesothelioma is much lower [10, 25, 28, 29]. In the general population, peritoneal malignant mesothelioma is more

likely to occur in men, whereas it is more common in women who have BAP1-TPDS [10]. It appears that patients with BAP1-TPDS are more likely to develop malignant mesothelioma when also subjected to environmental asbestos exposure [30, 31]. However, unlike *BAP1*-associated melanomas and renal cell carcinomas, patients with BAP1-TPDS and malignant mesothelioma tend to have a longer survival than the general population with malignant mesothelioma, especially with pleural involvement [27, 32–34]. The reason for better prognosis for pleural mesothelioma with germline *BAP1* mutation is unclear, particularly when germline *BAP1* mutation is a poor prognostic feature for other cancer (e.g., uveal melanoma, renal cell carcinoma).

Cutaneous melanoma is the third most common cancer associated with BAP1-TPDS, occurring in approximately 13% of patients with the syndrome [3]. Because cutaneous melanoma is so common in the general population, it is rare for patients with isolated melanoma or even familial melanoma to have *BAP1* germline mutations. It is common for cutaneous melanoma in the setting of BAP1-TPDS to be diagnosed at an earlier age than in the general population and for multiple primary cutaneous melanomas to be present in an individual. Data on the tumor aggressiveness compared to patients without BAP1-TPDS are inconsistent [17, 35–37].

The fourth most common cancer associated with BAP1-TPDS is renal cell carcinoma, particularly the clear cell variant [5, 10, 38]. While approximately 1–1.5% of patients with renal cell carcinoma have *BAP1* germline mutations [39, 40], 10% of patients known to have BAP1-TPDS have been diagnosed with renal cell carcinoma. Cancer diagnosis tends to occur at a younger age with a more aggressive course of disease and decreased survival [33, 41–45]. Histology of *BAP1*-associated renal cell carcinoma is higher grade without the somatic *PBRM1* pathogenic variants which are common in renal cell not associated with *BAP1* [46].

There are several additional lesser characterized tumor associations with BAP1-TPDS. Basal cell carcinoma has been shown to be associated [47–49], with multiple primary tumors being common. Basal cell tumors also tend to occur at a younger than usual age [10]. Meningioma is associated with BAP1-TPDS, in particular a high-grade rhabdoid subtype found in 8.5% of *BAP1* probands and 2.2% of relatives with *BAP1* germline mutations [4, 29, 49–51]. Cholangiocarcinoma has been associated with BAP1-TPDS and has been identified in 1.4% of probands [10, 14, 49, 52]. Germline *BAP1* mutations have been identified in 0.5% of patients with hepatocellular carcinoma, and hepatocellular carcinoma has been diagnosed in 0.7% of probands and 1.6% of relatives with known BAP1-TPDS [10, 12].

Unconfirmed associations include tumors that have been observed in patients with *BAP1* germline mutations, but with limited or inconsistent evidence that they are truly part of the predisposition syndrome. The tumors include breast cancer [2, 5, 14, 53], neuroendocrine tumors [4, 54], non-small-cell lung adenocarcinoma [4, 14, 54, 55], thyroid cancer [5, 56], and urinary bladder carcinoma [57].

It is expected that BAP1-TPDS prevalence data and associations with various cancers will be better elucidated in the near future through the work of the BAP1 Interest Group (BIG) Consortium. This is an international group of researchers working together to compile data from probands and their family members. This

collaboration may help in determining statistical significance for some of the less clearly associated tumors and should decrease or eliminate some of the problems that come with ascertainment bias of researchers approaching the syndrome from the perspective of their tumor of interest.

The diagnosis of BAP1-TPDS relies on a high index of suspicion combined with genetic testing. While there are no defined clinical diagnostic criteria, there are several patient characteristics that should alert the clinician to the possibility of the syndrome. It has been determined that 90% of patients with confirmed germline *BAP1* mutations had either two of the above confirmed tumors or one of the confirmed tumors plus a first or second degree relative with one of the confirmed tumors [36]. These criteria exclude the use of two cutaneous melanomas and/or basal cell carcinomas because of their high frequencies in the general population. BAP1-TPDS might also be considered for individuals whose demographics lie outside of the norm for their diagnosis. For example, testing may be considered for unusually young patients with confirmed tumors or for women with peritoneal mesotheliomas.

Knowledge of the presence of a *BAP1* germline mutation is important for several reasons. At-risk family members should be counseled and tested for the variant detected in the family. Preventive screening for family member with mutation should be carried out for uveal melanoma, cutaneous melanoma, renal cell carcinoma, and mesothelioma. Knowing the presence of a *BAP1* mutation allows for more careful examination of other organ systems that may be affected by the disease and thus increased potential for early tumor diagnosis. In addition, for a patient with a tumor diagnosis, the identification of germline *BAP1* mutation may be helpful in determining prognosis. For several cancers, prognosis is worse for patients with *BAP1* mutation, but in malignant mesothelioma, prognosis may be better. In the case of uveal melanoma, knowing that a *BAP1* mutation is present upgrades any tumor to a high-risk prognostic equivalent (e.g., of monosomy 3, Castle class 2 gene expression profile), resulting in more frequent systemic monitoring.

If *BAP1* genetic testing is considered, it is strongly recommended to work with a genetic counselor. *BAP1* genetic testing can be accomplished through two different approaches. When there is a patient with a *BAP1*-associated malignancy and suspicion for a germline mutation, panel testing may be considered. For any given malignancy, there are often multiple panel options available, and the clinician must choose the most appropriate panel based on the cancer phenotype in the family. A somewhat targeted approach is often best if the clinician knows which genes are most likely to be relevant. Next-generation sequencing (NGS) panel can detect both sequence variation and gene deletion. In most clinical laboratories, NGS panel is run as part of whole exome sequencing so assessment of other genes not included on the panel could be requested. Alternatively, if there is high suspicion for *BAP1* germline mutation, single-gene sequencing may be performed. However, the cost of single gene testing is usually as high or higher than a cancer panel. *BAP1* deletion/duplication analysis can be performed at the time of sequencing or if sequencing does not reveal mutation and the index of suspicion remains high. This is critical since large deletions in *BAP1* may be present which may be missed on other testing

modalities [22, 58]. Single-site sequencing is carried out for a family member of a subject with pathogenic variant in *BAP1*.

The differential diagnosis of germline *BAP1* mutation includes several additional genes. For uveal melanoma, other genes to consider include *BRCA1*, *BRCA2*, *MBD4*, *PALB2,* and *MLH1* [59–64]. Malignant mesothelioma may be associated with *CDKN2A* variants [65]. Cutaneous melanoma has been linked to predisposing variants in *CDKN2A*, *CDK4*, *TERT*, *ACD*, *TERF2IP*, *POT1*, *MITF*, and *MC1R* [66]. Renal cell carcinoma may also be associated with *CHEK2*, *VHL*, *XP11*, *FH*, *FLCN*, *MET*, *SDHA*, and *SDHB* [40].

Once the diagnosis of BAP1-TPDS has been made, there are several screening recommendations that may allow for early detection and treatment of associated tumors. Dermatologic evaluation may allow for identification of BIMT lesions, cutaneous melanomas, or basal cell carcinomas. Skin exams should begin at the age of 18 or at the time of diagnosis if later. Exams should be performed by a dermatologist specializing in melanoma. If lesions are identified, whole-body imaging should be considered for the purpose of monitoring the lesions for change. BIMT lesions do not need to be biopsied where there is no concern for melanoma, but with their atypical nature and the potential for malignant transformation, excisional biopsy may be considered especially where there are few lesions [67]. Management of other suspicious melanocytic lesions and basal cell carcinomas should proceed according to established guidelines for each condition.

Patients with BAP1-TPDS should be screened for uveal melanoma beginning at age 11 with a dilated eye exam and baseline fundus imaging. Any suspicious lesions should prompt referral to an ocular oncologist for further workup, monitoring, and treatment if necessary. If a uveal melanoma is diagnosed, it should be followed with a high-risk surveillance protocol because of the increased aggressiveness of these tumors and high potential for metastasis [14, 68].

There is no consensus for screening for malignant mesothelioma. It is recommended that patients at least undergo yearly physical examination beginning at age 30 with attention to symptoms of chest pain, cough, fever, shortness of breath, dysphagia, hoarseness, weight loss, upper body and face edema, abdominal pain, nausea, vomiting, or constipation and exam findings suggestive of pleural inflammation, pleural effusions, peritonitis, or ascites. If imaging is obtained for renal cell carcinoma screening, it may be extended to involve the pleura and peritoneum. *BAP1*-associated mesothelioma may allow longer survival than mesothelioma in the general population, and it may respond better to chemotherapy, so it is important that treatment is provided by an oncologist familiar with *BAP-1* malignant mesothelioma and ongoing chemotherapy trials.

There are better-defined guidelines for screening for *BAP1*-associated renal cell carcinoma. Beginning at age 30, patients should undergo yearly abdominal exam and investigation of any suspicious symptoms. MRI of the abdomen and chest should be performed every 2 years, alternating yearly with ultrasound. Early detection of renal cell carcinoma is critical, as excision of localized tumors is often curative. Treatment of any identified tumors or metastases should proceed according to current renal cell carcinoma guidelines.

There is limited evidence of the effectiveness of *BAP1*-tumor prevention, but there are several measures that are likely helpful and certainly low risk. Patients with BAP1-TPDS should avoid unnecessary imaging that involves radiation, particularly during screening exams, as it is theoretically possible that radiation exposure could increase the risk of tumor formation. Limiting sun exposure and wearing adequate UV protection decrease the risk of cutaneous malignancies. Arc welding is associated with uveal melanoma and should be avoided where possible. It is unclear whether eyewear protecting against UV exposure reduces the risk of uveal melanoma, but at the very least it reduces risk of cutaneous eyelid lesions. Smoking and asbestos exposure increase the risk of malignant mesothelioma and should be avoided.

Currently there is no targeted treatment for BAP1-TPDS, but this is a theoretical possibility and a goal for the future. Some targeted molecular treatments have been used to treat patients with *BAP1* mutations. Histone deacetylase inhibitors have been shown to counter the excess histone ubiquitination that results from loss of BAP1 function and have been effective in treating *BAP1*-associated uveal melanoma and mesothelioma [69, 70]. Enhancer of zeste 2 polycomb repressive complex 2 subunit (E2H2) inhibitors are potential therapies because this enhancer is upregulated in BAP1-deficient tumors [71, 72]. An E2H2 inhibitor has been at least partially effective in a trial of *BAP1* mutation-associated mesothelioma [73, 74]. Tumors with somatic *BAP1* mutations have been identified as candidates for poly-adenosine diphosphate-ribose polymerase (PARP) inhibitor treatment because PARP inhibitors preferentially affect cells with defects in DNA repair, such as those affected by BAP1 loss. There are currently several ongoing PARP inhibitor clinical trials enrolling patients with metastatic uveal and cutaneous melanoma, renal cell carcinoma, cholangiocarcinoma, as well as mesothelioma harboring pathogenic *BAP1* alterations [75–78].

There has been recent interest in immunogenic phenotypes of patients harboring *BAP1* mutations, with suggestion that *BAP1* mutation may confer susceptibility to certain immune-mediated treatments. It is well accepted that increased inflammatory tumor infiltration by T cells and macrophages is associated with increased risk of uveal melanoma metastasis. The shift in tumor and immune cell markers appears to result in an inflammatory response that suppresses the normal immune response to tumor growth [79, 80]. In the case of BAP1 loss in uveal melanoma, this appears to occur through upregulation of regulatory T cells, with increased expression of HLA-D4, CD38, and CD74, making these markers potential targets [81]. Patients with mesothelioma and *BAP1* mutations have increased immune infiltration and increased immune checkpoint activation, which has raised the question of whether these tumors may be susceptible to immune checkpoint inhibitors [82, 83]. CCR5 is an immune regulatory marker that is elevated in *BAP1*-associated renal cell carcinomas, allowing immune suppression and tumor infiltration. Therefore, CCR5 blockade has been suggested as a treatment for *BAP1*-deficient renal cell carcinoma [84].

Genetic counseling is important for patients diagnosed with BAP1-TPDS and for their relatives. Knowledge of this condition comes with the burden of

lifelong monitoring, specialized treatment of malignancies, and awareness that multiple family members are likely also affected. The patient should be encouraged to share their diagnosis with family members so that each relative can make informed decisions regarding testing. Young patients diagnosed with BAP1-TPDS might alter their family planning with this knowledge. Preimplantation and prenatal testing are available, and these very personal decisions should be made with a professional who can help guide a patient through the risks and benefits.

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Chapter 4
Pathology of Uveal Melanoma

Patricia Chévez-Barrios

General Characteristics

Uveal melanomas are malignant neoplasms arising from the melanocytes of the choroid, ciliary body, and iris. Uveal melanoma is the most common primary intraocular malignancy of adults. Different from the skin melanomas that are mostly associated with BRAF mutations, the uveal melanomas have different genetic mutational events that involve the GNAQ pathway as initial step (see previous chapters) [1]. Some patients have associated predisposing characteristics such as congenital melanosis oculi, combined form of Sturge-Weber and Phakomatosis pigmentovascularis, and BAP1 syndrome (see Chap. 3) [2]. Metastasis occurs in about 50% of patients, and the type of treatment has no influence on outcome. Most metastatic tumors go to the liver. The eye is an immune privilege site and lacks lymphoid tissue and lymphatic vasculature, apart from some lymphatic-like circulation in the ciliary body, and the tumors escape the eye through blood vessels [3].

Anatomy and Histology

Uveal melanomas arise from the dendritic melanocytes – derivatives from the neural crest – of the uveal tract. The uveal tract is divided in three portions by their location inside the eye: the iris (anterior), ciliary body (middle), and choroid (posterior). The uveal tract is the vascular coat of the eye with fenestrated and higher-density vessels in the choroid. The ciliary body also contains the smooth muscle that

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regulates the tension of the zonules that control the shape of the lens for accommodation. The iris is the anterior diaphragm of the eye that controls the amount of light that enters through the pupil by contracting either the true smooth muscle – sphincter muscle or the myoepithelial dilator muscle. The stroma contains mesenchymal cells and melanocytes alternating with the specialized vessels. The vessels have perivascular collagenous fibers that prevent the vessels to collapse during the dilatation of the iris. The surface of the iris is composed by the same mesenchyme stroma and melanocytes without a specialized layer forming the undulating valleys and elevations (Fig. 4.1a–f). Any type of cells or membranes layering the surface of the iris is abnormal and clinically and grossly visualized as a flat surface of the iris.

Location of Tumor, Surgical Procedures by Site, Specimen Type

The treatment of uveal melanoma depends on the site and size of the tumor, the decision of the patient to keep the eye, and the ocular oncologist's ability to treat and preserve vision. Outcomes for metastasis are not altered by type of treatment but predominantly by the volume, biology, and molecular features of the tumor [4].

The surgical approach employed to obtain the specimen determines the surgical pathology specimen type.

Iris melanomas, the least frequent representing 4% and the least aggressive of the uveal melanomas, may be obtained by segmental excision called iridectomy or if at the root of the iris an iridocyclectomy that includes the ciliary body. In these cases, margins should be checked by the pathologist because, although most iris melanomas are low-grade, they may recur if not completely excised (Fig. 4.2a).

Ciliary body melanomas represent 6% of the uveal melanomas, are the most aggressive by site, are usually of large size, and may have extraocular extension. These tumors are treated often with brachytherapy or if too large or causing glaucoma by enucleation (Fig. 4.2b).

Choroidal melanomas are the most common (90% of all uveal melanomas) and most often treated with brachytherapy if the size and the location allow for a plaque. Those tumors that are large and have associated neovascular glaucoma or extraocular extension are enucleated. In cases treated by brachytherapy and wherein the patient has consent for molecular prognostic testing, a fine-needle aspiration biopsy is performed to obtain the tumor sample before the radiation plaque is placed. See below for specifics of tumor sampling and cytopathologic adequacy evaluation (Fig. 4.2c).

Macroscopic Examination and Grossing

Macroscopic examination and adequate dissection of the specimen received are essential for precise diagnosis and recognition of histopathologic risk factors.

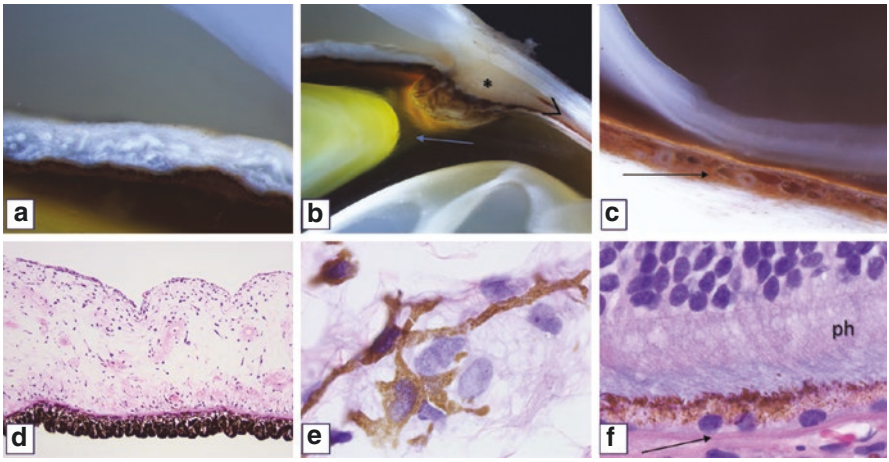

Fig. 4.1 Anatomic and histologic features of the uveal tract: (**a**) Macroscopic view of the mid portion of the iris in a patient with light-gray eye color. The anterior surface of the iris (top) is wavy with some tan color, and the cut surface of the iris stroma shows the specialized vessels (white). The posterior surface is covered by the pigmented layer of neuroepithelium. Top right is the cornea; between the cornea and the iris is the anterior chamber. Behind, posterior to the pigment epithelium of the iris is the posterior chamber, and the yellow structure in the bottom left is the lens. (**b**) Macroscopic view of the ciliary body in the same patient as in (**a**). The root of the iris joins in the back the ciliary body pars plicata (undulations of the epithelium) with the smooth muscle (*) and the pars plana posterior to this (arrowhead). The ciliary body is connected to the lens via thin strings of basement membrane – the zonule (arrow) – to regulate the shape of the lens for accommodation. The lens (yellow) at the left and a detached retina in the vitreous cavity (white membrane at the bottom) are seen. (**c**) The posterior choroid (brown vascular layer) and retina on top (white membranous layer) with the sclera in the inferior (outside white thick layer) portion are seen in the macroscopic picture. The choroid has the vessels of different sizes (arrow) and is covered by retinal pigment epithelium (RPE), seen as a light brown layer between the choroid and the retina. (**d**) Microscopic view of the iris shows the condensation of lightly pigmented melanocytes on the undulated anterior surface of the iris. The vessels are seen surrounded by a thick collagenous (eosinophilic pink) layer in the center of the stroma. The posterior non-pigmented myoepithelium (dilator muscle) is between the stroma and the pigment iris epithelium in the back. Melanocytes are present through the iris stroma and may give rise to melanocytic lesions (hematoxylin and eosin (HE) stain, original magnification 10X). (**e**) High magnification of the choroidal stroma shows the typical dendritic melanocytes between the vessels and stroma. Notice the fine globular pigment. These are the melanocytes that may develop into nevi or melanoma (HE stain, original magnification 100X). (**f**) In contrast with the uveal melanocytes, the RPE cells are cuboidal in a monolayer over Bruch's membrane (arrow) and under the photoreceptors from the retina (ph). Notice the different type of pigment in the RPE that is darker and elongated football shape (HE stain, original magnification 100X)

Iridectomies and iridocyclectomies: The specimen should be oriented by the surgeon with eye laterality and site (location by clock hours) to allow for adequate interpretation and evaluation of margins. The margins that are reported include the peripheral margin (toward or at the scleral resection) and the iris stromal margins. Pupillary, anterior, and posterior margins are not clinically relevant because they are not attached to any tissue (Fig. 4.2a) [5].

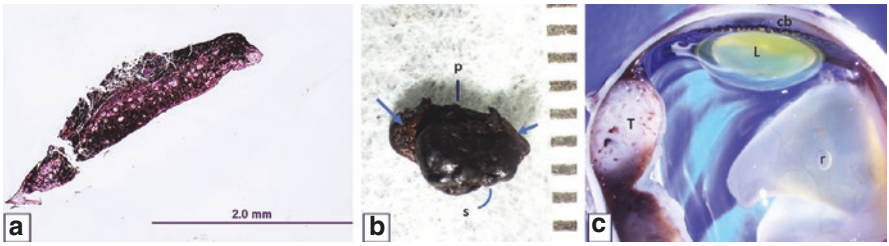

Fig. 4.2 Uveal melanoma specimens by location. (**a**) Low magnification of histopathology of iridectomy specimen with melanoma. The iris stroma is defaced by a darkly pigmented invasive spindle melanoma obliterating the surface or deep non-pigmented epithelium and with a plaque of tumor on the surface (superior). The peripheral margin (bottom left) is free of lesion (HE stain, original magnification 1.25X). (**b**) Macroscopic view from the posterior surface of an iridocyclectomy specimen. Notice the ciliary body melanoma (dark irregular nodule) and the posterior surface of the iris (arrow). The scleral (s) margin and the pupil (p) are marked for orientation. (**c**) Central section of the enucleated eye with choroidal melanoma, mostly amelanotic with areas of vascular congestion (T). There is associated retinal detachment (r), clear lens (L), and unremarkable ciliary body (cb)

Enucleation: The resection of the eye with a segment of optic nerve but without conjunctiva and extraocular muscles and fat represents an enucleation. The relevant margins for histological evaluation for uveal melanoma enucleation are the vortex veins (vascular drainage of the uveal tract), usually four located in the back of the eye exiting the sclera between each of the four quadrants of the eye. The optic nerve is not a relevant margin because uveal melanoma rarely tracts the optic nerve in contrast with retinoblastoma that has a special affinity for the optic nerve. If there is extraocular extension of the tumor then inking the surface at this site is important to be able to microscopically evaluate for the presence of tumor at the inked margin of resection. Transillumination of the eye with localization of the tumor mass is necessary to section the eye including the tumor in the optic nerve-pupil section (P.O.) (Fig. 4.3). For fresh tumor retrieval at time of enucleation, the vortex veins are obtained first. Then a scleral window, at least 2 mm from the optic nerve, is performed at the edge of the tumor shadow (identified through transillumination) parallel to the plane of section that will obtain the P.O. section after fixation. Ideally the amount of tumor left in the small cap created by the scleral incision is the tumor that will be harvested, avoiding unnecessary trauma to the remainder of the tumor and the intraocular structures (Fig. 4.3) [5].

Adequate fixation of the eyes in 10% formalin is essential to obtain adequate sections. Ideally, the eye should be fixed in formalin that is five to ten times the volume of the eye for 48–72 h to allow adequate penetration and fixation. If the eye was opened fresh for tumor retrieval, then the fixation could be shortened to 24–48 h. If the eye is not opened fresh to retrieve tumor, then there is no need to open the eye or inject formalin because these procedures, especially when not done by the pathologist, may alter the intraocular structures and add artifacts misleading final interpretation.

After adequate fixation, the eye is then opened to obtain the central P.O. section that would include most of the tumor and the two calottes (peripheral caps). The central P.O. section should have the intact optic nerve and the calottes only the

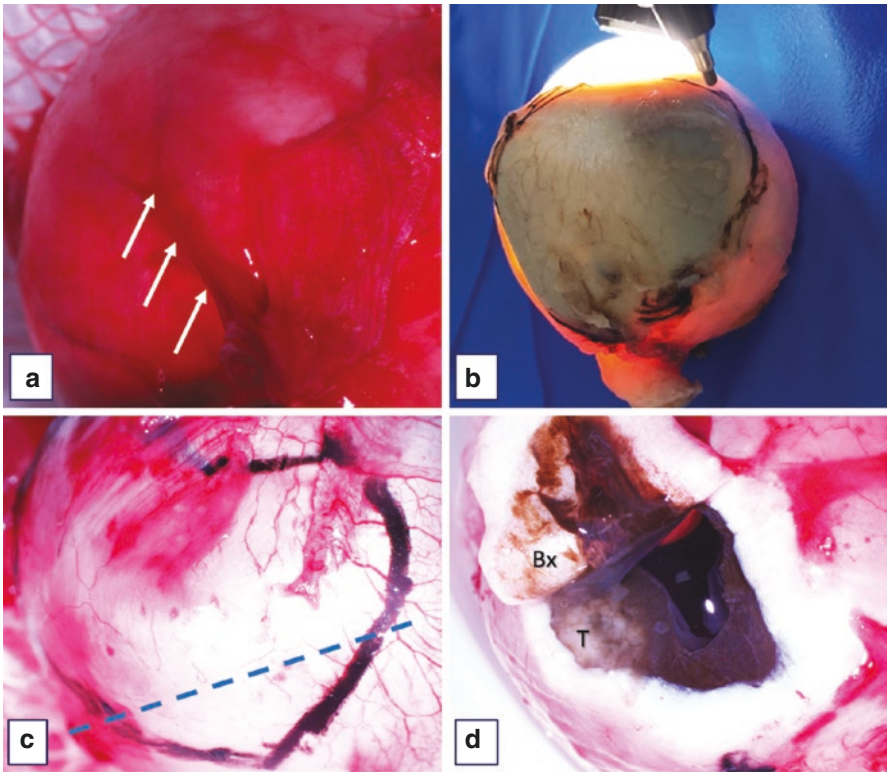

Fig. 4.3 Dissection of enucleations containing melanoma. (**a**) Freshly enucleated eye is oriented for laterality, and then dissection of the vortex veins (arrows) is performed to evaluate intravascular permeation of tumor. (**b**) Transillumination of the eye discloses the tumor mass. Drawing the limits of the mass allows for planning of the location of the incision for tumor retrieval and final sections. (**c**) After transillumination, the eye is open at the edge of the shadow (dashed line) forming a small scleral window. (**d**) After the scleral window is performed, biopsies of the tumor (T) are obtained for molecular studies. In this example, the tumor that was in the scleral window was harvested (Bx)

sclera and intraocular tissues. The calottes are further sectioned in a bread loaf manner to examine the choroid/sclera in more sections and avoid missing microscopic areas of extraocular extension (Fig. 4.4). In total, three to seven cassettes (3 for the eye and 4 cassettes one for each of the four vortex veins - if none or not all vortex veins are found then at least the 3 cassettes for the eye are needed) (1) P.O., (2) one calotte in segments, (3) the other calotte in segments, and (4–7) vortex veins, each in one cassette labeled by quadrant (Fig. 4.4) [5].

Growth Pattern

The growth pattern of the tumor is associated to prognosis. The patterns of growth are identified after the eye is sectioned. Iris melanomas with ring-type growth have a diffuse growth over the surface and invade the anterior angle structures in the 360°

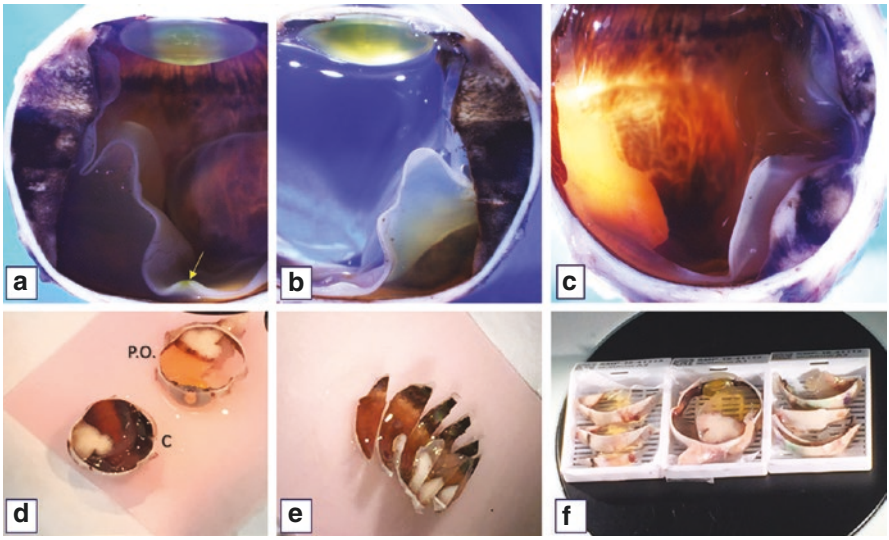

Fig. 4.4 Sectioning and processing the eye with melanoma. The eye is sectioned anteriorly-posteriorly to obtain a central section that contains the pupil and the optic nerve (**b**, **d**) plus the tumor and two calottes (**a**, **c**, **d**). In this eye, seen is in (**a**–**c**), the sections were made vertically to obtain a temporal (with half of the macula seen - arrow) calotte (**a**) and a nasal calotte (**c**) were obtained. The tumor heterogeneous with amelanotic and pigmented areas and it is diffuse type (23 mm at the base) involving from the posterior choroid to the ciliary body anteriorly. It is present in the three sections of the eye associated with retinal detachment. (**d**) The central section called pupillary optic nerve (P.O.) is seen by the calotte before further sectioning (**e**) to allow thorough examination of the choroid and sclera. The entire eye is then submitted for histologic processing and examination in the three cassettes (**f**)

forming a ring. These tumors are composed by epithelioid, high-grade-type cells and are the exception to the good prognosis of the iris tumors as they carry high risk of metastasis. Most commonly the choroidal melanomas have a solid well-defined nodular pattern, or, if they are of medium to large size, they may have a mushroom shape. Mushroom shape is the result of the tumor breaking through Bruch's membrane and growing rapidly into the subretinal space. The diffuse pattern of choroidal melanomas is associated more often with extraocular extension and spontaneous necrosis of tumor (Fig. 4.5) [6].

Scleral Invasion and Extraocular Extension

If the tumor invades the sclera, this could have a direct intrascleral component or follow the pathway of the intrascleral vessels and nerves (emissary channels) and reach the extraocular tissues through these channels. The extension, direct through the sclera or through the emissary channels, may be microscopic or macroscopic where a large nodule is identified at the time of grossing of the eye. The latter carries adverse outcomes (Fig. 4.6).

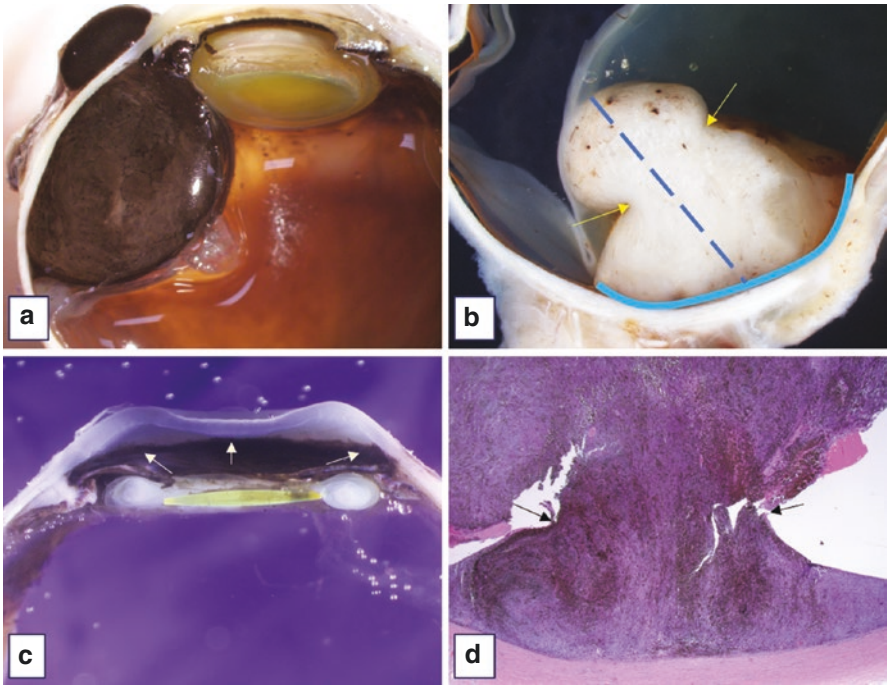

Fig. 4.5 Growth pattern and extraocular extension in uveal melanoma. (**a**) Nodular growth pattern in the anterior choroid is dome shaped. In this case, the tumor involves focally the ciliary body and extends into the extraocular tissue. (**b**) Mushroom-shaped tumor with rupture of Bruch's membrane (arrows). The diameter of the base of the tumor in contact with the sclera is an important prognostic feature (solid blue line). Height of the tumor is also measured at the steepest point (dashed line). This tumor is amelanotic and detaches the retina. (**c**) Iris ring-type melanoma invades the surface and root of the iris and invades angle structures of the anterior chamber (arrows) without forming a mass. (**d**) Histologic features of a mushroom-shaped tumor show the ruptured Bruch's membrane that allows the nodular growth of the tumor into the subretinal space (top of the picture) (HE stain, original magnification 2X)

Size

The tumor volume is a marker for prognosis in uveal melanoma. The measurement that has more prognostic value is the basal diameter (measurement of the tumor in contact with the sclera), but the maximum height is also important, and both should be recorded (Fig. 4.5) [7]. See below for staging.

Microscopic Features with Prognostic Value

Tumor cellularity type: Uveal melanomas are classified by the proportion of each cell type of the tumor composition. This is the basis for the modified Callender classification [8]. Although not without controversy, for the precision and interobserver

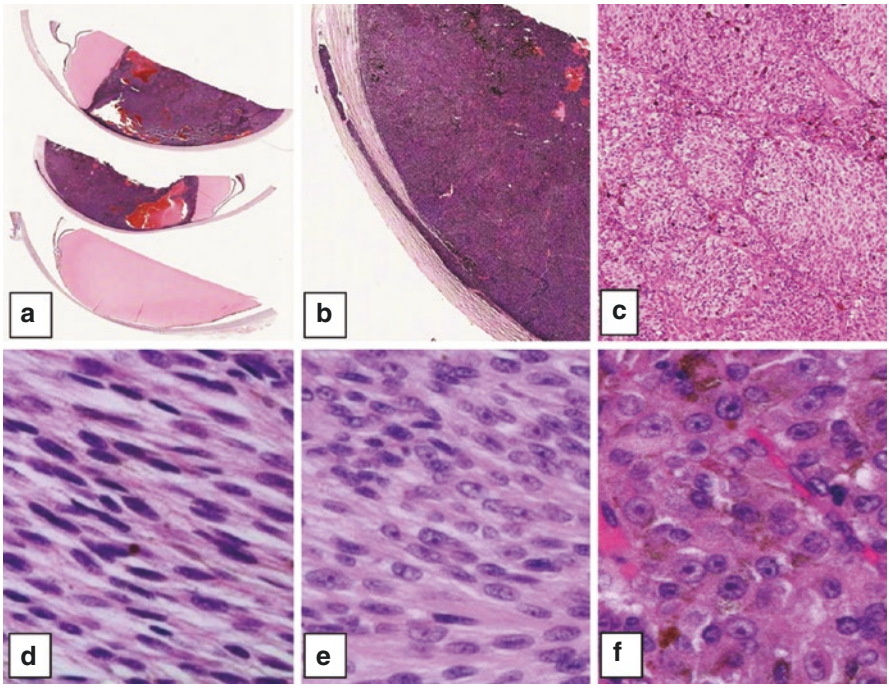

Fig. 4.6 Microscopic findings in uveal melanoma. (**a**) Often the calottes that show more choroidal and scleral surface contain the features of invasion. The middle section of the calotte shows intravascular vortex vein invasion (HE stain, original magnification 1.25X). (**b**) Close-up to this area shows the tumor thrombosis of the vessel (HE stain, original magnification 4 X). (**c**) Presence of vasculogenic mimickers is better seen using PAS stain. Notice the lines crossing and anastomosing in the tumor. (PAS stain, original magnification 10 X). (**d**) Spindle type A melanoma cells show no nucleoli and a central nuclear fold. (**e**) Spindle type B display nucleoli and oval nuclei. (**f**) Epithelioid-type melanoma cells are round with prominent nucleoli and abundant cytoplasm. (**d–f**) HE stain, original magnification 100X)

validity of the classification, it is widely used for assessment of prognosis. The epithelioid melanomas are the ones that have more value for prognosis. The classification is based on the amount and percentage of spindle cells: A (slender cells with elongated nuclei without distinct nucleoli and with a central groove) and/or B (elongated cells with oval nuclei and prominent nucleoli). If tumors were made of pure spindle A cells, these would represent *melanocytic nevi*. Tumors with both A and B spindle cells or predominantly B spindle cells are the *spindle melanomas*. Epithelioid cells are round, medium-to-large cells that have a round nucleus with prominent nucleolus. These cells may be binucleated. If the tumor is made up of a mixture of spindle and epithelioid cells, then it is called *mixed melanoma*. Although controversial, those tumors with more than 90% epithelioid cells are classified as *epithelioid melanomas* (Fig. 4.6). The importance of the epithelioid cells is that they are statistically significantly associated with increased risk of metastasis. Molecular mutations in the BAP1 gene have been identified in tumor cells with epithelioid morphology

and not only in uveal melanoma but in other tumors such as mesotheliomas [9]. BAP1 mutations are also associated with adverse outcome in uveal melanoma (see Chap. 3).

Cellular proliferation: Mitotic figures are not easy to identify in uveal melanomas, and in large tumors, it may be necessary to find a mitosis and then start the counting from this index mitosis (amount of mitoses per 40 high-power fields are counted). The higher the number, the worse the prognosis. The proliferation index and the result of cells in proliferation phase labeled by Ki-67 (Mib-1) by immunohistochemistry are not often used. However, it highlights much more cells in proliferation phase than mitotic figures may be found (Fig. 4.7).

Tumor milieu: The formation of circulation channels by the malignant uveal melanoma cells is recognized as vasculogenic mimicry because these are not composed by vascular cells. Histologically, these vasculogenic patterns may be better seen using periodic acid-Schiff (PAS) stain as they have extracellular matrix material that is highlighted by this stain. If there is tubular formation, these tubes may contain blood cells. The vasculogenic patterns may be loops or networks, simple or complex. The more aggressive tumors form these vasculogenic mimicries and its prognostic value for metastasis and poor outcomes have been associated to gene expression profiles of adverse outcome (Fig. 4.6) [10, 11].

Tumor-infiltrating lymphocytes and plasma cells represent an immunologic sign of probable micrometastases. The eye is an immune-privileged site, and the presence of lymphocytes and especially plasma cells indicates that the tumor has exited this privilege site and has elicited an immune response toward the tumor cells. However, the prognostic value of these lymphocytes infiltrating the tumor has fallen out of statistical value in recent publications. Nevertheless, the presence of lymphocytes and plasma cells infiltrating the main mass should be reported if found. The infiltration by macrophages has also been reported as a probable adverse prognostic factor (Fig. 4.7) [12].

Post-treatment effect: Occasionally the eye is enucleated after brachytherapy often because of complications of radiation (neovascularization, retinopathy, neovascular glaucoma, and hemorrhages), and evaluation of the tumor and sclera is

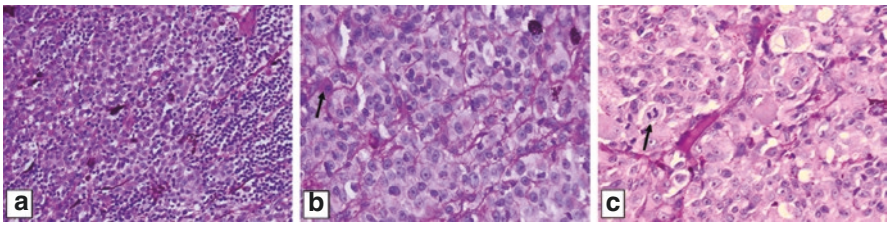

Fig. 4.7 Adverse histopathologic features in melanoma. (**a**) Tumor mostly composed by small epithelioid melanoma cells is infiltrated by lymphocytes (PAS stain, original magnification 20X) (**b**). Vasculogenic mimickers seen between groups of epithelioid melanoma cells and rare plasma cells infiltrating the tumor (arrow). (PAS stain, original magnification 40X). (**c**) Mitoses (arrow) are often found in areas rich in epithelioid cells. Notice also the vasculogenic mimicry with lumen to the right of the mitosis in bright pink – PAS positive (PAS stain, original magnification 40X)

necessary to exclude extraocular extension. The tumor may show signs of necrosis or infarction with hemorrhage. The sclera may be thinned and may contain pigment-laden macrophages but no melanoma cells, and this is an important differentiation often made with the help of immunohistochemistry (see below).

Immunohistochemistry: The location, cellularity, and overall histopathologic features of uveal melanoma are very distinctive, and most of the time the diagnosis is made without the aid of immunohistochemistry. However, in small biopsies, cytology samples, atypical tumors with predominance of epithelioid cells, or metastatic tumors (often to the liver), immunohistochemistry may be necessary to confirm the diagnosis (Fig. 4.8). The most useful markers for identification of the melanocytic lineage are MART1/Melan-A, HMB45, and SOX10. S100 protein is positive in approximately 70% of the uveal melanomas; thus, it is not recommended as first-line antibody to prove the tumor lineage. Although not used routinely, the nuclear proliferation marker (Ki-67/Mib-1) may be used to assess the proliferation rate of tumors. Most often, though, Ki67 is used to confirm that a melanocytic lesion is proliferating differentiating a melanoma from a nevus (in dormant state). This is usually employed in cell blocks of fine-needle aspiration biopsies (FNAB) where there is scant number of cells to evaluate pattern of growth and other features. When using Ki67 for this purpose, it is recommended to perform a double immunostain with either Melan-A or HMB45, both cytoplasmic melanocytic markers, to confirm the melanocytic origin of the proliferating cell. Usually Ki67 is paired with a brown (DAB) chromogen and Melan-A or HMB45 with a red chromogen (Fig. 4.8). Red chromogens are used frequently when using melanocytic markers to distinguish the stain from the melanin pigment. If a tumor is too pigmented, immunohistochemistry may be performed after the tissue is bleached of the melanin pigment. Immunostaining for tumor-infiltrating lymphocytes, macrophages, or for BAP1 and PRAME expression has recently been added in some practices to aid in assessment of prognosis [12]. The positivity for the cancer-testis-antigen PRAME (preferentially expressed antigen in melanoma) has been identified as an independent prognostic marker because it identifies an increased metastatic risk in patients with uveal melanomas that have a low-risk molecular signature [13].

Cytology

Uveal melanomas differ from other tumor malignancies in that the diagnosis is made solely on clinical and imaging basis and treated without biopsy confirmation. This is based on the idea that FNAB may induce metastasis or local recurrence if tumor cells scape the eye. In addition, if the decision is to use brachytherapy, then all efforts are made to preserve vision by avoiding any complication of FNAB (hemorrhage, retinal detachment, endophthalmitis). However, since the recognition that uveal melanoma tumors have molecular prognostic features, biopsies to obtain tumor at time of placement of the radiation plaque have become the norm [14, 15]. Most ocular oncologists perform the biopsies and submit the entire sample to

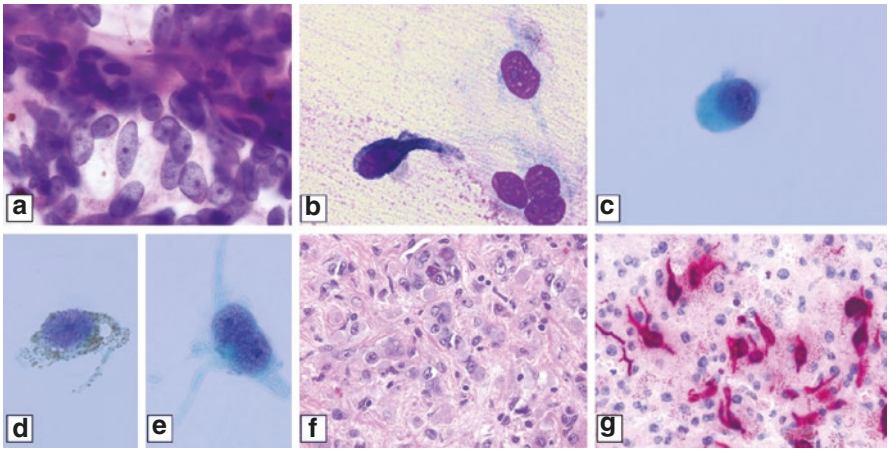

Fig. 4.8 Cytologic and Immunohistochemistry in uveal melanoma. (**a**) Touch imprint during tumor harvesting shows adequate sample for molecular studies. Notice the cells are slender or oval with small nucleoli in melanoma B cells with medium amount of cytoplasm and some pigment (HE stain, original magnification 100X). (**b**) Fine-needle aspiration adequacy evaluation shows spindle pigmented cell and some epithelioid cells with scant cytoplasm (Diff-Quik stain, original magnification 100X). (**c**) Alcohol-fixed preparations show more details of the cells in this epithelioid melanoma cell. Notice the abundant cytoplasm and the prominent nucleoli at the center. (Papanicolaou stain, original magnification 100X). (**d**) Spindle melanoma cell with dust-like fine pigment in the cytoplasm and elicit tentacular-like prolongations of the cytoplasm is seen (Papanicolaou stain, original magnification 100X). (**e**) Binucleated spindle B cells are often encountered (Papanicolaou stain, original magnification 100X). (**f**) Liver core biopsy in a patient with history of uveal melanoma with many epithelioid cells shows the epithelioid mostly amelanotic cells invading the liver. Some cells show pigment (top center) (HE stain, original magnification 40X). (**g**) Immunohistochemistry using a combined HMB45 (melanocytic cytoplasmic red stain) and Ki67 (proliferation nuclear brown stain) shows the cells infiltrating between the hepatocytes in the same biopsy seen in (**f**) (immunohistochemistry, HMB45 antibody with red chromogen, Ki67 antibody with DAB chromogen, original magnification 100X)

molecular testing without confirmation of cellularity. Ideally, though, confirmation of diagnosis and of adequate cellularity for molecular testing is recommended. Many of the molecular tests do not include sequencing that would confirm the presence of melanocytic cells but rather focus on finding the specific pathogenic variants; thus, any type of cells will be tested and results reported. If the biopsy contains blood, retina, other normal cells, or metastatic malignancy, the results will be reported as not having the high-risk molecular profile or the pathogenic variants, or in the case of metastasis, some high-risk molecular profiles can be identified. These results may be misleading and may defeat the purpose of the biopsy [16].

To confirm that what is genetically tested is the tumor and no other type of cell, two options based on cytopathology are available. The first option is to have a rapid on-site evaluation (ROSE) of the specimen that has been submitted for molecular testing. ROSE requires the presence of a cytopathologist at time of biopsy to evaluate the sample. ROSE is routinely available for other types of tumors in hospital-based

procedures (pancreatic, lung, and liver tumors that undergo FNABs) in terciary hospital settings. Ideally, the same sample used for molecular is evaluated by ROSE. To this end, the first and last drops of the needle biopsy may be looked at microscopically while the middle portion of the biopsy is submitted to molecular testing. Rinses of the needle, tubing, and syringes used into cytology fixative may be used to obtain a cell block. If the cytology specimen looked at during the procedure has no tumor cells, then the surgeon can perform a second FNAB or more until obtaining tumor [17]. The second option is for those ocular oncology practices that do not have the availability of cytopathologists to perform ROSE. This option is like the one used for ROSE, but, instead of putting the first and last drops on the slides, they are put directly into the cytology fixative together with the rinses. The fixative is then submitted (mailed or referred) for cytologic evaluation. This second option confirms the diagnosis and validates the results of the molecular testing if melanoma cells are present.

Rarely, atypical tumors with uncertain clinical and imaging features need to be biopsied to confirm diagnosis. In these cases, enough cells are required to prepare the cell block that will be used for immunohistochemistry and possible for molecular testing.

Cytopathologic features of uveal melanoma include a hypocellular sample that is deceivingly bland. The same modified Callender classification may be applied to cytologic findings. Most samples have a combination of spindle cells. The cells may be pigmented or amelanotic and single or most often in groups with delicate elongated cytoplasm and occasional nucleoli. If epithelioid cells are present, they are characteristically medium to large size and oval to round, with a round prominent nucleus and/or two nuclei [18]. Cell blocks may be immunostained to confirm diagnosis and to evaluate for presence of other cells such as tumor-infiltrating lymphocytes (Fig. 4.8).

Differential Diagnosis

The most common differential diagnosis is with metastasis because metastasis is the most common intraocular malignancy in adults. The presentation of metastasis is usually very typical by clinical features, and history and biopsies are often omitted. In cases in which FNAB is needed, immunohistochemistry is essential to prove the cell of origin. The other common differential diagnosis, especially in small lesions in the iris and choroid, is nevus. Nevi lack the presence of spindle B-type melanoma cells and proliferating cells. Cell block and immunohistochemistry using Ki67 and Melan-A or HMB45 are helpful in assessing proliferation; however, if the sample is paucicellular and because uveal melanoma has a low proliferation rate, the absence of Ki67 co-staining may be inaccurate, and diagnosis mostly relies on cytologic features alone. Other frequent differential diagnosis of choroidal melanoma is with old hemorrhage associated with subretinal neovascular membranes. Melanocytoma, hemangiomas, retinal pigment epithelium adenomas/adenocarcinomas, and

bilateral diffuse uveal melanocytic proliferation (BDUMP) – a rare paraneoplastic intraocular disease – may be in the differential diagnosis. These lesions and tumors have distinct histopathologic and cytologic features [19].

Pathologist's Report

The pathology and cytopathology reports should include confirmation of diagnosis and type of tumor. The pathology report in enucleations and segmental excisions should be based on what the 8th edition of the American Joint Committee on Cancer

Table 4.1 Example of tumor synoptic report in uveal melanoma following the AJCC 8th Edition

Anatomic site of cancer:	Left eye
Tumor type:	Malignant melanoma of the uvea
Procedure:	Enucleation
Specimen size:	
Anterior posterior diameter	24.0 mm
Horizontal diameter	24.0 mm
Vertical diameter	24.0 mm
Length of optic nerve:	5.5 mm
Diameter of optic nerve:	4.5 mm
Tumor site by transillumination and macroscopic examination:	Peripapillary to limbus, inferonasal quadrant
Tumor size after sectioning:	
Base at cut edge:	23.0 mm
Height at cut edge:	4.0 mm
Greatest height:	4.0 mm
Tumor involvement of other ocular structures:	Sclera, ciliary body
Growth pattern:	Diffuse
Histopathologic type:	Mixed-type melanoma
Histologic grade:	G3
Other:	Areas of necrosis and regression
Margins:	No melanoma at margin
Pathologic staging	
Primary tumor:	pT4b (more than 18 mm at base + ciliary body involvement)
Regional lymph nodes:	pN0
Distant metastasis:	pM0
Staging:	IIIB

Additional findings: Mitotic rate of 7 mitoses per 40 high-power fields, vasculogenic mimickers present, tumor-infiltrating lymphocytes and plasma cells
Gene expression profiling: Class 2, PRAME positive

(AJCC) has put together for reporting essential features for prognosis (Table 4.1) [20]. These include location of tumor in the uvea, growth pattern, size (base and height) of tumor, scleral and extraocular extension, type of cellularity of the melanoma (modified Callender classification), mitotic count in 40 high-power fields, presence of vasculogenic mimicry, and presence tumor infiltrating lymphocytes, plasma cells, or macrophages. The staging is primarily based on the size of tumor in combination with the other features. Molecular genetic findings should be included in the staging if available.

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Chapter 5
Uveal Melanoma: Epidemiology of Uveal Melanoma and Potential Clusters

Asghar A. Haider and John O. Mason III

Incidence of Uveal Melanoma

The incidence of uveal melanoma varies by age, sex, race, and country [1, 2]. The National Institutes of Health (Maryland, USA) have a Surveillance and Epidemiology and End Result (SEER) program that collects and provides reliable population-based incidence data on a wide range of cancers, including uveal melanoma in the US population [3].

In the United States, the median age of diagnosis is approximately 62 years and peaks in the seventh decade of life [3]. At 70 years, the incidence of uveal melanoma is 24.5 per million in males and 17.8 per million in females [3]. There has been a minor variation of age-adjusted incidence of uveal melanoma over time, but the age-adjusted incidence has roughly remained approximately 5 per million, with the 2010 to 2015 SEERS data demonstrating an overall incidence of uveal melanoma of 4.637 per million (95% CL, 4.458–4.821) in the United States [4].

The reported incidence of uveal melanoma varies drastically in different countries. Asian countries like Japan, South Korea, and Taiwan have incidence rates of uveal melanoma ranging from 0.3 to 0.42 per million [5–7], whereas European countries have an incidence rate of around 5 to 10 per million [8–10]. Of note, countries with predominantly white populations and those exposed to higher ultraviolet light levels like Australia and New Zealand have similar incidence rates of uveal melanoma to their European counterparts [11].

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Risk Factors for Uveal Melanoma

Race and skin color are the most significant host risk factors for the development of uveal melanoma, with non-Hispanic whites being the most affected group accounting for 98% of reported cases in the United States [3]. When compared to African-American patients, the relative risk of uveal melanoma is 1.2 (0.5–3.2, 95% CI) for Asian and Pacific Islanders, 5.4 (2.5–11.5, 95% CI) for Hispanics, and 19.2 (0.7–39.0, 95% CI) for non-Hispanic whites [1].

Light irides have similarly been shown to increase the risk for uveal melanoma, with one meta-analysis by Weiss et al. showing 1.75 times increased chance of developing uveal melanoma [12]. The cause of this increased susceptibility is unclear. Some authors postulate that lighter irides' reduced pigmentation results in greater transmittance of ultraviolet light to the fundus and subsequently contributes to increased uveal melanocytic damage [9, 13]. This hypothesis has been challenged as sunlight exposure, outdoor leisure activities, and birth latitude have all been shown to be inconsistently associated with the development of uveal melanoma [14].

Naymar et al. highlighted several other risk factors for the development of uveal melanoma in a systemic review of published meta-analyses [15]. This included atypical cutaneous nevi (OR 2.82, 95% CI 1.10–7.26), common cutaneous nevi (1.74, 95% CI 1.27–2.39), propensity to sunburn (OR 1.64, 95% CI 1.29–2.09), iris nevi (OR 1.53, 95% CI 1.03–2.27), and cutaneous freckles (OR 1.27, 95% CI 1.09–1.49) [15].

As our understanding of uveal melanoma genetics grows, more genetic predispositions to uveal melanoma are identified [15]. Germline *BAP1* mutations were initially identified in patients with familial uveal melanoma and familial cutaneous melanoma and in families with uveal and cutaneous melanomas and have been consistently shown to increase the risk of uveal melanoma [16, 17]. Additional germline genetic mutations in genes such as *BRCA2* and *CDKN2A* have also been reported in patients with uveal melanoma and may be associated with increased susceptibility to uveal melanoma, though additional research is necessary [18–21].

Finally, it is important to highlight increased susceptibility to uveal melanoma with syndromes such as oculodermal melanocytosis, familial atypical mole, and melanoma syndrome of neurofibromatosis type 1 [22–25]. Of the known syndromes associated with uveal melanoma, oculodermal melanocytosis, which has hyperpigmentation of the skin, episcleral, uvea, orbit, and meninges, is the most significant and has been found to have an estimated risk of uveal melanoma in about 1 in 400 in white individuals [26]. The additional melanocytes in patients with oculodermal melanocytosis may explain the increased susceptibility to uveal melanoma development [23]. Recent genetic studies have provided a possible genetic explanation between oculodermal melanocytosis and uveal melanoma by identifying a *GNAQ* mutation in many of these patients [27].

Potential Uveal Melanoma Cluster Populations

According to the Centers for Disease Control (CDC), a cancer cluster is defined as a greater-than-expected number of cancer cases that occurs within a group of people in a geographic area over a period of time. Traditional epidemiological approaches to identify environmental risk factors have been difficult in uveal melanoma due to the rarity of the condition and difficulty obtaining reliable historical information [28]. Even so, multiple reports in the literature have reported increased incidence of uveal melanoma in specific populations [5, 28, 29].

In 1980, Albert et al. reported five choroidal melanoma cases diagnosed in the 1970s at a chemical facility in Belle, West Virginia [29]. An ophthalmologic survey was done with 847 active and retired employees and 302 subjects as control comparisons. No choroidal melanomas were observed in the control comparison group. The authors noted that these findings represented a significantly greater choroidal melanoma occurrence in the study population than expected in the general population. Thirteen substances were initially identified as suspected carcinogens by the National Institute for Occupational Safety and Health, but due to incomplete exposure histories and multiple agent exposures, the authors felt it was inappropriate to highlight any specific chemical exposure as causative [29].

Louria et al. reported another possible uveal melanoma cluster in 1982, with three cases of choroidal melanoma, detected over 2.5 years in a small community of 3592 [30]. This study estimated this small potential cluster represented an incidence of about 20 times that expected ($P = 0.0006$) [30]. The community had an isolated water supply; however, the three patients who developed uveal melanoma had no other common exposures. Analyses of air and water from the involved community by mass spectroscopy, chromatography, and mutagenicity tests were noncontributory [30].

More recently, Orloff et al. identified three new potential uveal melanoma clusters in Alabama, New York, and North Carolina [28]. The authors investigated patients in these potential cluster populations by identifying patients who had (1) unusual age at the time of diagnosis (younger than 40 years old), (2) diagnosis of uveal melanoma in close proximity, and (3) exposure to environmental toxins or uncommon infections [28].

Dr. John Mason III, MD (Director of Retina Services and Ocular Oncology, UAB Department of Ophthalmology), and Dr. Fred Kam, MD (Medical Director Auburn University), are currently leading the Auburn Ocular Melanoma Study. A total of 31 ocular melanoma patients were located who spent time in Auburn, Alabama, as students, professors, or family members. Approximately half were female, and half were male. The average age of diagnosis was 42. The medium time spent at Auburn was 1987–1988. 25 of the 31 remain living, and the geospatial analysis is evaluating potential areas of environmental concern in the Auburn area. While initially delayed due to the COVID-19 pandemic, IRB-approved protocols for genetic testing of the remaining 25 living patients are now underway. Whole-genome sequencing will be performed, examining serum as well as pathology

specimens for specific mutations (BAP1) as well as molecular environmental signature mutations of carcinogens (J. Mason, personal communications, June 26, 2021).

In Huntersville, North Carolina, five young women were identified who all resided in Huntersville during a time overlapping in 2005 and were diagnosed with uveal melanoma from 2008 to 2014. The women were aged 22 to 30. Three of these patients attended the same high school, Hopewell High School in Huntersville, NC [28]. Dr. Mike Brennan, MD, a former president of the American Academy of Ophthalmology, was selected to coordinate and evaluate the genetic testing, geospatial survey, and environmental testing. Dr. Brennen identified at least two dozen patients that had spent time in and around Huntersville, NC. These patients' geospatial survey did not find a "hot spot" or concentrated area where these ocular melanoma patients spent time. Therefore, no environmental testing was performed. The genetics aspect of the study was inconclusive as well. Officials at the North Carolina Department of Public Health Occupational and Environmental Epidemiology Branch (OEEB) published a report stating these cases were random, albeit tragic, events. Furthermore, the OEEB noted that an analysis of NC Central Cancer Registry (CCR) data in 2015 did not find a higher than expected number of ocular melanoma cases in Mecklenburg County, Huntersville, or Huntersville/Cornelius (M. Brennan, personal communication, November 6, 2020).

Fourteen patients were identified in a 15-mile radius along the Susquehanna River in New York [28]. These towns include Owego, Apalachin, Vestal, Endicott, Johnson City, and Binghamton and are located in Broome and Tioga counties. Eight of the fourteen patients are women with a median age at diagnosis being 52 years. The New York state cancer registry was informed about these patients, and an investigation was requested. The respective agencies calculated the standard incidence ratios (SIR) of uveal melanoma in the identified populations. SIR can be used to determine whether the suspected accumulation of cases represents a statistically significant increase in the ratio of observed to expected cases [28]. There were concerns regarding the under-reporting of identified cases in the investigations, partly attributed to patients being diagnosed out of state or living out of the state at the time of diagnosis [28]. The SIRs calculated for the identified populations were not statistically significant except the ratio calculations for males and females (2.00, 95% CI 0.91–3.9) and females alone (3.33, 95% CI 1.34–6.87) in Tioga county.

Summary

Uveal melanoma is the most common intraocular malignancy with a progressively rising incidence with age and affects males more than females. Host risk factors include fair skin, non-Hispanic white race, and older age. Environmental risk factors have been challenging to identify. Reports of potential cluster populations are promising, to hopefully elucidate an environmental, genetic, or combination of genetic and environmental carcinogens responsible for uveal melanoma. Although the Huntersville population results were inconclusive, the Auburn Ocular Melanoma

Group investigation is currently underway. Hopefully, it will yield results that will allow us to better understand uveal melanoma cancer genomics and causative environmental insults. While these particular groups do not meet the criteria for cancer clusters, investigating these patients is an essential first step to better understand the epidemiology and pathogenesis of uveal melanoma.

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Chapter 6
Gene Expression Profiling in the Management of Uveal Melanoma

Zelia M. Correa

Introduction

Historically, one of greatest challenges in the management of uveal melanoma (UM) has been management of metastatic disease. Because UM is the most prevalent primary malignant intraocular tumor in adults and carries a significant risk of metastases, which has shown to be mostly unresponsive to available systemic therapy [1], very little progress has been made over the years in improving survival. Thus, researchers have searched for prognostic indicators (initially clinical [2], then pathological [3], chromosomal [4], and finally genomic [5, 6]) to identify patients at increased risk for developing such metastasis in order to optimize surveillance testing and early treatment of metastatic disease. New insights on molecular pathways have shown multiple events to be dysregulated during the multistep process of oncogenesis indicating potential novel therapeutic approaches with promising clinical applications [7].

It has been shown that prognosis of UM can be most accurately predicted by genetic profiling of a fine-needle aspiration biopsy (FNAB) aspirate from the primary tumor before treatment. Currently, research is also looking at next-generation sequencing, single-cell sequencing, and ancestry to further enhance the identification of high-risk patients for clinical trials that may lead to target-based therapies for metastatic disease and adjuvant therapy which aims to prevent metastatic disease [8, 9].

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Historical Relevance of Gene Expression Profiling in UM Prognosis

Conceptually, cancer is believed to develop from a series of genomic aberrations. Conversely, it remains unclear when these metastases determining aberrations occur in the process of tumor evolution [8]. Prognostic assessment of UM was historically inaccurate likely because this tumor's evolution was poorly understood. Several chromosomal abnormalities in UM have been used for prognostication, including loss of 1p, 3, 6q, 8p, and 9p and gain of 1q, 6p, and 8q. Various techniques have been investigated to detect these changes, including standard karyotyping, fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH), spectral karyotyping, microsatellite analysis (MSA), multiplex ligation-dependent probe amplification (MLPA), and single-nucleotide polymorphisms (SNPs) [4, 10]. While loss of chromosomal heterozygosity (LOH) was identified in 63% of tumors, loss of one copy of chromosome 3 (monosomy 3 or LOH3) occurs in 52% of all UMs and has shown to be the most prognostically significant of these chromosomal markers [6, 10]. The importance of monosomy 3 alone was misrepresented although some clinicians started using it as a prognostic marker [11]. Although cytogenetic alterations afforded an important step toward the development of accurate prognostic markers for uveal melanoma, they hold significant drawbacks in using this information in routine clinical practice. These methods were developed from uveal melanomas' specimens obtained from enucleation that yields large amounts of tumor tissue. However, about 90% of uveal melanomas are now managed by plaque brachytherapy and not by enucleation, in which case the only way to obtain tumor tissue without severely damaging the patient's vision is by needle biopsy. Unfortunately, the amount of tumor material obtained by needle biopsy is often insufficient for chromosomal assay techniques [10, 12, 13]. Further problems with chromosomal prognostic testing include sampling error resulting from intratumoral heterogeneity and the complicated combination of chromosomal changes and clinicopathologic information that are needed to maximize prognostic accuracy [11].

Clinical management of UM began to change with the discovery that molecular classification based on gene expression profiling (GEP) of the primary tumor was superior to monosomy 3 and clinicopathologic prognostic factors for predicting metastasis, and it is feasible even in small tumor aspirates [14]. GEP is strikingly different from chromosomal analysis because it provides a functional "snapshot" of the tumor's microenvironment that is more consistent across the tumor [10, 15, 16]. The GEP test consists of reverse transcription (RT)-PCR-based assay comprising 12 discriminating genes and 3 control genes performed on a microfluidics platform used routinely in clinical practice on very small tumor samples from fine-needle aspiration biopsy (FNAB) [16]. GEP-based assignment of UMs to Class 1 (low risk for development of metastasis) or Class 2 (high risk for development of metastasis) was validated in a prospective, multicenter study [17] and is now routinely performed for clinical use in many centers [12, 18, 19].

As previously mentioned, GEP was developed to be used ideally on fresh tumor samples obtained from FNAB [20]. However, GEP can also be tested on formalin-fixed, paraffin-embedded tissue (Fig. 6.1) [18]. Once the genetic material is extracted, RNA samples quantified using the Nanodrop 1000 spectrophotometer are

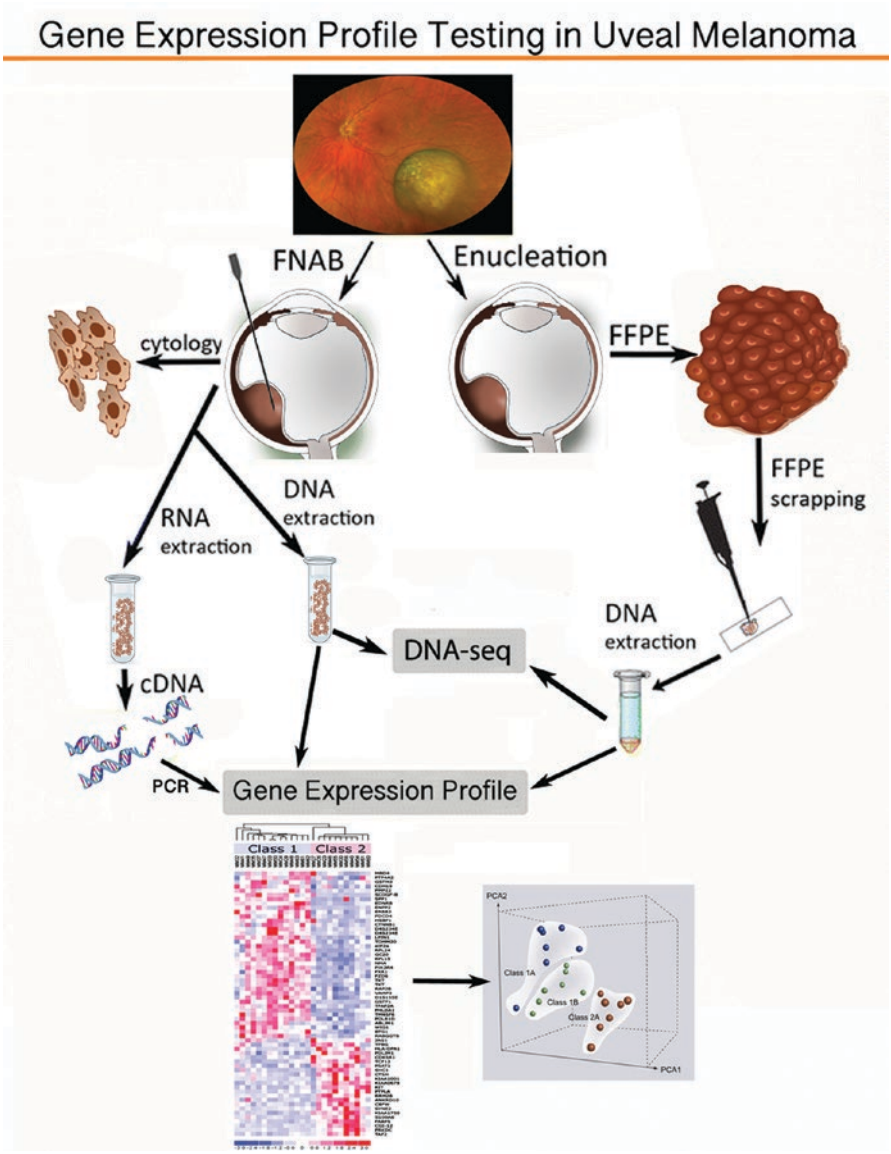

Fig. 6.1 Gene expression profiling testing in uveal melanoma. Schematic drawing depicts how tumor sampling can be used for prognostic testing – fine-needle aspiration biopsy (FNAB) and enucleation

converted to cDNA using the High-Capacity cDNA Reverse Transcription kit. The technique used for GEP testing of UM has been described in great detail [10]. Separate from its prognostic value, GEP has provided critical insights into the pathobiology of UM. It has been shown that GEP of Class 1 tumors closely resembles that of normal uveal melanocytes and low-grade uveal melanocytic tumors, whereas GEP of Class 2 tumors shows reduced expression of melanocytic genes and instead resembles the transcriptome of primitive neural/ectodermal stem cells.

After the GEP test became available commercially, clinicians started using it routinely to determine the frequency of surveillance testing since clinical evidence confirmed that most of UM metastases occurred in patients with Class 2 tumors. However, a small number of Class 1 tumors were retrospectively identified to also develop delayed metastases. Further investigation based on a retrospective analysis of expression data from the 12-gene classifier on Class 1 tumors that metastasized revealed a subgrouping of Class 1 tumors into "1A" and "1B" based on the expression of two of these genes (CDH1 and RAB31). Class 1A tumors had low CDH1/RAB31 expression while Class 1B tumors had high expression. This subgrouping has been used as a provisional indicator of Class 1 patients who may be at increased risk of metastasis [18]. The further pursuit to recognize additional more accurate biomarkers for metastasis in Class 1 tumors led to a genome-wide integrated transcriptomic and chromosomal analysis in a cohort of Class 1 tumors. The cancer-testis-antigen PRAME (preferentially expressed antigen in melanoma) was shown to be a biomarker for increased metastatic risk in Class 1 tumors. This finding showed that PRAME provides additional discriminating power among Class 1 patients and provides a potential pathway for stratification of patients for clinical trials involving adjuvant and targeted therapies [19, 21].

Practical Clinical Application of GEP in UM

After the GEP assay was validated, it was transitioned from a high-density microarray platform to a 15-gene (quantitative) qPCR-based assay that is now performed in a College of American Pathologists (CAP)-accredited Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory routinely on tumor aspirates and on archival formalin-fixed specimens [18, 22].

Multiple groups have published their findings after the GEP test for uveal melanoma became commercially available [18, 19, 23]. The GEP assay remains the only prospectively validated tool that can be used for routine clinical prognostic testing of UM and for stratifying patients into high or low risk for development of metastasis [17]. The reliable results and accessible logistics have made the commercially available Decision-DX UM® (Castle Biosciences, Inc.) the most used prognostic test for uveal melanoma in the United States [22].

Because most patients with a class 2 tumor will develop detectable metastasis within 3 to 5 years after primary tumor diagnosis despite successful treatment of the primary tumor, enrolling these patients into clinical trials at the time of primary

tumor diagnosis can hypothetically reduce the number of patients needed to treat and the length of follow-up needed to detect a difference in outcomes. The identification of BAP1 and other driver mutations as well as PRAME expression, all of which strongly associated with tumor prognosis, may soon lead to the discovery of new targeted therapies for clinical trials [9].

By and large, GEP is used to assess clinically diagnosed uveal melanoma at the time of (or immediately prior to) treatment to access individual risk for development of metastases and tailor surveillance testing in order to allow early detection of tumor spread and timely management as seen on Fig. 6.2. Patients with a Class 1 PRAME-negative tumor (or Class 1A) are recommended to have annual surveillance tests consisting of liver and lung imaging that may range from abdominal ultrasound and chest X-ray to CT of the chest and abdomen for at least 5 years. Patients with Class 1 PRAME-positive tumor (or Class 1B) should have biannual surveillance testing similar to other Class 1 patients also for at least 5 to 7 years. Patients with Class 2 tumor (independent of their PRAME status) are suggested to have surveillance imaging consisting of MRI of the abdomen every 3–4 months and chest X-ray every 6 months.

Curiously, clinicians started to use the test as a "diagnostic surrogate" to recommend treatment after the prospective validation of GEP. This unexpected use of the

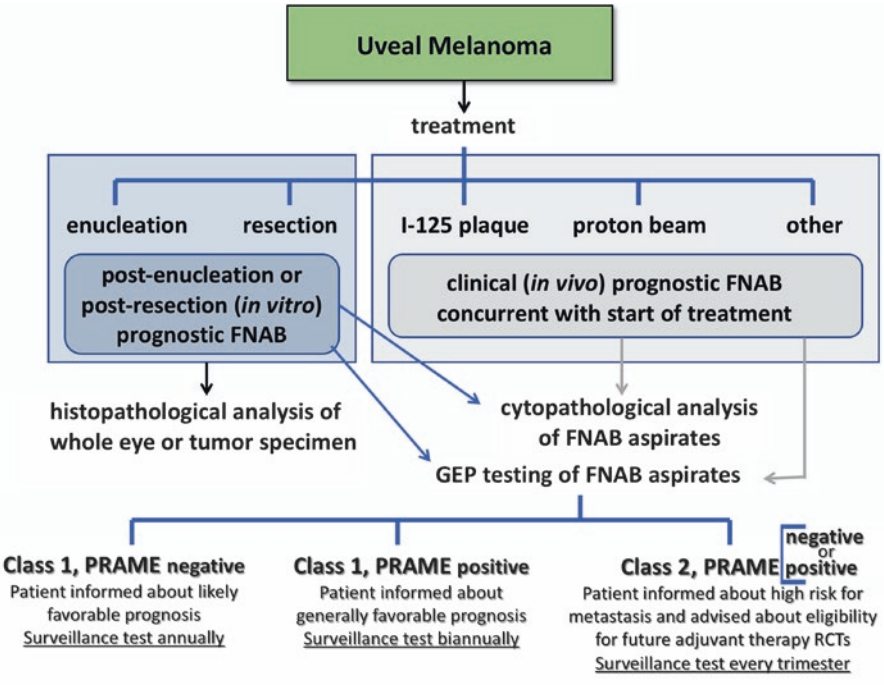

Fig. 6.2 Clinical application of gene expression profiling (GEP) in clinically diagnosed uveal melanoma. In these cases, GEP has a prognostic indication, and it is used to guide surveillance testing and future adjuvant treatment for high-risk patients

test was driven by the need ocular oncologists have to confirm early tumor diagnosis, the size overlap between small melanomas and large nevi, and the fact that GEP testing needs fewer cells than cytology to yield a conclusive test result [12, 24]. Despite personal clinical preferences, most clinicians follow a certain patter illustrated on Fig. 6.3. When an indeterminate uveal tumor is detected, it prompts 1 out of 3 options, observation for documented tumor progression, diagnostic FNAB to determine management, or treatment (that may concur with a prognostic FNAB). If FNAB is performed, cytology and GEP test may be obtained. If cytology is performed, it is used as the first diagnostic point. In cases which the FNAB yield renders an inconclusive cytology assessment (benign cells or insufficient cellular aspirate) or if cytology is not performed, GEP is solely used as a surrogate diagnostic test to recommend treatment. Patients with high-risk tumor (Class 2/PRAME positive or negative) and those with moderate risk (Class 1B and/or PRAME-positive) are treated promptly. Those with low risk (Class 1A/PRAME-negative) may or may not be treated depending on tumor location and risk for vision loss, patient age, and overall health. This information allows deferral of treatment and safe observation of patients for tumor progression and malignant transformation.

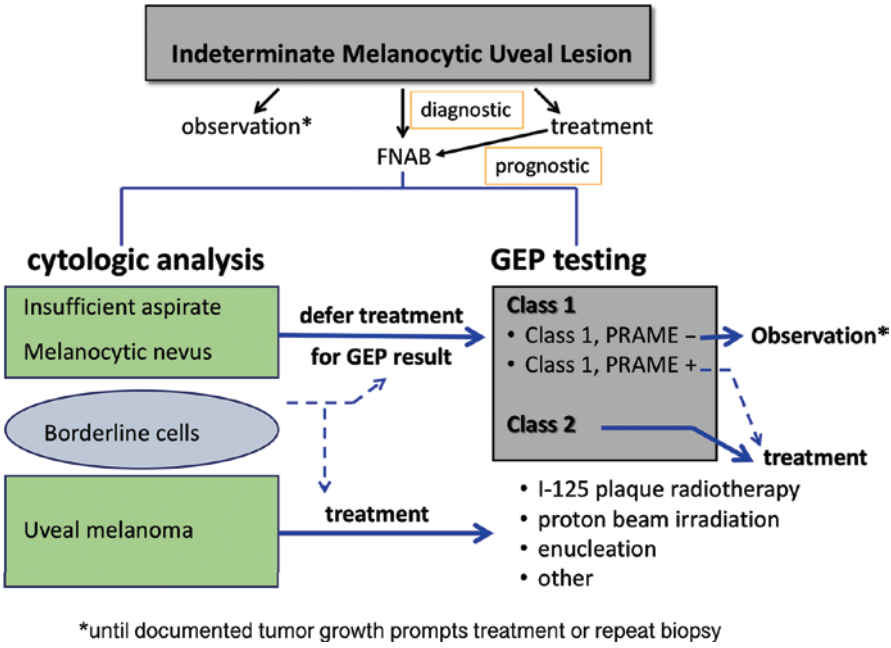

Fig. 6.3 Clinical application of gene expression profiling (GEP) in clinically indeterminate uveal melanocytic lesion. In these cases, cytology and GEP (or GEP alone) are used to indicate tumor management. If cytology is performed, it is the first diagnostic point. If cytology is inconclusive or not performed, GEP is solely used as a surrogate diagnostic test to recommend treatment. *Until documented tumor growth prompts treatment or repeat biopsy

Future Clinical Applications of GEP in UM

Research into molecular prognostic testing in uveal melanoma continues to advance as new technologies are becoming available. Rigorous research is essential to guarantee accuracy and reproducibility of any assay. While there are other tests used currently around the world, the great variability in methodology and quality requires critical assessment to identify the most accurate, accessible, and cost-effective test to move research forward with clinical trials for high-risk patients in the adjuvant and metastatic settings. Similar to the standard for other forms of medical genetic testing, centralized testing facilities are necessary to achieve high quality-control standards and provide worldwide access to this technology [10]. Continued effort is pointing to new classes of compounds, including MEK, protein kinase C, histone deacetylase inhibitors, and more recently Lag 3 inhibitor that may be tested as adjuvant therapy for high-risk patients identified as Class 2, as well as in the setting of advanced disseminated disease [25]. Consensus in methodology and multi-institutional collaborations are critical to achieve these goals and provide reliable and timely progress in managing patients with uveal melanoma.

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Part II
Management of the Eye

Chapter 7
Surgical Considerations of Radiation Plaques and Enucleation

Amy C. Schefler and Hannah J. Yu

Introduction

The management of uveal melanoma was a controversial topic among physicians until the late 1980s/early 1990s [47]. Through much of the 1900s, enucleation was the standard method of treatment among patients with uveal melanoma, but by the early 1990s, after the results of the Collaborative Ocular Melanoma Study (COMS) were released, clinicians began to turn toward radiation therapy as a proven method of tumor control [18]. Today, both plaque brachytherapy and enucleation are valid methods of management for cases of uveal melanoma, though careful assessments must be made in each individual patient case to determine the best method of treatment [47]. In this chapter, we will review the results of the COMS, discuss the indications for plaque brachytherapy and enucleation, and review the surgical techniques and considerations for both methods of tumor management.

Collaborative Ocular Melanoma Study

In the 1980s and 1990s, the Collaborative Ocular Melanoma Study (COMS) was designed to assess the effectiveness of two treatment modalities for choroidal melanoma: enucleation and plaque brachytherapy [9]. Patients included

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in the study had unilateral, treatment-naïve, medium- or large-sized choroidal melanoma without apparent metastases. The efficacy of radiation therapy compared to enucleation was unknown and controversial, and the COMS trial was initiated to evaluate and compare the two techniques in patients with choroidal melanoma.

Brachytherapy Versus Enucleation in Medium-Sized Choroidal Melanoma

The primary COMS trial focused on the management of medium-sized choroidal melanoma, defined as tumors 2.5 to 10 mm in apical height and ≤16 mm in largest basal diameter (LBD) [8, 9]. These patients were randomized 1:1 to one of two cohorts. The first cohort was treated with enucleation in a single surgical session; the second cohort was treated with radiation over 5–7 days for a total of 85 Gy of radiation to the tumor apex. The radiation was administered through iodine-125 (I-125) seeds in a gold plaque surgically placed on the sclera over the tumor base. Following the completion of scheduled radiation treatment, the plaque was surgically removed.

Through 5-year follow-up, the COMS trial for medium-sized choroidal melanoma demonstrated no statistically significant difference in survival rates between the enucleation and brachytherapy cohorts [18]. Among eyes treated with plaque brachytherapy, 10.3% of eyes demonstrated local treatment failure (defined as tumor recurrence) or extrascleral extension) and 12.5% of eyes required enucleation due to local treatment failure, pain, loss of vision, or other reasons [28]. Tumor thickness and distance to the fovea were found to be significantly associated with risk of local treatment failure or enucleation.

As for preservation of vision, the COMS trial reported visual acuity outcomes in study eyes through 3 years of follow-up [12]. Following treatment with plaque brachytherapy, eyes had a median visual acuity of 20/125 compared to 20/32 at baseline; 34% had a visual acuity of 20/40 or better, and 45% had a visual acuity of 20/200 or worse compared to 70% and 10% at baseline, respectively. An estimated 43% to 49% of eyes had meaningful visual impairment in their study eye through 3 years of follow-up.

Following the results of the COMS trial, treatment of uveal melanoma began to shift meaningfully in the direction of radiation therapy, though management still depends significantly on each individual case [47]. Today, though plaque brachytherapy is the favored method of treatment for many patients, enucleation is still a valid treatment for some. Tumor characteristics such as size and location must be carefully assessed to determine the best treatment plan and to decide which treatment is appropriate.

Pre-enucleation Radiation in Large-Sized Choroidal Melanoma

Subjects with large-sized choroidal melanoma (tumors 10 mm in apical height or >16 mm in LBD and juxtapapillary tumors 8–10 mm in apical height that could not feasibly be treated with brachytherapy) enrolled in COMS were randomized to either enucleation or enucleation preceded by external beam radiation [9]. Radiation was applied over 5–8 days with five fractions of 4 Gy applied daily for a total of 20 Gy daily radiation; enucleation was performed within 80 h of the last radiation treatment. At the 5-year endpoint, enucleated subjects had an estimated 5-year survival rate of 57% compared to 62% for subjects with pre-enucleation radiation, though there was no statistically significant nor clinically meaningful difference observed in survival rates or local complication rates between cohorts at 5 or 10 years [10, 11, 26]. The COMS study group did report that older age and larger LBD were predictors of time to death among subjects with large-sized melanoma.

Trends in Small-Sized Choroidal Melanoma

The COMS study also followed a cohort of patients with small-sized choroidal melanoma (1–3 mm in apical height and 5–16 mm in LBD) for 5 years to determine mortality rate and to analyze factors predictive of growth [9]. Throughout the observational study, subjects appeared to have a low risk of dying within 5 years from metastatic melanoma with an estimated 6% all-cause mortality rate [45]. Factors predictive of growth and treatment were greater initial tumor thickness and diameter, presence of orange pigment, absence of drusen, and absence of areas of retinal pigment epithelial changes adjacent to the tumor [44].

COMS Quality of Life Study

The COMS study team also conducted a quality of life study following the trials for medium- and large-sized tumors to investigate the quality of life in patients treated with brachytherapy versus enucleation through 5 years of follow-up [15]. Although patients treated with brachytherapy did report better vision-related quality of life in regard to ability to drive and peripheral vision in the first 2 years following surgery, patients treated with enucleation had less anxiety about cancer recurrence in the years following treatment [31]. However, both the difference in visual function and in anxiety between the treatment groups decreased in the long term, especially following the release of the mortality results of the COMS trial. Nevertheless, patient anxiety and overall quality of life are important for physicians and patients to consider when determining a course of treatment.

Brachytherapy

Plaque brachytherapy for ocular melanoma was first described in 1930 by Moore [32], who inserted radon seeds directly into ciliochoroidal melanoma. This study introduced the idea of suturing radioactive isotopes to the eye to treat ocular cancers. Since then, cobalt-60, ruthenium-106, iodine-125, and palladium-103 have all been used in plaque brachytherapy for uveal melanoma and other ocular cancers [24, 36, 37, 43]. Brachytherapy offers an important alternative treatment to enucleation, allowing for patients to save their eye and maintain some level of visual acuity.

Indications for Radiation Therapy

The use of plaque brachytherapy as a first-line treatment for uveal melanoma remains controversial in some cases, but it is generally accepted that brachytherapy is appropriate for patients with small-sized melanomas that are growing and exhibiting malignant behavior and in patients with medium-sized melanomas [47]. Plaque brachytherapy may be contraindicated in some cases of small- and medium-sized uveal melanoma if the tumor has been shown to have extrascleral extension that cannot be covered by the plaque.

Preoperative Planning

Prior to surgical placement of plaques, tumor size and location must be measured and assessed. Patients should have a full dilated ophthalmic examination, optical coherence tomography imaging, color fundus photography, and B-scan ultrasonography. The largest basal diameter of the tumor and the greatest apical tumor height should be measured to accurately plan the radiation dosage and plaque size.

These measurements allow physicians to properly choose a plaque and radiation dosage for each individual patient case. During the COMS trials, seven radioactive plaques were designed using iodine-125 (I-125) seeds placed inside a gold plaque to shield sensitive ocular structures from radiation. Most COMS plaques were circular in shape with limited diameters [14]. Recent advancements in plaque design, however, have provided physicians with a larger spectrum of plaque sizes and shapes to choose from [38, 20]. Additionally, the development of plaque planning software that uses tumor dimension and location measurements has provided physicians with more information and options regarding plaque type, placement, and dosage during preoperative planning [39]. This new software has also allowed for the assessment of radiation dosages to other ocular structures such as the retina, lens, and optic nerve before surgical placement of the plaques. For more information on types of radiation plaques and dosimetry, please see Chap. 9.

Surgical Placement and Removal of Plaques

The placement of radioactive plaques usually occurs in an operating room with local or general anesthesia. The affected eye should be dilated and examined with indirect ophthalmoscopy to confirm the previously measured tumor characteristics.

The surgeon should perform a 180° to 270° peritomy and enter Tenon's capsule. The rectus muscles should be isolated for traction with silk sutures. The surgeon should then use transillumination 180° away from the tumor to visualize its location (Fig. 7.1a). Surgeons may also use an indirect ophthalmoscope, condensing lens, and scleral depressors to visualize tumors. A sterile marking pen or diathermy should be used to mark the border of the tumor on the sclera (Fig. 7.1b). The largest basal diameter should be measured to ensure that the pre-selected plaque is properly sized. For posterior tumors, calipers may also be used to measure and mark the location of the tumor and the predetermined location of the plaque border based on presurgical measurements (Fig. 7.2a, b).

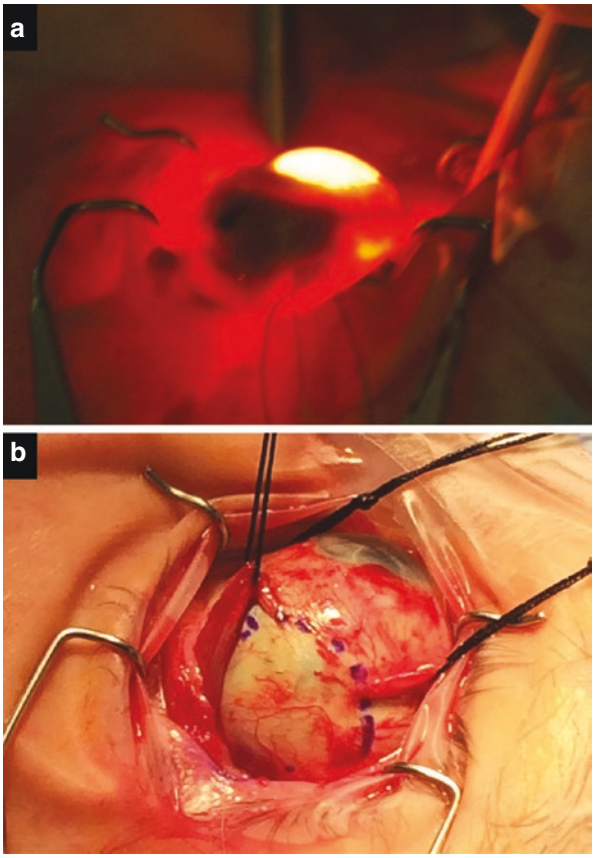

Fig. 7.1 Localization of an anterior melanoma by (**a**) transillumination and (**b**) marking on the sclera with a surgical marker

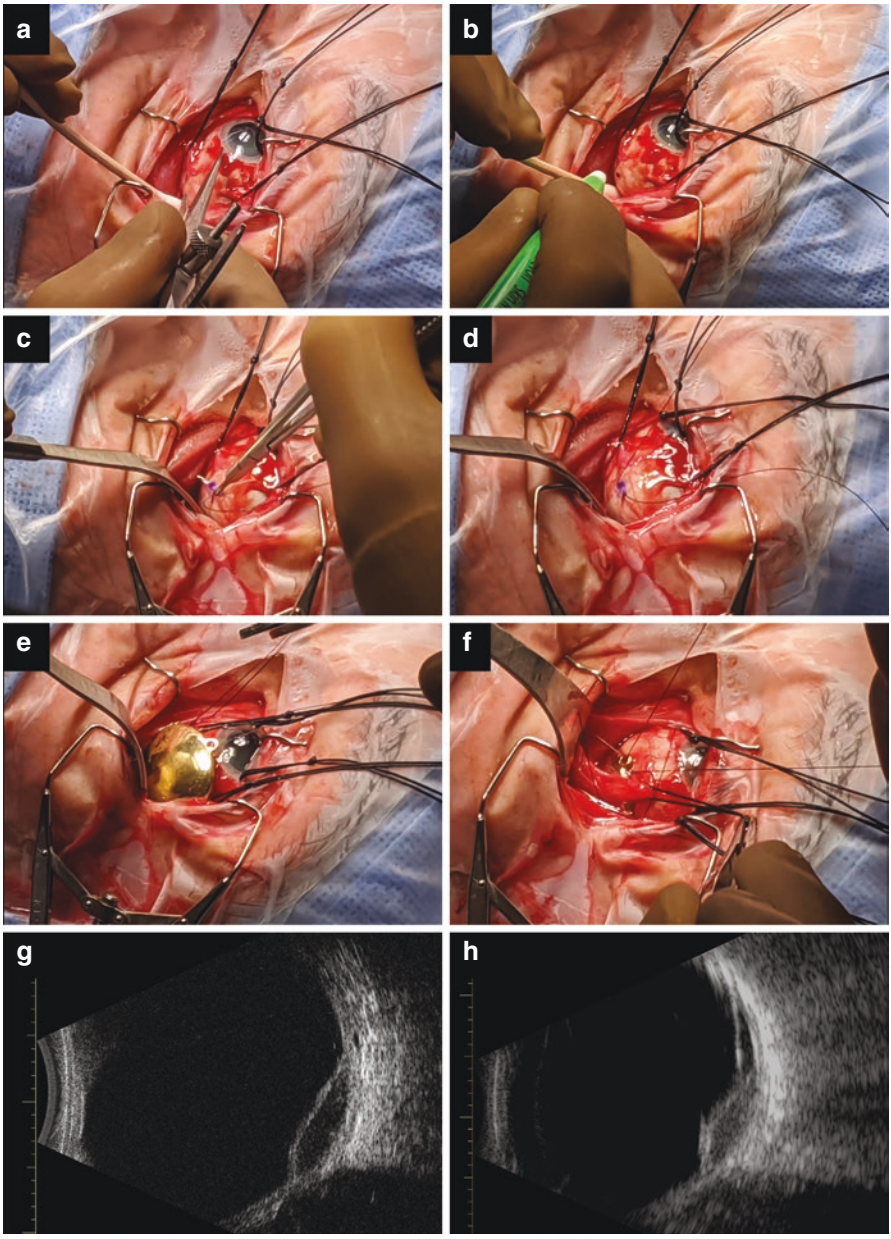

Fig. 7.2 Surgical placement of a gold plaque with iodine-125 seeds for a juxtapapillary tumor. (**a**) The surgeon uses calipers to measure to the expected location of the plaque based on preplanning measurements. (**b**) The locations for pre-placed sutures are then marked using a surgical marker. (**c**) and (**d**) The surgeon pre-places sutures for the plaque. (**e**) Following confirmation of the number of seeds in the plaque, the surgeon places the plaque in the pre-specified tumor location and (**f**) sutures the plaque against the sclera. The accurate localization of the plaque is confirmed and compared to the (**g**) presurgical ultrasound using an (**h**) intraoperative ultrasound

After confirmation of the plaque size, the surgeon may place the gold plaque or a plaque template over the tumor location. The border of the plaque may be marked with a sterile marking pen as well to further confirm the 2 mm margin around the tumor. The surgeon may then preplace nylon sutures with or without a plaque template/dummy plaque (Fig. 7.2c, d). These sutures should be strong enough to support the plaque without perforating the sclera. An intraoperative ultrasound can be used to confirm the proper localization of the template over the tumor, and adjustments to the plaque placement can be made if necessary.

Once plaque localization is confirmed and the preplaced sutures are complete, the plaque template may be removed and replaced with the gold, radioactive plaque using the preplaced sutures to secure it to the sclera (Fig. 7.2e). The location can be rechecked again by transillumination, indirect ophthalmoscopy, or ultrasound (Fig. 7.2g, h). The surgeon can then check the tumor for bleeding and the optic nerve for adequate blood flow.

In some cases, rectus muscles may overlap with the site of the tumor and need to be disinserted. This should occur following the marking of the tumor border before nylon sutures are preplaced. After the radioactive plaque is secured to the sclera, the surgeon may reattach the rectus muscle with a preplaced, temporary 6-0 Vicryl suture (Fig. 7.2f). This suture should be left long and tied at the end so the muscle isn't lost during the treatment period and the muscle may be disinserted again during plaque removal.

Following plaque placement and rectus muscle reattachment, the eye is irrigated with antibiotic solution, and the conjunctiva is closed with Vicryl sutures. The eye is then patched and covered throughout the treatment period.

After the designated treatment period, the plaque is removed under local anesthesia monitored by an anesthesiologist. The conjunctival sutures are removed, the plaque is visualized, and the rectus muscle is again disinserted in cases in which it was disinserted during insertion. The nylon sutures securing the plaque are then cut and the plaque is removed. The plaque is examined to ensure that all seeds remain intact; some facilities may also use a Geiger count to confirm removal of radiation from the affected eye. Finally, the rectus muscle is sutured permanently to its original position.

Complications and Postoperative Considerations

Though rare, complications may occur during the surgical placement or removal of the radioactive plaque. Perforation of the globe, central retinal artery occlusion, and subretinal or vitreous hemorrhage can occur. As with any major surgery, anesthetic complications may occur.

Physicians must also consider the effect of plaque tilt following completion of treatment with brachytherapy. During the course of radiation plaque therapy, some

plaques may tilt slightly on the sclera possibly resulting in a change in radiation dosage to the tumor. Although intraoperative ultrasound has improved the localization of the plaque during surgical placement, many plaques may have >1 mm of plaque tilt at plaque removal. In a prospective study by Alimony et al., 162 patients were treated with plaque brachytherapy, and intraoperative ultrasound was used to localize the plaque during placement and removal. At placement, 9% of patients had >1 mm of plaque tilt which was then adjusted for optimal localization. Although optimal localization was ensured, 53% of patients had >1 mm of plaque tilt at the time of removal. Male sex, juxtapapillary tumors, the use of notched plaques, and episcleral hematomas were all factors that were associated with increased risk of plaque tilt at the time of removal. Alimony et al. also found that plaque tilt resulted in a >10% decrease of radiation dose to the tumor apex in 23% of patients; only three patients experienced local treatment failure, though all three had >1.95 mm of plaque tilt at removal. These results emphasize the need for physicians to use intraoperative ultrasound at the time of plaque placement and to be aware of the possible effects of tilt on patient outcomes.

In the long term, plaque brachytherapy or any radiation therapy to the orbit may result in visual function-related complications. Although the gold plaque is designed to shield other ocular structures from damaging radiation, it is possible and common that other important structures such as the optic nerve, retina, and lens may be affected by the exposure to radiation. For more information on these long-term complications of radiation therapy, please see Chap. 10.

Plaques are typically kept in place for 4–7 days; in some states, patients are monitored as inpatients, and in others, patients are able to return home while the plaque is in place. Patients are advised to have minimal contact with others and no contact with pregnant women and children.

Enucleation

For decades prior to the COMS trial, enucleation was a common method of treatment for uveal melanoma [47]. Following the results of the COMS trial, however, many physicians began to move toward radiotherapy as the standard treatment for most medium-sized tumors and for some small-sized tumors. Nevertheless, enucleation is still an important method of treatment for many cases of uveal melanoma, and a few key indications must be considered when deciding tumor management.

Indications for Enucleation

The primary indications for enucleation in case of uveal melanoma include large tumor size, extrascleral extension, and optic nerve invasion. The COMS trials did not study the effectiveness of plaque brachytherapy in large tumors (defined as

tumors >10 mm in apical height or >16 mm in diameter at the base) because the investigators determined that the radiation required to treat these tumors would result in too small a therapeutic ratio [9]. Since the COMS trial, newer radioactive plaques have allowed for the treatment of some large tumors, though the use of plaque brachytherapy is still controversial among ocular oncologists. Notched plaques have also allowed for the treatment of tumors close to the optic nerve.

Enucleation may also be indicated for patients with eye pain, a very low probability of retaining vision, and neovascular glaucoma. For this population, plaque brachytherapy may not provide enough visual benefits to justify radiation treatment. Some patients may prefer enucleation over plaque brachytherapy to decrease their own anxiety over concern for cancer recurrence [31]. For these reasons, it is important that physicians thoroughly review the risks and benefits of both plaque brachytherapy and enucleation so that patients can make an informed decision.

Types of Implants After Enucleation

Orbital implants are inserted into the socket following the removal of the glove to restore volume and improve appearance. Optimal implants allow for postoperative motility, have low complication rates, and are cost effective. Generally, intraorbital implants are classified as either nonporous or porous. Porous implants are made from materials such as polyethylene, aluminum oxide, or, the most common, hydroxyapatite [46]. These porous implants allow for fibrovascular ingrowth which may increase stability of the implant and decrease the risk of migration or exposure [5]. Porous implants may also have a rough surface that can lead to tissue degradation and conjunctival thinning. Nonporous implants, commonly made out of solid materials such as glass, acrylic, silicone, or polymethyl methacrylate, do not promote fibrovascular ingrowth but can be wrapped in a biomaterial that does promote ingrowth such as donor sclera [29].

In the past, porous implants were sometimes “pegged” for attachment to prosthetics. Pegging increases horizontal motility of the prosthetic and creates more lifelike movement [5, 46]. However, pegged implants have fallen out of favor in recent years due to a much higher risk for complications such as infection or extrusion [30].

Surgical Technique for Enucleation

Enucleation is generally performed in a major operating room under general anesthesia but can be performed under sedation in certain cases if general anesthesia is contraindicated. To begin, the surgeon performs a 360° conjunctival peritomy with blunt Westcott scissors and dissects the conjunctiva and Tenon’s layer from the sclera with Stevens scissors. The rectus muscles are isolated using a Von Graefe muscle hook and secured using a 2-0 silk suture. Each rectus muscle is disinserted

from the eye with Westcott scissors; the superior and inferior rectus muscles are disinserted flush with the globe, and the medial and lateral rectus muscles are disinserted with a stump left on the globe. The superior oblique muscle is identified with a muscle hook and cut. The inferior oblique muscle insertion is identified with a muscle hook and transected with cautery. Any remaining soft tissue attachments are identified with a muscle hook and cut.

Enucleation scissors or a long curved hemostat is then used to palpate the optic nerve. Enucleation scissors are then used to transect the optic nerve. If extrascleral extensions are suspected, use the quadrant opposite the extension to introduce enucleation scissors. Following transection, the posterior Tenon layer is also cut and the globe is removed using the enucleation scissors (Fig. 7.3). Pressure is placed with a 4x4 gauze wrapped around a test tube or digital pressure to achieve hemostasis. Cautery or pharmacologic adjuncts are rarely needed to achieve hemostasis.

The implant is then inserted into the orbit, using an injector or periosteal elevators. For porous implants that are not wrapped or have windows cut into the wrapping, the rectus muscles are sutured to the implant using a 5-0 Vicryl suture. After placement of the implant and attachment of the rectus muscles, the anterior Tenon layer is closed using 5-0 Vicryl sutures, and the conjunctiva is closed using 6-0 plain sutures. The surgeon then places antibiotic ointment and a small or medium plastic conformer in the socket. A pressure patch is then placed to minimize lid edema.

Postoperative Care

Enucleations require minimal postoperative care. Patients are instructed to use ophthalmic ointment two to three times per day for 1–2 weeks. After approximately 1 month, patients are cleared to be fitted by an ocularist for a prosthesis.

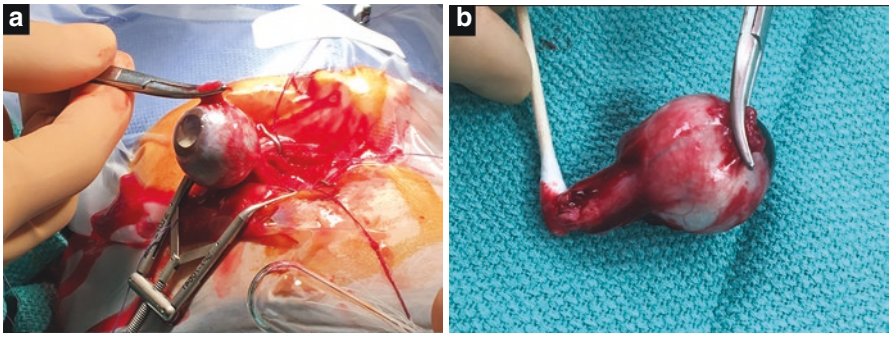

Fig. 7.3 Enucleation of the globe for uveal melanoma. (**a**) The rectus and oblique muscles are disinserted to remove the globe. (**b**) The optic nerve is severed and the globe is removed

Complications of Enucleation

Complications after enucleation are very rare. Pyogenic granuloma formation, conjunctival inclusion cyst formation, orbital or conjunctival hemorrhage, conjunctival wound dehiscence, or orbital infection rarely occur, usually with trauma or poor patient hygiene after surgery [11]. Cosmetically, patients can experience socket contracture, poor motility, or poor alignment. In the first 6 weeks after surgery, patients in the COMS trial who received standard enucleation surgery had a low rate of complications at 4% [11]. The rate of complications from standard enucleation reported at the 5-year follow-up were even lower, with resolution of most previously reported complications. The most commonly reported complication at the 5-year follow-up was poor motility of the prosthetic eye. This was more common in patients treated with pre-enucleation radiation.

Fine Needle Aspiration Biopsy

Fine needle aspiration biopsy has also become a common surgical technique performed for patients with uveal melanoma to help establish or confirm a diagnosis [1–3]. For patients receiving plaque brachytherapy, fine needle aspiration biopsy is often completed prior to plaque placement. Biopsy samples can also be sent for genetic testing which can provide prognostic information to guide patient management.

Preoperative Planning for Biopsy

Prior to biopsy, patients should be counseled about the cytologic and genetic testing that the biopsy sample will undergo and the prognostic information that can be gained. The physician should explain the implications of specific gene expression profiling (Class 1A, 1B and Class 2) and how the patient's management may be altered according to these results [21–23, 25, 33].

Patients should also have a full ophthalmic examination prior to surgery including a dilated fundus exam, color fundus photography, B-scan imaging, optical coherence tomography, and fluorescein angiography. Tumor characteristics should be thoroughly documented, and the physician should have a full understanding of tumor location and size. If the biopsy is preceding the placement of a radioactive plaque, these measurements should already be available from preoperative planning for plaque placement.

Surgical Preparation

For tumors not involving the iris, eyes should be dilated and general anesthesia is recommended. Eyes should be prepped with povidone-iodine solution and draped in a sterile fashion. A lid speculum should be used.

A 25- or 27-gauge long needle should be connected to a 10 cc syringe using a connecting tubing (Fig. 7.4). Connecting tubing is preferred by the authors as it minimizes accidental movement transmitted to the surgeon by the surgical assistant. As an option, the biopsy syringe may be placed in a suction control device or syringe holder for better control.

The presence of an intraoperative ocular pathologist may also be useful to confirm the quality of biopsy samples following retrieval (Fig. 7.5).

Transcorneal Technique

For anterior uveal melanoma, such as those affecting the iris, a transcorneal biopsy technique should be performed using a surgical microscope. Following eye preparation, a paracentesis wound is made through the cornea 120 to 240° away from the tumor. The biopsy needle should be prepared as described above (see Sect. 5.2).

Once the paracentesis wound has been made, the surgeon advances the biopsy needle through the wound and is progressed carefully into the geographic center of the tumor. As an option, the needle may be pre-marked with a surgical marking pen at a length just less than the greatest apical height of the tumor to guide necessary needle insertion depth. The surgical assistant then creates suction by withdrawing the plunger of the syringe or by pulling the handle of the suction control device. Once the assistant feels adequate resistance, the tubing is clamped with a hemostat

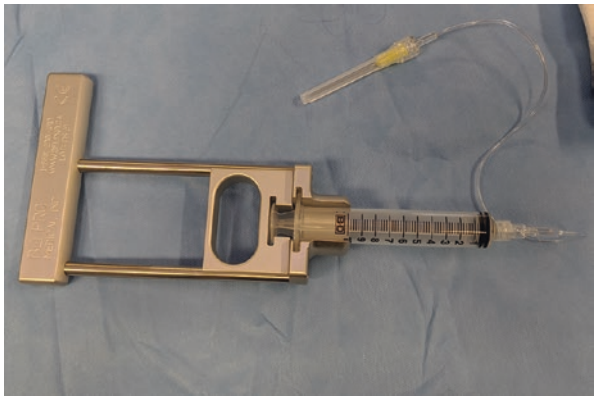

Fig. 7.4 Biopsy needle connected to a syringe via connective tubing with a syringe holder (Belpro Medical Inc., Anjou, Quebec, Canada)

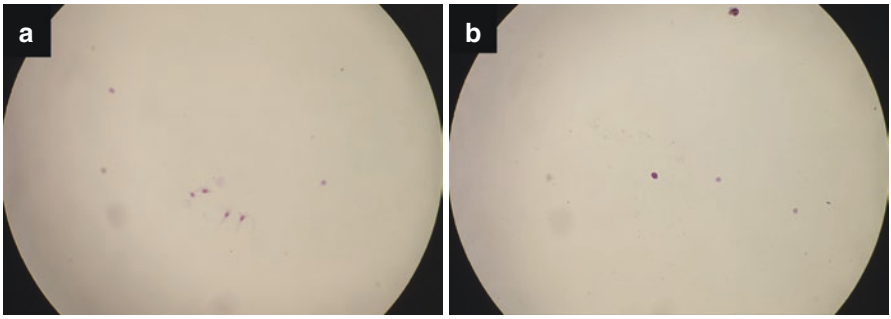

Fig. 7.5 Intraoperative staining of tumors cells taken from one melanoma in two distinct tumor locations (see Fig. 7.6). (**a**) Spindle cells and (**b**) epithelioid cells

and the needle is withdrawn. This process may be repeated to retrieve multiple biopsy samples. The paracentesis wound is hydrated with a balanced salt solution. If necessary, the surgeon may use a 10-0 nylon suture to close the wound and bury the knot.

As an alternate to the fine needle aspiration biopsy technique, the surgeon may also use end grasping forceps or curved retinal scissors to retrieve a tumor sample.

Transscleral Technique

For uveal melanoma anterior to the equator, such as those affecting the ciliary body, a transscleral biopsy technique should be used; for tumors near the equator, a transscleral technique can be considered. Following eye preparation, a partial peritomy is performed to displace the conjunctiva from the sclera directly over the tumor. Using a transilluminator or indirect ophthalmoscope, the tumor border is marked on the sclera with a surgical marking pen or diathermy probe (Fig. 7.1). The biopsy needle should be prepared as described above (see Sect. 5.2). In our experience, a scleral cutdown is not necessary and does not lead to higher tumor yields. The surgeon then advances the biopsy needle through the sclera in the geographic center of the tumor as guided by the previously marked tumor border. For thin tumors, the surgeon may advance the needle at an angle. Once the needle has been advanced through the tumor, the assistant retrieves the biopsy sample as described above. The surgeon should then remove the needle, applying cryotherapy to the base of the needle as it exits the sclera to reduce risk of extrascleral extension. This process may be repeated as necessary to retrieve multiple biopsy samples. After all samples have been retrieved, the surgeon may close the conjunctiva with a 6–0 plain gut.

Transvitreal Technique

For uveal melanoma posterior to the equator, such as those affecting the choroid, a transvitreal technique should be used; for tumors near the equator, a transvitreal technique may be considered. For transvitreal biopsies, surgeons may choose to use either an indirect ophthalmoscope or a surgical microscope to visualize and perform the biopsy. The indirect ophthalmoscope option may be preferred because it can result in a quicker procedure, but it also requires the surgeon to perform the procedure upside down and backward with significantly less magnification than a surgical microscope. Alternatively, the surgical microscope-guided procedure may be a slower process, but it provides a better view and can result in better sampling.

For an indirect ophthalmoscope-guided procedure, the surgeon should use calipers to measure 3.5–4 mm from the limbus and mark the site of the pars plana. The biopsy needle should be prepared as described above (see Sect. 5.2) and should be inserted 90° to 180° opposite the tumor in pseudophakic patients and 90° in a phakic patient. The surgeon advances the needle through the pars plana, using the indirect ophthalmoscope to visualize the progression. The biopsy needle should then be advanced into the geographic center of the tumor. Once the needle is at the necessary depth, the surgical assistant retrieves the biopsy sample as described above. The surgeon can then remove the needle, applying cryotherapy to the base as it exits to reduce risk of extrascleral extension. This process may be repeated as necessary.

For a surgical microscope-guided procedure, the surgeon should make a beveled sclerotomy 3.5–4 mm posterior to the corneoscleral intraocular location using a 25-gauge trocar-cannula. Insert and visually confirm the location of the infusion cannula in the vitreous cavity before securing and turning it on. In considering the location of the tumor, ensure that the infusion cannula is not placed directly above the tumor. Though infusion cannulas are generally placed inferotemporally, it should be placed more inferiorly for inferotemporal tumors. Make two additional 25-gauge sclerotomies for the light pipe and the biopsy needle.

The light pipe – or chandelier depending on surgeon preference – should be placed 180° from the tumor location. For example, for superotemporal tumors, the light source should be placed inferonasally. The biopsy trocar should be placed depending on the lens status of the patient. For phakic patients, the surgeon should avoid crossing the midline for biopsy. If the tumor is located in the superior region, the biopsy trocar should be placed at 12 o'clock; if the tumor is located inferotemporally or inferonasally, the biopsy trocar should be placed superotemporally or inferonasally, respectively. For pseudophakic patients, if the tumor is located temporally, the biopsy trocar should be placed superonasally; if the tumor is located nasally, the biopsy trocar should be placed superotemporally.

The biopsy needle should be prepared as described above (see Sect. 5.2). Following the placement of the infusion cannula and the light source, the biopsy needle should be inserted through the trocar and advanced into the geographic center of the tumor (Fig. 7.6a). Once at the necessary depth, the surgical assistant should retrieve the biopsy sample as described above. The biopsy needle can then

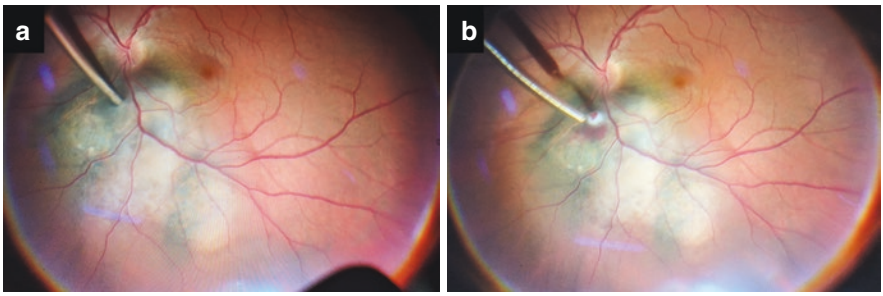

Fig. 7.6 Fine needle aspiration biopsy of a posterior uveal melanoma in a melanotic location. (**a**) The tumor was sampled and (**b**) treated with diathermy and intraoperative laser to prevent bleeding and retinal detachment. The tumor was then biopsied again in an amelanotic area to ensure a thorough sampling

be removed from the eye. This process may be repeated for additional samples. Following the sampling, the surgeon may choose to increase the intraocular pressure to minimize bleeding. Intravitreal diathermy may also be used (Fig. 7.6b).

A 25- or 27-gauge cutter may be used to retrieve the biopsy sample instead of fine needle aspiration, depending on surgeon preference. In the cause of a cutter, a needle should be used to create the retinotomy before the vitrector is inserted into the cannula and advanced into the geographic center of the tumor. The cutter should be set to a high aspiration/low cut rate and activated until the sample has been retrieved. The authors prefer the fine needle aspiration biopsy as it is quicker and allows for more control. Though the cutter technique may result in higher tissue yield, it also increases risk for iatrogenic retinal tear. To account for bleeding following the biopsy or to prevent iatrogenic retinal complications, the surgeon can choose to use endolaser at the retinotomy site after the biopsy sample has been collected. The light source and infusion cannula should then be removed, followed by the trocars. For the biopsy trocar, cryotherapy should be performed upon removal to reduce the risk of extrascleral extensions. The trocar sites are typically sutured with a 7-0 Vicryl suture if leaking is observed.

Postoperative Care

If the patient does not undergo a plaque placement following the biopsy procedure, the following postoperative care should be given. At the end of the surgery, subconjunctival injections of antibiotics and steroids should be given, and the lid speculum is removed. The eyelids and surrounding skin should be irrigated with a balanced salt solution, and an antibiotic ointment should be applied to the eye. Cover the eye with two sterile patches and an eye shield.

The eye patches and shield can be removed the following day. The patient should be instructed to administer antibiotic, steroid, and dilating eye drops. The patient should avoid strenuous activity for a week.

Remaining Controversies in Local Treatment

Although enucleation and plaque brachytherapy are both well-understood treatment modalities for uveal melanoma, controversy remains among physicians regarding the optimal management strategy for different cases. Since the publication of the COMS trial results, many physicians have chosen to decide on a treatment strategy according to COMS tumor size guidelines – small tumors observed, medium tumors treated with radiation, and large tumors treated with enucleation. In recent years, though, these standards have been questioned; should physicians still be determining treatment strategy based on size, or should other risk factors, such as genetic, anatomic, and histopathologic features be the main deciding factor [27]?

Small Tumors

For small tumors, some physicians have argued that early treatment with plaque brachytherapy would cause more harm than good for surrounding ocular tissues and that observation is best with a relatively low mortality rate among these tumors. Nevertheless, others have argued the benefit of early treatment in preventing micrometastases that may occur years before active management of the primary ocular tumor [16, 19, 42]. With no large-scale prospective study regarding treatment of small uveal melanoma with brachytherapy, however, decisions in small tumor management have been left up to the clinicians discretion based on the risk factors associated with the tumors and clinician preference.

Along those lines, recent studies in genetic profiling have provided physicians with another biomarker apart from anatomic tumor characteristics to identify risk of metastasis. A 15-gene expression profile (GEP) test was developed and validated for uveal melanoma (DecisionDX-UM, Castle Biosciences, Friendswood, Texas) which evaluates 12 discriminating genes and 3 control genes and classifies uveal melanoma as Class 1A, 1B, or 2 [6, 33–35]. Class 1A tumors are considered low risk with an estimated 2% chance of metastasis over 5 years, and Class 1B tumors are considered intermediate risk with an estimated 21% chance of metastasis over 5 years [17, 34]. Class 2 tumors are at high metastatic risk with a 72% chance of metastasis over 5 years. The PRAME (preferentially expressed antigen in melanoma) antigen was also found to be prognostically valuable in both Class 1 and Class 2 tumors; the overexpression of PRAME in uveal melanoma cells has been shown to be associated with increased metastatic risk [21, 22]. Multiple studies have validated the accuracy and prognostic value of GEP classification and PRAME identification over the more historical TNM classification [6, 35].

As these genetic markers have become better understood, oncologists have begun to use these commercially available tests to assess risk of metastasis in their patients with small melanoma as opposed to evaluating tumors based on size or growth rate. Currently, there are more ongoing trials to further validate and investigate genetic markers for metastatic risk and mortality rate among patients with all sizes of uveal melanoma.

Large Tumors

In the COMS trials, protocol dictated that large tumors were always eventually treated with enucleation, a management strategy that continued among physicians following study results for many years. Some oncologists, however, have chosen to further investigate the use of plaque brachytherapy and other radiation treatments in patients with uveal melanoma [40, 41]. Advocates of radiation for large tumors argue that by the time a large tumor has been diagnosed, the probability of metastasis already having occurred is high enough that the benefits of enucleation may not outweigh the benefits of the eye- and vision-saving brachytherapy treatment. Additionally, with the development of larger, more versatile plaques, brachytherapy has become a viable option for more patients [38]. Multiple small studies have demonstrated similar survival benefits of radiation therapy compared to enucleation in patients with large tumors [4, 7, 40, 41], but to date, no large-scale prospective study has validated these results.

Among these patients, the risk to the retina and other important ocular tissues must also be carefully evaluated before deciding to treat with radiation. Tumors with large apical heights require a much larger dosage of radiation which may result in exposing surrounding tissue to increased radiation damage and thus reduced visual acuity in the long term [11, 13, 28]. Studies in radiation retinopathy have demonstrated an association between thicker tumors and incidence of retinopathy following radiation therapy as well (see Chap. 10). Thus, treating oncologists must consider and compare the benefits of globe salvation with the risk of metastasis or radiation damage with patients before local treatment decisions can be made.

Conclusion

Overall, in the last few decades, huge strides have been made in the local treatment of uveal melanoma. The COMS trials demonstrated excellent tumor control and survival rates among patients treated with plaque brachytherapy, revolutionizing the treatment of ocular cancers and providing an important alternative treatment for

patients. Today, both brachytherapy and enucleation are still employed by physicians for local tumor management in uveal melanoma, each with their own indications, risks, and benefits. There are still controversies among physicians regarding optimal treatment strategy in some cases of melanoma, and current literature is limited in these cases. Future large-scale, prospective trials will be helpful in elucidating risks and benefits of brachytherapy and enucleation in cases of small and large melanoma. During surgical intervention, patients may also undergo a fine needle aspiration biopsy which can provide important diagnostic and prognostic information to physicians and can help guide future tumor management. Recent advances in genetic profiling have allowed for a much better understanding of metastatic risk factors in uveal melanoma and have also shed light on management of controversial melanoma cases.

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Chapter 8
Uveal Melanoma: Imaging

Jose R. Davila and Prithvi Mruthyunjaya

Introduction

Imaging is pivotal to the diagnosis and management of intraocular uveal melanoma (UM). Interpreted in the context of thorough clinical history taking and examination, imaging serves to objectively document key clinical findings and enhances our understanding of tumor characteristics including chronicity and propensity for metastasis, facilitating serial evaluation and informing medical and/or surgical approaches to treatment. Results of imaging furthermore help to refine the differential diagnosis in cases of diagnostic uncertainty and may also offer important information regarding patient prognosis.

Several imaging modalities are relevant to UM and may be found in clinical ophthalmology settings. Specifically, these include color photography of the anterior and posterior segments, ocular ultrasonography (US), and ultrasound biomicroscopy (UBM), autofluorescence (FAF), fluorescein angiography (FA), indocyanine green angiography (ICG), optical coherence tomography (OCT), and optical coherence tomography angiography (OCTA). In some cases, computed tomography (CT) or magnetic resonance imaging (MRI) may be used as an adjunctive strategy, although these are usually reserved for cases where metastatic disease is suspected or where access to ophthalmic evaluation is not available.

Successful application of imaging in the management of UM will frequently involve multiple imaging modalities used serially over time, each offering a different piece of information for the provider [1]. This chapter will review the most common imaging modalities used in the management of intraocular UM.

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Photography

The primary imaging modality in the clinical management of UM is color photography. Color photography of UM is obtained with slit-lamp biomicroscope-based cameras for anterior iris lesions and traditional, wide-field, or ultra-wide field color fundus cameras for choroidal lesions. Imaging of iris melanomas (IMs) is ideally achieved without pupillary dilation, maximizing the visible iris surface area, while imaging of choroidal melanomas (CMs) typically requires pupillary dilation for optimal visualization. In addition to providing objective documentation of lesion appearance and basal size for diagnosis and serial examination, color photography may be used to educate the patient about the findings of their examination.

Iris melanoma most often appears as a pigmented lesion or mass on the anterior surface of the iris. Important clinical features of IM include degree of pigmentation, lesion size, and lesion location. These features impact the clinical diagnosis of melanoma and provide information regarding the risk of extraocular metastasis. For example, IM involving the iris root or anterior chamber angle has an increased risk of developing extraocular metastatic disease, elevating the risk of death [2]. Overall, IMs are more common in light irides than in dark irides, as are amelanotic or lightly melanotic melanomas [3]. Other features captured with color photography and indicative of malignant growth include ectropion iridis, corectopia, prominent episcleral vessels, visible intrinsic tumor vessels, and hyphema (Fig. 8.1) [2]. Anterior

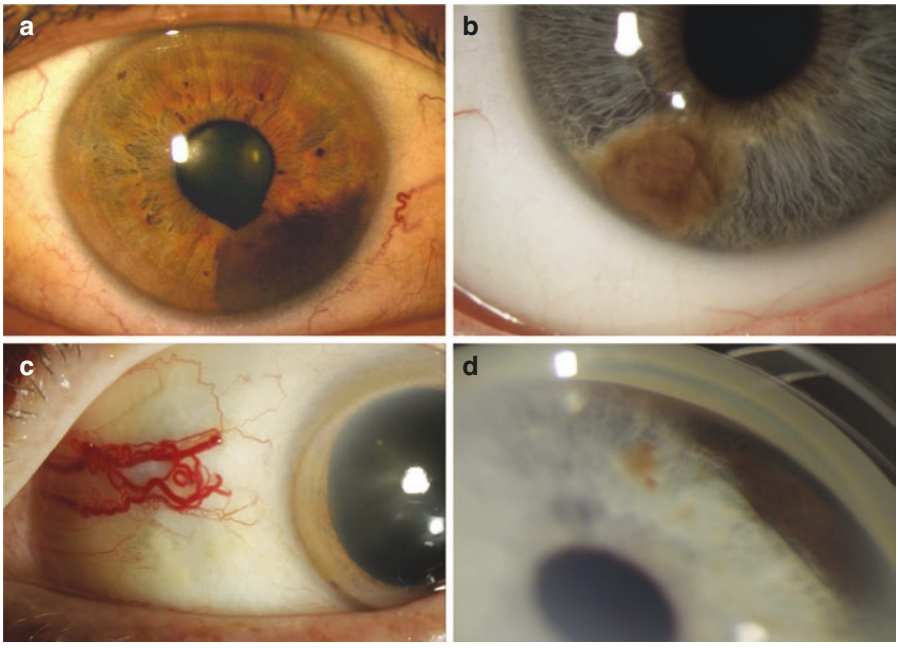

Fig. 8.1 Anterior segment color photographs showing a pigmented iris melanoma with secondary corectopia (**a**), a lightly pigmented iris melanoma (**b**), prominent episcleral vessels feeding into a cilio-choroidal melanoma (**c**), and a melanoma of the iris, angle, and ciliary body visible with gonioscopy (**d**)

segment photography under high magnification with angled illumination or sclerotic scatter may be used to highlight subtle surface features of an IM. Photography of lesions, on the posterior surface of the iris, in the ciliary body, or in the far peripheral choroid, is difficult to obtain, even for the most experienced ophthalmic photographers and may require assistance of mirrored lenses.

Choroidal melanomas often appear as variably pigmented subretinal lesions in any of three typical configurations – dome, diffuse, or mushroom (Fig. 8.2) [4, 5]. Slightly more than half of CMs are darkly pigmented, while approximately one-third show mixed pigmentation and approximately 15% are amelanotic [5]. Regarding tumor location, a large retrospective study of CM cases found that there was no predilection for a specific quadrant of retina but did find that 70% of diagnosed melanomas are located between the equator and the macula [5]. While dome and diffuse configurations represent sub-Bruch membrane tumor, a mushroom configuration occurs when tumor breaks through Bruch membrane and into the subretinal space (Fig. 8.2d) [4]. Over time, approximately one-half of CMs will disrupt Bruch membrane and assume a mushroom configuration [4]. Although binocular examination with a slit lamp or indirect ophthalmoscope is needed for true stereoscopic visualization, apical tumor prominence can be inferred from clues on fundus photography, such as shadowing from the tumor or blurring of the tumor apex relative to the retinal surface. In small pre-malignant lesions, photographic features

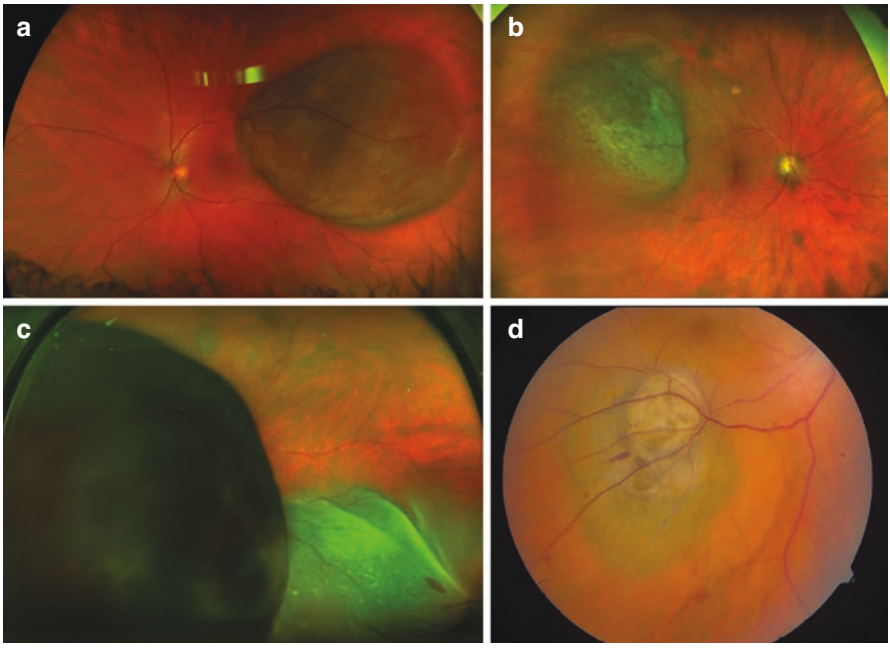

Fig. 8.2 Fundus color photographs showing an elevated choroidal melanoma as seen with widefield imaging (**a**), a melanoma of mixed pigmentation with overlying drusen seen with widefield imaging (**b**), a heavily pigmented melanoma extending into the ciliary body with associated exudative inferior retinal detachment seen with ultra-widefield imaging (**c**), and a melanoma of mixed pigmentation with mushroom configuration seen with conventional 50-degree fundus imaging

indicating a higher probability of melanoma and risk of future growth include orange pigmentation from lipofuscin and proximity to the optic nerve head with lesion margin touching the disc, both of which can be documented with color fundus photography [6]. The presence of drusen, which are yellow-white subretinal pigment epithelium (RPE) deposits, may indicate a more chronic lesion and thus a lower risk of malignancy.

The ideal imaging modality for a CM depends on the size and location of the tumor. While standard fundus cameras are capable of imaging 30–50° of retina in a single image, wide- and ultrawide-field cameras, recently defined by consensus as imaging anterior to the vortex vein ampullae in all four quadrants [7], can capture the posterior pole and nearly 120° of the peripheral retina in a single image. In this context, ultrawide-field fundus images may be the most appropriate modality for CM of the far retinal periphery. Although WF and UWF imaging systems are capable of non-mydriatic fundus photography, pupillary dilation reduces light artifact and thus maximizes image quality particularly in more peripheral lesions.

Ultrasonography

Ultrasonography (US) is a second clinic-based imaging modality that is fundamental to the diagnosis and management of UM. Similar to color photography, US serves to document key findings of UM and is used to monitor for change in tumor size over serial evaluations. Two ultrasound techniques are used for UM. The first is ultrasound biomicroscopy (UBM), which uses higher frequency ultrasound to image the anterior segment in detail, and the second is ocular US, which uses lower frequency ultrasound to image the posterior segment. Either modality should be performed with the ultrasound probe placed directly on the anesthetized ocular surface to optimize signal strength and thus image quality. Both modalities are capable of producing A-scan tracing, a unidimensional tracing of tissue echogenicity, and B-scan imaging, a bidimensional image of tissue echogenicity that can be used to measure the dimensions of a tumor. Proper and consistent technique is critical to obtaining high-quality US images, which are used for making clinical decisions. For this reason, many ocular oncology centers will have a dedicated ocular ultrasonographer.

UBM uses an ultrasound frequency of 20 but up to 40–50 MHz which allows for high-resolution imaging of the anterior segment [8]. Specifically, the lateral resolution of UBM approaches 20 μm. This allows accurate measurement of tumor dimensions as well as visualization of tumor sub-structures associated with high-risk morphology, including internal tumor vascularity (Fig. 8.3a) [8, 9]. Although surface vessels on anterior IMs can be observed on slit-lamp examination, internal vasculature is not directly visible but is present in over 90% of IMs [9]. Internal vascularity is seen as large-lumen hyporeflective vessels or small-lumen hyperreflective vessels within the tumor tissue. UBM is also used to determine the posterior extension of IM. Disruption of the iris pigment epithelium, which is seen as a

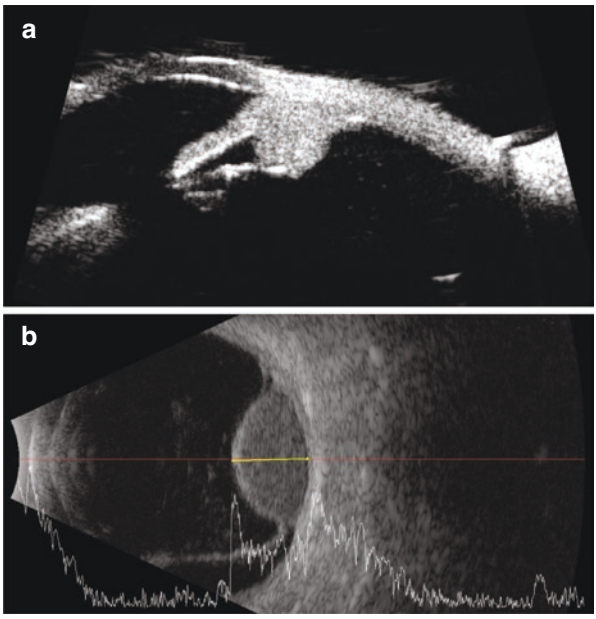

Fig. 8.3 Ultrasound biomicroscopy of a ciliary body melanoma showing disruption of the iris pigment epithelium and invasion into the anterior chamber angle (**a**); B-scan ultrasonography of a choroidal melanoma with associated retinal detachment and overlying A-scan tracing with characteristic low-medium internal reflectivity (**b**)

continuous hyperreflective band at the posterior iris margin, may be disrupted in IM [9]. Furthermore, IM that shows extension into the angle or iris root is associated with increased risk of extraocular metastasis [2]. Other features visible on UBM of iris and ciliary body melanoma include surface plaques and extraocular extension [9]. Surface plaques are hyporeflective bands of densely compacted cells that are common to iris nevi and melanomas [9].

Conventional ocular B-scan US is used to image CM and, in addition to color photography, is the most important tool for determining tumor dimensions and surveilling for growth (Fig. 8.3b). Several studies evaluating risk factors for metastatic death in cases of CM have found that tumor size, and in particular largest tumor basal diameter and tumor apical height, is highly correlated with extraocular extension and death [10–12]. On A-scan US, CM classically exhibits medium-to-low internal reflectivity, also referred to as acoustic hollowness. When present in choroidal nevi, this finding is associated with an increased risk of progression to CM [13]. Internal vascular pulsations within a CM may also be seen with A-scan US and, although not unique to CM, can be an important feature distinguishing UM from other simulating lesions such as large choroidal hemorrhage. Features visible on B-scan US include associated serous and/or exudative detachment of the neurosensory retina, vitreous seeding of tumor, choroidal compression, acoustic shadowing of the orbital fat directly posterior to the CM, and extraocular extension [14]. Furthermore, ocular US can be used to confirm tumor breakthrough across Bruch membrane, which exhibits the mushroom configuration on B-scan and a hyperechogenic “cap” with a hypoechogenic base on A-scan. Far peripheral CM approaching the ora serrata or extending into the ciliary body can be imaged with a mixed

technique using both B-scan ocular US and UBM. The use of UBM in such lesions offers the advantage of detailed evaluation of the ciliary body to assess the extent of the tumor margin, which may not be visible via direct examination [15].

Ocular Coherence Tomography

Ocular coherence tomography (OCT) has made a profound impact on imaging of many anterior and posterior segment diseases, including uveal melanoma. The advantage of OCT over prior imaging modalities lies in its ability to noninvasively capture images of deep structures with high resolution, often likened to in vivo histopathology. Image resolution from spectral-domain or swept-source domain OCT is sufficient to screen for subtle pathology, such as mild subretinal fluid, which is not consistently possible with ocular US. Imaging of iris or ciliary body tumors is done with anterior segment OCT (AS-OCT), while choroidal tumors are imaged with enhanced depth imaging OCT (EDI-OCT).

AS-OCT is known in ocular oncology for its use in imaging suspicious conjunctival lesions as a way to screen for signs of ocular surface neoplasia. In IM, its use is limited to providing high-resolution images of the anterior surface of a tumor (Fig. 8.4a). AS-OCT does not perform as well as UBM when it comes to imaging the posterior margin of a tumor, imaging tumors on the posterior surface of the iris or in the ciliary body, and is not as reliable when measuring the full dimensions of

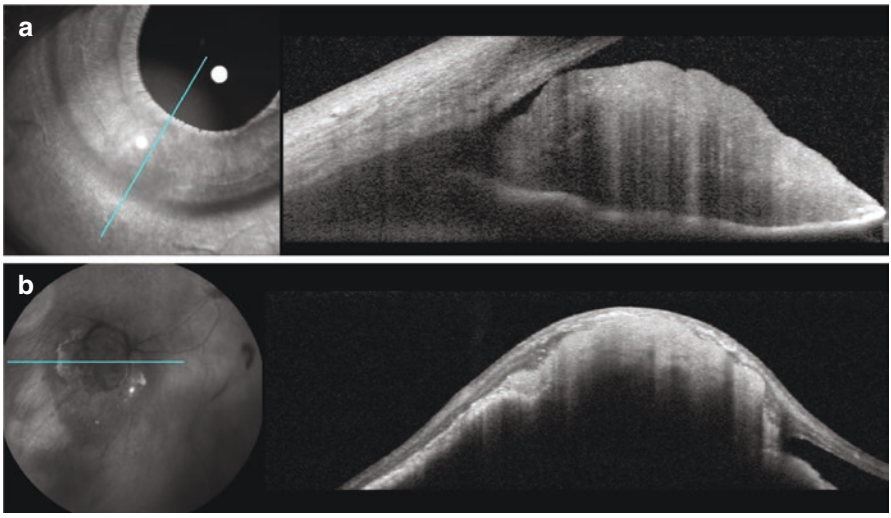

Fig. 8.4 Anterior segment optical coherence tomography of an iris melanoma depicting the anterior tumor margins with clarity (**a**); optical coherence tomography of a choroidal melanoma showing compaction of the choriocapillaris, deep optical shadowing, tumor breaking through Bruch membrane, and associated subretinal fluid (**b**)

an iris or ciliary body melanoma. These conclusions are supported by studies directly comparing the performance of AS-OCT with high-quality UBM [16, 17]. The reason for imaging limitations of AS-OCT is significant posterior imaging shadowing in cases of large or pigmented lesions. Nevertheless, AS-OCT can be used to confirm and document tumor parameters including the anterior surface dimension and any effect on adjacent structures such as the angle.

Choroidal melanoma is imaged with use of EDI-OCT, an adaptation of OCT that uses of spectral domain or swept-source technology to image deep ocular structures including the choroid and sclera. As compared to B-scan ocular ultrasonography, EDI-OCT offers a higher resolution (4 microns versus 100–300 microns) but is not able to penetrate as deep into tissue (SD-OCT about 2.5 mm). Features of CM that may be seen on EDI-OCT include thinning or complete compaction of the choriocapillaris and deep optical shadowing (Fig. 8.4b) [18, 19]. Features observed in the retina overlying a CM include disruption of the retinal photoreceptor layer, presence of subretinal fluid with or without lipofuscin deposition, and presence of intraretinal fluid [20]. Perhaps the most useful finding when imaging suspicious choroidal lesions with EDI-OCT is the presence of subretinal fluid [21]. Early subretinal fluid that is not apparent on clinical examination can be detected with EDI-OCT and is an important sign of malignancy [13, 21]. In estimating tumor thickness, OCT has demonstrated a higher level of accuracy when compared to ocular ultrasound, which has been shown to overestimate actual tumor thickness by up to 55% in small CMs [20]. Nevertheless, ocular ultrasound continues to be the gold standard for measuring tumor size, as it is the imaging modality used in landmark studies establishing treatment protocols by tumor size [12].

Fundus Autofluorescence

Fundus autofluorescence is an adjunctive tool for imaging of CM. There are two primary methods for obtaining fundus autofluorescence (FAF), one with the use of a standard fundus camera equipped with internal filters and one with a confocal scanning laser ophthalmoscope (cSLO). The advantage of FAF obtained with WF fundus cameras is the ability to image a large area of the retina at once and to compare intralesional autofluorescence to background autofluorescence from surrounding retina. By contrast, cSLO is limited to imaging a single plane of retina and thus may miss areas of elevated retina due to choroidal mass effect or retinal detachment [22].

The source of fluorescence seen in FAF is lipofuscin stored within the cells of the retinal pigment epithelium (RPE). Lipofuscin is a by-product of photoreceptor degradation and can accumulate in RPE cells in areas of diseased retina, giving a hyperfluorescent appearance [22]. By contrast, dead or absent RPE will appear hypofluorescent. Accumulation of lipofuscin in the RPE can come secondarily from disease processes in the adjacent choroid. CM tends to have overlying hyperautofluorescence, with brightness increasing in pigmented tumors, larger

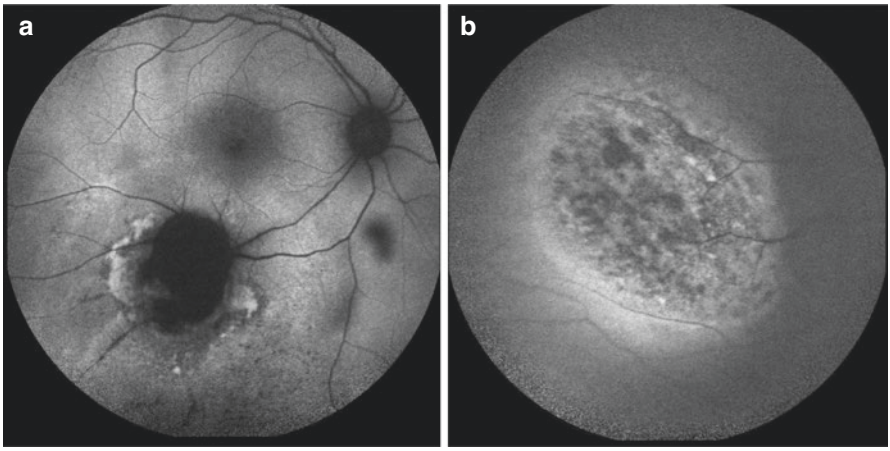

Fig. 8.5 Fundus autofluorescence showing mixed hyper- and hypo-autofluorescence of two distinct choroidal melanomas (**a** and **b**) - hyperautofluorescence from subretinal fluid adjacent to the tumor margins (**a**) as well as hypoautofluorescence from retinal pigment epithelium death internal to the tumor margins (**a**); mixed autofluorescence with few areas of strong hyperautofluorescence consistent with orange pigmentation characteristic of choroidal melanoma (**b**)

tumor, or tumors with disruption of the RPE (Fig. 8.5) [23]. The presence of hypoautofluorescence in within the margins of a CM may indicate tumor chronicity. Orange pigmentation from lipofuscin, which is a feature predictive of CM, is also strongly hyperautofluorescent on FAF [23]. Similarly subretinal fluid in the area of a CM often appears as hyperautofluorescent. As a result, many CMs exhibit a mixture of hypo- and hyperautofluorescent regions. Although not essential to diagnosis, FAF provides information regarding the health of the RPE overlying CMs and may aid in making a diagnosis of melanoma in cases of suspicious pigmented choroidal lesions.

Fluorescein Angiography and Indocyanine Green Angiography

Fluorescein angiography (FA) is an imaging test that involves administration of intravenous or oral sodium fluoride dye, which passes through the systemic and ocular circulation, allowing visualization of the ocular arteries and veins as the dye passes through. FA works by illuminating sodium fluoride with an excitation light of approximately 490 nanometers (nm) wavelength and capturing images through a yellow-green filter of approximately 525 nm. These images are captured with use of anterior segment or fundus cameras for iris and retinal lesions, respectively. In FA imaging, fluorescence appears relatively white compared to the dark background of the iris or fundus. In a healthy eye, the circulation of the iris and retina will appear to trace the normal anatomical structure of arteries, veins, and capillaries.

Meanwhile, the choroidal circulation will fluoresce in a mild, diffuse pattern that is deep to the retina. Abnormalities in arterial or venous circulation can be seen as telangiectasias, areas of increased vascularity, enlarged vessels, and patches of hyperfluorescence or hypofluorescence. When hyperfluorescence is seen in a pattern that increases in intensity and size over time, it is often indicative of vascular leakage, which is seen in cases of immature or neovascular vessels. Hypofluorescence, either in patches of retina or choroid, or in drop-out of the tracing of normal arteriovenous anatomy, is indicative of vascular occlusion or ischemia, or can be secondary to blockage of transmission by blood or other media opacities.

Anterior segment FA can be used as an adjunctive tool for imaging IMs. As with most malignant tumors, melanomas of the iris can show early and intense hyperfluorescence consistent with internal neovascularization and increased vascularity [14, 24]. Physiologically, these findings indicate active tumor growth. Although not specific for IM, when present, early and abnormal hyperfluorescence raises suspicion for a malignant lesion. By comparison, melanocytoma, a benign pigmented tumor, will show blockage of fluorescence from underlying iris vasculature by its intensely pigmented surface [24]. Unlike IM, melanoma of the ciliary body cannot be visualized by fluorescein angiography, which relies on direct photography of a lesion.

Fundus FA can be used to investigate retinal vascular abnormalities secondary to CM. In general, FA imaging captures the greatest detail at the level of the retina and lacks sufficient resolution of the choroid to be able to visualize the internal vasculature of a CM. As a result, there is no pathognomonic finding for CM on FA imaging [14]. Nevertheless, certain findings are common on FA. For example, most CMs show a mottled pattern of early hyper- and hypofluorescence that results from atrophy of the retinal pigment epithelium and with patchy visualization of the underlying intralesional vessels (Fig. 8.6) [14, 25, 26]. CMs with orange pigment deposition

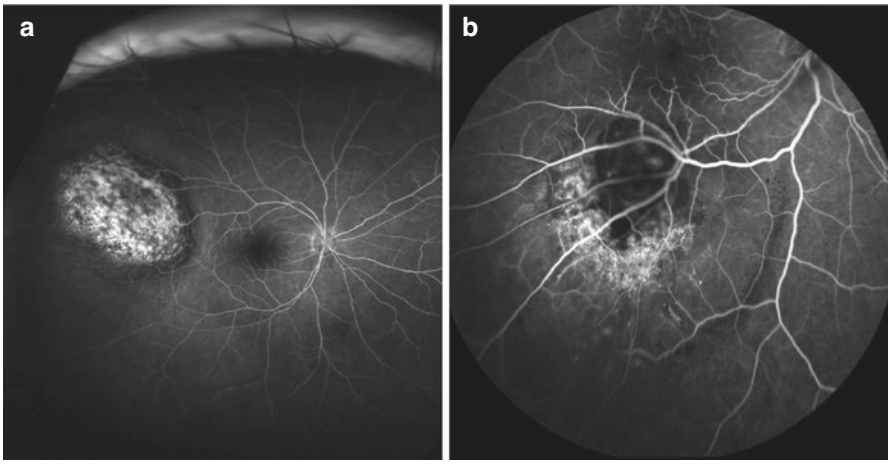

Fig. 8.6 Fluorescein angiography of two distinct choroidal melanomas depicting the characteristic mottled mixture of hyper- and hypo-fluorescence representing atrophy of the retinal pigment epithelium and patchy visualization of the underlying intralesional vessels (**a** and **b**)

will show areas of hypofluorescence in the areas of orange pigment, which block fluorescence from the underlying retina and choroid. Later images may show increasing hyperfluorescence over the lesion, which is due to leakage and accumulation of fluorescein dye over the choroidal tumor [14]. In cases where serous subretinal detachment is present, pooling hyperfluorescence is observed in the area of the detachment.

Indocyanine green angiography (ICGA) similarly uses intravenous injection of a dye, but with indocyanine green (ICG), which absorbs and emits light in the near-infrared range [27]. This characteristic allows light from ICG fluorescence to be captured without interference from common ocular pigments in their physiologic concentrations [27]. The molecule is primarily protein-bound and therefore stays within the choroidal and retinal vessels [27]. As a result of these properties, ICGA is most commonly used to visualize the choroidal vasculature, which is difficult to observe with FA. ICGA of CM is variable but most commonly shows hypofluorescence due to blockage of ICG fluorescence signal from melanin contained within the choroidal tumor and inconsistent visualization of the inherent tumor vasculature [28]. This is true for melanotic as well as for amelanotic CM [28]. As a result, imaging of the vasculature within a CM is limited, even with ICGA. When the internal vasculature of CM is visible, it is described as a "double circulation," with one circulation visible within the tumor borders and another visible in the surround choroid [29]. Within this context, a potential use of ICG is in excluding non-melanomatous choroidal tumors, such as choroidal hemangiomas or choroidal metastases, which show early and prominent hyperfluorescence and bland isofluorescence, respectively [28]. ICGA is not routinely used to image anterior segment tumors.

Optical Coherence Tomography Angiography

Optical coherence tomography angiography (OCTA) is a newer, noninvasive adaptation of OCT that allows imaging of the ocular vasculature. It can be used for imaging of pathology in both the anterior and posterior segment. As with OCT, the ability of OCTA imaging to penetrate deep ocular tissues is limited. Unlike FA, OCTA does not provide a dynamic representation of blood flow with features like leakage or pooling of dye but rather highlights areas of tissue where blood flow is present.

Iris surface melanomas can be imaged with anterior segment OCTA (AS-OCTA). Similar to findings seen in FA, AS-OCTA of IMs shows abnormal intralesional vessels. This finding stands in contrast to benign lesions such as iris cysts or nevi, which do not have abnormal internal vessels [30]. Screening for abnormal vessels as a sign of malignancy in suspicious iris lesions is one potential role for AS-OCTA. However, not all AS-OCTA devices are able to reliably image vessels within an IM. In a study comparing the performance of 840 nm with 1050 nm

wavelength AS-OCTA, only 1050 nm AS-OCTA was able to visualize the internal vasculature of an IM [30]. As AS-OCTA technology further develops, it may be used to routinely screen anterior segment tumors for vascular abnormalities suggestive of malignant growth.

OCTA imaging of the retina has shed light on secondary retinal pathology in eyes with CM. Specifically, OCTA has demonstrated that eyes with CM exhibit an enlargement of the deep foveal avascular zone and a decrease in capillary vascular densities in the superficial and deep layers of parafoveal retina when compared with their healthy fellow eye [31]. These changes in retinal vasculature seem to be more common in eyes with larger tumors and subretinal fluid and implicate proinflammatory factors in the pathogenesis of vision loss in CM [31]. Furthermore, changes in retinal vessels appear to be a distinguishing feature of CM that may be used to distinguish suspicious lesions, such as choroidal nevi, from melanoma [32].

Imaging of the choroidal vasculature with standard OCTA is limited by the ability of spectral domain technology to penetrate beyond the retina. However, the most recent commercially available version of OCTA, swept-source OCTA (SS-OCTA), has been used to capture images of the choroidal vessels. In one case report, this technology was used to differentiate choroidal neovascularization secondary to choroidal nevus from age-related macular degeneration [33]. In another case series including 22 patients with CM, SS-OCTA was able to detect the intrinsic microvasculature in all cases regardless of their degree of pigmentation, size, location, or history of prior treatments [34]. Although not yet widely available, SS-OCTA may certainly become a part of routine noninvasive testing completed when screening choroidal lesions for malignancy.

Conclusion

Imaging is fundamental to diagnosis and management of uveal melanomas. Historically, color photography and ultrasonography have been the cornerstone of uveal melanoma imaging. Although their utility has not diminished, several other imaging modalities have joined their ranks as adjunctive imaging technologies for providers taking care of patients with uveal melanoma. These include fluorescein angiography, indocyanine green angiography, and optical coherence tomography angiography, which capture the integrity of the ocular vasculature, as well as optical coherence tomography, which helps to screen for secondary changes in the retina, and autofluorescence, which highlights the health of the retinal pigment epithelium. Together with excellent clinical history taking and examination, imaging of uveal melanoma offers objective documentation for serial comparison over time and gives the provider a more comprehensive understanding of the dynamic changes occurring within the eye.

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Chapter 9
Radiation Therapy in Ocular Melanoma

Andrew J. Wong and Bin S. Teh

Significance of Radiotherapy in Uveal Melanoma

Radiotherapy plays an important role in uveal melanoma (UM) treatment. Historically local treatment of UM was managed with enucleation. A series of Collaborative Ocular Melanoma Study (COMS) trials established eye plaque brachytherapy as a standard treatment option for most UMs, demonstrating no significant difference in outcome metrics while preserving the globe and visual function. Numerous external beam radiotherapy modalities and techniques (photon-fractionated and stereotactic radiosurgery, proton and particle-based radiotherapy) further complement plaque brachytherapy with an array of definitive or adjunct treatment options.

A fundamental balancing act for radiotherapy in UM is achieving both excellent tumor control and sparing nearby critical structures to preserve vision and maintain quality of life. Local recurrence in UM increases the risk of metastasis and disease-specific mortality [1, 2]. Thus, an understanding of the different radiotherapy modalities and factors to optimize local control has impactful ramifications.

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Indications for Radiotherapy

In this chapter, the COMS staging is the basis for guiding the radiation treatment paradigm approach based on tumor size. This staging classification was utilized in a series of key trials that established treatment approaches and baseline outcome characteristics. In particular, a COMS small tumor is defined as between 1 and 3 mm in height and between 5 and 16 mm in basal diameter, a medium tumor is 3.1–8 mm in height and no more than 16 mm in basal diameter, and a large tumor is more than 8 mm in height or more than 16 mm across. The American Joint Committee on Cancer (AJCC) 8th edition staging (2017) utilizes tumor dimensions in addition to presence of ciliary body involvement and extraocular extension to describe a tumor size category. Prognostic stage groups are determined by tumor size category in addition to presence of nodal or distant metastasis [3]. Please refer to the AJCC staging manual for details.

Radiotherapy for Small Versus Medium Versus Large Tumors

Small, melanocytic tumors with an uncertain diagnosis for uveal melanoma or with few risk factors for growth can be observed [4]. Two-thirds of such lesions may represent choroidal nevi and do not grow, with approximately one-third of lesions progressing at 5 years [5]. Observation is particularly applicable for small tumors near the fovea, optic nerve, or critical structures in which radiation-induced vision toxicity is likely. Radiation therapy is indicated for small tumors when significant growth is demonstrated after conservative management – observation or local treatments such as local resection, photodynamic laser photocoagulation, or transpupillary thermotherapy.

For medium-sized tumors, eye plaque brachytherapy, particle/proton radiotherapy, and enucleation are all options. COMS report No. 28 was a prospective randomized trial that compared enucleation and iodine-125 eye plaque brachytherapy for select cohort of medium-sized choroidal melanomas. It found no difference in survival rates through 12 years of follow-up [6]. These results established eye plaque brachytherapy as the standard treatment approach for most medium-sized tumors, allowing for equivalent control while sparing both globe and vision.

Enucleation and particle/proton radiotherapy are options for local treatment to large tumors. If extraocular extension is present at the time of enucleation, particle beam or photon beam to the orbit can be considered. Of note, neoadjuvant external beam radiation therapy preceding enucleation did not improve outcomes over enucleation alone [7].

Local Recurrence and Metastatic Disease

Plaque brachytherapy and particle beam therapy are definitive options for treating intraocular recurrences. Re-irradiation can be considered for recurrent lesions in the

absence of metastatic disease. In the setting of limited or symptomatic hepatic disease, photon-based radiotherapy can be utilized for palliation.

What Are Other Options Preferred Over Radiotherapy?

Conservative management (observation, local treatment) is preferred for small COMS tumors as described above. Enucleation is considered for the following: salvage setting, large tumors involving substantial intraocular volume, extrascleral extension, nonfunctional eye, compromised (e.g., marked neovascularization) or symptomatic (e.g., painful) eye, or poor predicted functional outcome from other treatment options.

Radiotherapy Modalities

Episcleral Plaque Brachytherapy

Indication/Contraindications

Episcleral plaque brachytherapy (EPB) is a common, effective, vision-sparing method for treating uveal melanoma. Applications for plaque therapy have expanded to include most uveal melanoma of varying sites (including iris, ciliary body, choroid, subfovea, juxtapapillary, circumpapillary) and sizes (small and large tumors as well as those with limited extrascleral extension (i.e., AJCC T1, T2, T3, T4a-d)) [8]. EPB is appropriate for tumors $\leq$18 mm in largest base diameter or $\leq$10 mm in thickness as definitive therapy [9] and for small tumors that progress after observation or local recurrence after a prior local therapy. EPB is contraindicated for large tumors with extraocular extension (AJCC T4e) or with dimensions that exceed the size limit of brachytherapy, in blind painful eyes, and in cases with no light perception vision [8].

Overview of Treatment Planning and Delivery

Performing eye plaque brachytherapy is a multidisciplinary team effort involving an ocular oncologist, radiation oncologist, and brachytherapy physicist.

Preoperative plaque treatment planning takes into consideration several patient, tumor, and radiotherapy characteristics. A fundus diagram describing geometry and orientation of tumor within the eye, derived from ophthalmic examination, ultrasound findings, and photographic images (CT or MRI), is utilized as the basis for treatment planning. The appropriate radionuclide, prescription dose, dose rate, and eye plaque are selected for optimal treatment delivery as determined by a computerized treatment planning system. See Fig. 9.1 for representative images in plaque

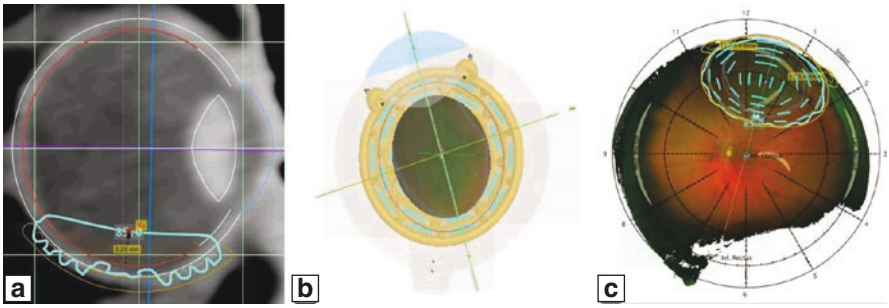

Fig. 9.1 3D treatment planning of plaque brachytherapy using plaque simulator software. (**a**) Fusion of thin-cut CT image set to 2D and 3D models of the affected eye, plaque, tumor, and dose distribution. (**b**) 3D reconstruction of the eye with plaque placed in relation to critical structures, including optic nerve and lens. (**c**) Retinal diagram with plaque, its coordinates, and isodose distribution. *CT* computed tomography, *2D* two-dimensional, *3D* three-dimensional

treatment planning. Eye plaques may be gold-plated with grooves to seat radiation sources (iodine-125, palladium-103, cesium-131) or solid beta-emitting plaques (ruthenium-106, strontium-90) [10]. Eye plaques can be customized by shape and size to accommodate sparing of important visual structures. For instance, notched plaques can be utilized for peripapillary tumors.

Particulars of plaque surgery are detailed in the corresponding chapter in this text. Under regional or general anesthesia, tumor localization is performed using transillumination, indirect ophthalmoscopy, or light pipe diathermy. A safety margin is often incorporated about the target volume. A dummy plaque may be used to verify position, followed by plaque placement and suturing. Imaged guidance may be utilized at the time of plaque placement to validate full spatial coverage of the target. The tumor is treated for a predetermined set period (typically 4–7 days) for which a lead eye shield is worn, followed by plaque removal under regional or general anesthesia.

Dose Prescription Considerations

For COMS iodine-125 plaques, the standard dose prescription is established as 85 Gy to the tumor apex (or 5 mm from internal surface of sclera if height is <5 mm) with ≥2 mm margin around tumor edge if possible, at a dose rate of 0.6–1.05 Gy/hr. The largest commercially available eye plaque is 22 mm in diameter. Factoring in a 2 mm margin on the tumor edge, generally the maximum allowed tumor dimensions for an eye plaque are 18 mm basal diameter and 10 mm thickness (8 mm if optic nerve is involved). For other radioisotopes, including non-COMS iodine-125 plaques, there are variations of dose (generally 60–100 Gy at low dose rate), prescription location (apex versus base), plaque margin, and maximum allowed plaque size [9].

Real-world practice patterns generally adhere to guidelines yet vary among institutions. For instance, a survey among nine high-volume institutions utilizing eye plaque brachytherapy overall found good agreement in practice patterns. The survey reported clinical margins and planning systems to be similar among institutions, while prescription dose, dose rates, and dosimetry varied [11].

Dose De-escalation

85 Gy has been the standard dose prescription for iodine-125 plaque brachytherapy as it was the minimum dose prescribed in COMS report No. 19 (2002), the first prospective randomized clinical trial comparing iodine-125 plaque brachytherapy with enucleation for patients with medium-sized tumors. Dose modifications may be appropriate to account for different tumor sizes. Dose de-escalation may result in better visual and treatment convocation outcomes without compromising tumor control, especially with smaller tumors. Perez et al. reported their analysis of dose on disease control and visual outcomes with iodine-125 plaques and suggested a dose to tumor apex <85 Gy especially for tumors <5 mm in height [12]. Prospective dose de-escalation trials using ruthenium-106 brachytherapy, proton beam, and gamma knife radiosurgery have demonstrated favorable results. For iodine-125, dose de-escalation has yet to be investigated via randomized, prospective trials [13].

Radionuclides in Plaque Brachytherapy

Iodine-125 is the most commonly utilized isotope for eye plaque treatment, particularly in North America. This may be a consequence of its use in COMS trials establishing eye plaque brachytherapy as a treatment modality. Ruthenium-106 has been long established in Europe for eye plaque brachytherapy. As a beta-emitter, ruthenium-106 has limited dose penetration and generally demonstrates a lower ocular toxicity profile compared to iodine-125. As a consequence, it is well suited for smaller tumors [14] although limited to treatment of tumors <6 mm in thickness. Some centers performing ruthenium-106 plaque brachytherapy have also added transpupillary thermotherapy to supplement coverage for larger tumors [15]. Ruthenium-106 may also be utilized for treatment of juxtapapillary and circumpapillary choroidal melanoma due to sharper dose falloff. Palladium-103 has also been implicated to deliver lower radiation dose to normal ocular structures and more irradiation to tumor compared to iodine-125, which has translated to improved long-term visual acuity outcomes and reduced risk of radiation retinopathy [16, 17]. Other less commonly utilized radionuclides include strontium-90 and cesium-131. Table 9.1 summarizes key features of common radionuclides used in plaque brachytherapy (Fig. 9.1) [8].

Table 9.1 Common isotopes in plaque brachytherapy for uveal melanoma

Mode of emission	Half life	Regional use	Key facts
Photon			
Iodine-125	59.4 days	North America, Europe	Most commonly utilized isotope overall. Isotope used in COMS studies.
Palladium-103	17.0 days	North America	Lower dose to normal ocular structures compared to iodine-125, per some studies.
Cesium-131	9.7 days	–	–
Beta			
Ruthenium-106	371.8 days	Europe, Japan, Russia	Limited dose penetration and sharper dose fall off. Suitable for smaller tumors.
Strontium-90	28.8 years	Russia	–

Modified from The American Brachytherapy Society consensus guidelines for plaque brachytherapy of uveal melanoma, 2014

Particle/Proton Beam Radiotherapy

Indications for Use

Particle/proton beam radiotherapy is also commonly used in definitive treatment of uveal melanoma. The modality is additionally appropriate for tumors near the optic nerve/disc or macula (for which plaque brachytherapy may have difficulty balancing adequate coverage versus toxicity to surrounding tissue), for tumors with greater thickness and larger diameter (for which plaques cannot adequately penetrate depth or cover spatially), for tumors under the orbital muscles, after margin positive enucleation, and for intraocular/orbital recurrence.

Compared to plaque brachytherapy, there are a few drawbacks of particle/proton beam therapy. First, the eye can move independently of the external beam, which creates an uncertainty in dose deposition. Often, the patient is asked to fixate the eye at a specific point such that the intraocular target remains within the proton beam path. Secondly, the anterior entry dose tends to result in more anterior segment complications and is particularly a challenge for posteriorly located tumors. Finally, particle/proton beam therapy is more expensive.

Treatment Planning Considerations

Tantalum clips are utilized as fiducial markers for image guidance and verification. Volumetric planning with CT and/or MRI and selection of gaze angle, field collimation, and beam depth/width are performed to maximize delivery to the target volume and minimize dose to organs at risk. For proton beam therapy, general prescription is 50–70 CGyE in five fractions over 7–10 days. For carbon ions, dose prescription is 60–85 CGyE in five fractions [9].

Photon-Based Radiotherapy

Stereotactic Radiosurgery/Radiotherapy

Stereotactic radiosurgery (SRS) and stereotactic radiotherapy (SRT) are less often used radiation modalities in uveal melanoma, and few prospective studies have assessed efficacy and safety. Considerations for treatment planning include mobile intraocular target volume, as well as a greater dose inhomogeneity associated with photon-based planning when compared to protons [18]. The dose prescription is 18–45 Gy for single fraction and 45–70 Gy for multi-fraction treatment [9].

Conventional Photon Therapy

Photon beam radiotherapy has a role in the adjuvant setting following surgery for orbital involvement, particularly for patients at risk for local recurrence (i.e., positive margins, suspected residual subclinical disease) or regional recurrence. Adjuvant dosing is generally 20–30 Gy in five fractions. Additionally, photon beam radiotherapy is utilized for palliation for symptomatic distant metastases.

Table 9.2 summarizes key differences between the aforementioned radiotherapy treatment modalities (Table 9.2) [8].

Table 9.2 Comparison of plaque, proton, and stereotactic radiation therapy

Plaque brachytherapy	Proton/particle beam therapy	Stereotactic radiosurgery/ stereotactic radiotherapy
Mobile radiation field	Static radiation field	Static radiation field
Continuous low dose treatment	Daily high dose fractions	Daily high dose fractions
85 Gy for iodine-125 COMS plaques	50–70 CGyE in five fractions (protons)	18–45 Gy (single fraction treatment)
Generally 60–100 Gy at low dose rate over 3–7 days	60–85 CGyE in five fractions (protons)	45–70 Gy (multi-fraction treatment)
Most common and established	Additionally appropriate for tumors near optic disc/macula, larger tumors	Least used and studied
Size limitation	More expensive	Greater dose inhomogeneity when compared to protons
	More anterior segment complications	

Modified from The American Brachytherapy Society consensus guidelines for plaque brachytherapy of uveal melanoma, 2014

Outcomes Following Radiotherapy for Uveal Melanoma

Following definitive radiotherapy, the treated tumor tends to regress slowly over time. Reporting outcomes is challenging because, among different institutions, even with utilizing the same radiation modality, the method of plaque placement, radiotherapy planning, follow-up at reporting, and use of actuarial estimates can vary widely. We present below reported outcome metrics from a review of the literature as available at the time of this publication.

Local Control

Overall, for small to medium tumors, local control (LC) is in the range of 90–100% with 5-year overall survival (OS) >80%. For large tumors, LC is roughly 70% with 5-year OS about 70% [19]. Factors cited to impact local control include larger tumor dimension, extrascleral extension, close proximity to optic disc, lower radiation dose to tumor apex, lower radiation dose rate to tumor apex and base, and longer overall treatment time [1, 20].

Local Control Following Plaque Brachytherapy

Several reports in the literature report long-term outcomes for iodine-125 eye plaque brachytherapy for uveal melanoma. Local control rates upon literature review are reported in the range of 86.9–96% at 2–3 years, 80–98.3% at 5 years, and 93–94.4% at 8–10 years [21].

Image-guided brachytherapy at the time of plaque placement has been investigated and cited to be important in helping to decrease local failure rates. Tann et al. reported on early outcomes for uveal melanoma patients treated with iodine-125 brachytherapy utilizing intraoperative ultrasound guidance. The local control rate was 100% at a median follow-up of 21.6 months [22]. A 100% local control rate has since been maintained at a median follow-up of 36 months, for a larger patient cohort spanning across different stages, sizes, and GEP complications. Intraoperative image guidance likely contributed to accurate plaque placement, as evidenced by adequate coverage metrics (mean V85Gy of 96.8%) and image-guided repositioning utilized in almost one in six plaques [21].

A few studies have compared iodine-125 to other isotopes in eye plaque brachytherapy for uveal melanoma with respect to outcomes and toxicities. Takiar et al. performed a comparative analysis of iodine-125 and Ru-106, with actuarial 5-year local control at 83% for iodine-125 and at 97% for Ru-106 [14]. Leonard et al. reported on their institutional experience treating uveal melanoma with iodine-125, Ru-106, and Cs-131. In their literature review for which can be referenced, the

5-year local control rate for Ru-106 averaged over five select studies was 91.5%, and local control rate for Pd-103 averaged over two studies was 96.3% [23].

Local Control Following Particle/Proton Beam Therapy

Outcomes for proton and helium particle radiotherapy as modalities to treat uveal melanoma are also reported in the literature. Lin et al. examined the National Cancer Database in comparing proton beam radiotherapy versus episcleral plaque brachytherapy and found that 5-year local control rates for proton therapy ranged from 85% to 96%. They also concluded that proton beam radiotherapy reported inferior overall survival when compared to episcleral plaque brachytherapy (51% versus 77%, respectively) which was statistically significant [24]. Verma et al. sought to review outcomes of proton radiotherapy for uveal melanoma, drawing from 14 studies from 10 institutions. They found that 5-year local control rates exceeded 90%, which persisted at 10 and 15 years [25]. Mishra et al. reported on a prospective trial comparing helium particle radiotherapy to plaque treatment. Local control for particle versus plaque treatment was 100% versus 84% at 5 years and 98 versus 79% at 12 years ($P = 0.0006$). They concluded that helium particle therapy resulted in significantly improved local control, eye preservation, and disease-free survival [26].

Distant Metastasis

Overall for plaque brachytherapy and proton beam therapy, 5-year distant metastasis rate is 16–20% [19]. Larger tumor size is a risk factor for distant metastasis; for small choroidal melanoma treated with plaque brachytherapy, a study reported each millimeter of increasing thickness and diameter contributed risk for metastatic disease [27].

Local tumor recurrence is associated with a significantly higher risk of distant metastasis. Thus, effective initial treatment and long-term surveillance of treated uveal melanoma are necessary [1, 20]. Effective local treatment may eradicate locally aggressive disease prior to development of micrometastases, particularly in smaller tumors or tumors in transition from a less aggressive genotype to a more aggressive one.

The key prognostic factor for metastasis in uveal melanoma is the GEP (gene expression profile) class. This classification was developed to distinguish between uveal melanomas that have a low metastatic risk (class 1 tumors) versus high metastatic risk (class 2 tumors) [28]. GEP class has been validated as the strongest prognostic factor for metastasis, over TMN classification and the previous gold standard, chromosome 3 testing [29, 30].

Summary

Radiotherapy has an established role in multiple settings in the treatment of uveal melanoma, initially emerging as an effective treatment option that can spare both globe and visual function. Delivery modalities include eye plaque brachytherapy, proton/particle beam therapy, and photon-based treatments, each of which has select indications and limitations. Two principal goals in radiotherapy involve achieving excellent tumor coverage and control while sparing surrounding structures to preserve vision and quality of life.

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Chapter 10
Late Complications of Radiation Therapy in Uveal Melanoma

Hannah J. Yu, Andrew J. Wong, Bin S. Teh, and Amy C. Schefler

Introduction

Since reports from the Collaborative Ocular Melanoma Study (COMS), radiation therapy has become a primary treatment for uveal melanoma [13, 19]. It offers a favorable alternative to enucleation as patients are often able to maintain some visual acuity in their affected eye following treatment. Nonetheless, although plaque brachytherapy has similar survival rates compared to enucleation for small- and medium-sized tumors [19], radiation therapy results in significant side effects. Although some level of visual acuity may remain in the affected eye, in the years following radiation, visual side effects may be experienced by patients to various degrees, due to radiation damage to important ocular structures such as the iris, lens, optic nerve, and retina. Early studies in the treatment of ocular tumors demonstrated severe visual impairments in up to 50% of eyes in the years following radiation

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therapy; often, these visual side effects are not observed until several months or years after treatment.

The most commonly seen ocular complications from radiation therapy are cataracts, radiation retinopathy, and radiation optic neuropathy [35]. Five years after radiation, the COMS trial reported 83% of study eyes with cataract formation [15], and Gunduz et al. reported an estimated 42% and 8% of patients with nonproliferative and proliferative radiation retinopathy, respectively [36]. A retrospective study by Kinyoun et al. reported development of optic neuropathy in 55% of study eyes with a median follow-up time of 28 months [47]. Though these complications may be seen in any patient treated with radiation, the extent and effect of radiation damage depends heavily on the size and location of the tumor as well as the specifications of the radiation treatment itself.

Radiation Retinopathy

Radiation retinopathy, defined as changes to the microvasculature in the months or years following radiation treatment for ocular or head/neck cancers, is one of the most common side effects of radiation treatment. For a large proportion of patients, radiation retinopathy is a slowly progressive disease that can cause devastating and sometimes irreversible damage to their visual acuity. There are several risk factors that physicians should be aware of when planning radiation treatment and follow-up visits. Fortunately, there are promising treatments currently being studied to improve visual acuity damage and to potentially prevent damage from retinopathy.

Etiology and Clinical Features

During radiation treatment, the retinal vascular endothelial cells may be damaged, leading to a devastating domino effect on the retinal vasculature. The damage can result in one of the most common visual side effects of radiation therapy – radiation retinopathy. Radiation retinopathy, also known as radiation maculopathy, generally presents between 6 months and 3 years following treatment in a significant portion of patients [13]. In a previous study of patients treated for melanoma with plaque brachytherapy, 30% of patients presented with evidence of radiation retinopathy within 2 years of treatment [50]. Other studies have reported various rates of disease development, though it is generally understood that the risk of radiation retinopathy can rely heavily on type of radiation, fractionation schedule, tumor characteristics, and radiation dosage.

Similar to other diseases of the retinal vasculature, such as diabetic retinopathy, radiation retinopathy can present in two stages: nonproliferative and proliferative [48]. Most patients affected are classified as nonproliferative retinopathy in the

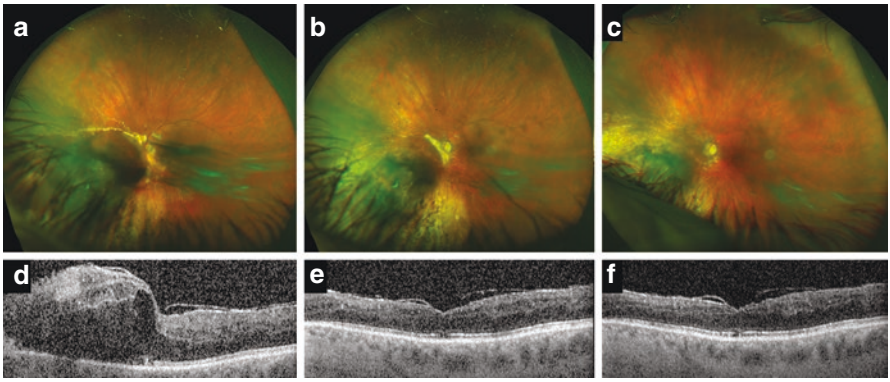

Fig. 10.1 Presentation and treatment of radiation retinopathy following treatment for choroidal melanoma. A 50-year-old male presented with a choroidal melanoma posterior to the equator in the left eye and was treated with Iodine-125 plaque brachytherapy. (**a** and **d**) Nineteen months later, the patient presented with cystoid macular edema, intraretinal exudates, retinal hemorrhage, and RPE atrophy. The patient then initiated treatment with monthly intravitreal injections of ranibizumab. (**b** and **e**) At 6 months, the patient presented with improved CME, improved intraretinal exudates, and resolved hemorrhage. (**c** and **f**) At 12 months, the patient presented with resolved CME, resolved exudates, and resolved hemorrhage

early stages of the disease; advancement to a proliferative classification occurs when growth factors are upregulated and induce neovascularization in the retina, a progression that occurs in a small portion of severe cases [7]. Upon presentation, clinical features of radiation retinopathy include intraretinal hard exudates, cotton wool spots, macular edema, microaneurysms, and retinal ischemia (Fig. 10.1) [21]. Atrophy of the retinal pigment epithelial (RPE) cells may also be observed.

Risk Factors

Physicians should be aware of several risk factors for radiation retinopathy when treating patients with radiation therapy. Risk factors can range from radiation-level factors to patient demographics (Table 10.1). In the years following radiation therapy, patients should be cognizant of any visual changes they experience; patients should also be regularly assessed by an ophthalmologist to rule out any early anatomical changes indicative of radiation retinopathy.

Types of Radiation

Although late complications from radiation have been observed across all types of radiation used to treat uveal melanoma (i.e., external beam radiation, plaque brachytherapy, proton beam radiation, gamma knife), incidence and extent of visual side

Table 10.1 Risk factors for radiation retinopathy

Risk factor	Details
Types of radiation	Gamma knife radiation – in severe VA loss in nearly all eyes External beam radiation and plaque brachytherapy – VA loss in approximately 40–60% of eyes
Radioactive isotopes	Cobalt-60 – high rates of retinopathy due to high energy gamma emission/poor shielding Ruthenium-106 and Strontium-90 – beta emitters, less VA loss due to rapid dose fall-off Iodine-125 and Palladium-106 – low-energy photon emitters. Palladium has a more rapid dose fall-off and thus may result in lower rates of radiation retinopathy compared to iodine
Dosimetry and fractionation	<70 Gy to the tumor and <40 Gy to the retina are associated with lower rates of retinopathy If possible, hyperfractionation can lower the risk of radiation damage
Tumor characteristics	Larger tumors require higher doses of radiation and are associated with higher incidence of retinopathy Proximity to the macula is associated with worse radiation damage
Patient demographics	Diseases of the vasculature such as diabetes, hypertension, or coronary artery disease increase risk of retinopathy

effects may differ between treatment modality. In the COMS study, among eyes with medium-sized tumors treated with plaque brachytherapy, 66% and 45% of eyes had a visual acuity worse than 20/40 and 20/200 3 years after radiation treatment, respectively [13]. A study by Gunduz et al. reported radiation retinopathy in 42% of eyes treated with plaque brachytherapy 5 years after treatment [36]. Among eyes treated with proton beam radiation for parapapillary choroidal melanoma, 68% of patients developed radiation papillopathy a mean of 1.5 years after treatment; 42% of these eyes, however, retained useful vision and a third spontaneously recovered vision over 5 years [45]. By far the modality with the highest rates of visual damage is gamma knife radiation. In a study of patients treated with gamma knife radiosurgery for choroidal melanoma, visual acuity loss was severe and highly prevalent with only one of 32 patients (3%) retaining vision of 20/40 or better after a median 38 months of follow-up [38].

Radioactive Isotopes

Type of radioactive isotope also plays a role in the amount of radiation damage to the eye. Several different isotopes have been studied and utilized in plaque brachytherapy over the last several decades with differing risks of radiation damage compared to others. In 1948, Stallard began treating uveal melanoma with Cobalt-60 radiation applicators, a technique that was used through the 1970s and 1980s for choroidal melanoma [80]. However, the popularity of Cobalt-60 fell out due to its high-energy gamma emission which made it challenging to shield from other important ocular structures and OR staff [76]. Today, Iodine-125, extensively studied in

the COMS trials [11], is one of the most popular radioactive isotopes used in plaque brachytherapy, especially in the United States. Iodine-125 emits low-energy photon radiation and has excellent tumor control and mortality rates when compared to enucleation [73].

Ruthenium-106 and Strontium-90 have also been studied for their effectiveness in treating ocular cancers, and Ruthenium-106 is commonly used in plaque brachytherapy in Europe. Both isotopes are classified as beta emitters and are known to have rapid dose fall-off which can help minimize unnecessary doses of radiation to adjacent ocular structures compared to other isotopes [62]. However, the use of Ruthenium-106 generally delivers a high dose of radiation to the sclera in order to fully penetrate the tumor and thus is not often used for tumors over 5 mm in apical height. Some have also reported inferior local tumor control rates when compared to Iodine-125, likely due to a lack of adequate radiation to the tumor apex beyond the area of radiation fall-off [6, 54, 70, 87]. Similar mortality rates have been reported when comparing treatment of thin tumors [54]. Despite this restriction, studies in Ruthenium-106 with properly chosen cases have demonstrated excellent long-term results for patients treated for ocular tumors with favorable results in visual acuity. In a large study by Bergman et al., a visual acuity of 20/200 or worse was observed in only 38% of patients at 3 years compared to 49% treated with Iodine-125 in the COMS study, with comparable heights of tumors between studies [6]. Thus, some physicians may opt for treatment with Ruthenium-106 for thinner tumors when given the option between isotopes.

Research has also been conducted for Palladium-106, categorized as low-energy photon radiation with a more rapid radiation dose fall-off compared to Iodine-125 [62]. It has been proposed as an alternative to Iodine-125 with the benefit of lower radiation doses to surrounding ocular structures. Additionally, Palladium-106 seeds are similar in size to Iodine-125 and thus can be used in identical plaques. Limited research has been conducted with this isotope, though lower rates of radiation retinopathy compared to Iodine-125 have been observed in single-center reports in the published literature [25]. Nevertheless, investigators must be wary that the rapid dose fall-off may result in increased rates of local treatment failure.

Radiation Dosimetry and Fractionation

Radiation dosimetry and fractionation are known to be important risk factors for late complications of radiation treatment. Recent advancements in technology have allowed radiation therapists to plan and calculate dosimetry to certain parts of the tumor and to surrounding structures prior to radiation treatment. Generally, lower total doses to the tumor (<70 Gy) and to the retina (<40 Gy) are associated with lower rates of radiation retinopathy and radiation complications. If possible, hyperfractionation of radiation doses with lower doses at each session can also lower the risk of radiation damage [60, 65]; for modalities such as plaque brachytherapy, however, hyperfractionation is not possible. Total dose reduction to an apex dose

less than that reported in the COMS is controversial and has been explored in non-randomized studies [66].

Tumor Characteristics

Several studies have reported on the association between specific tumor characteristics and rates of radiation complications as well. Tumors that are larger at baseline typically require higher doses of radiation for treatment and are thus associated with a higher incidence of radiation retinopathy and other complications [12, 14, 42]. Tumor location at baseline is also critical when assessing risk of radiation damage to other important ocular structures such as the retina, optic nerve, lens, sclera, and other structures. For example, for peripapillary tumors, the risk of optic disc edema or optic neuropathy is increased compared to equatorial tumors.

Patient Demographics

Patient-level factors can increase the risk of radiation retinopathy significantly when treated with radiation therapy. Patients with diseases of the vasculature such as diabetes, hypertension, or coronary artery disease [64, 85, 86] are already at risk for retinopathies and have increased incidence when treated with radiation to the eye.

Treatment for Radiation Retinopathy

Though radiation retinopathy has been a well-established side effect of radiation treatment for decades, there is no widely accepted management strategy for eyes affected by the disease. However, due to the clinical similarities to other exudative retinal diseases such as diabetic retinopathy and neovascular age-related macular degeneration, multiple similar treatment techniques have been studied. Although there is no treatment currently approved by the US Food and Drug Administration, studies in laser photocoagulation and intravitreal pharmacotherapy have produced promising results for patients affected by radiation retinopathy.

Laser Photocoagulation

Early research in the treatment of radiation retinopathy explored the efficacy of various laser photocoagulation techniques to resolve anatomical changes and improve visual acuity in patients. Overall, improvements in anatomic and visual outcomes appeared to be modest and short-lived among these patients. In 1996, Kinyoun et al. reported a small retrospective case series in which 11 eyes with proliferative

radiation retinopathy were treated with panretinal photocoagulation [48]. Through a mean follow-up time of 75 months, neovascularization had resolved in 91% of treated eyes, though these eyes still demonstrated poor visual acuity following treatment. In a study by Hykin et al., focal laser for radiation-induced macular edema modestly improved visual acuity in the short term though the one-time treatment did not seem to sustain visual acuity improvements through 1 or 2 years of follow-up [41]. The use of photodynamic therapy (PDT) was reported by multiple authors with promising improvements in neovascularization, visual acuity, and anatomic outcomes, though most of these studies were small, retrospective case series with limited follow-up time [3, 4, 51].

Intravitreal Steroids

Intravitreal injections of corticosteroids such as triamcinolone acetonide and dexamethasone have been theorized to help improve visual and anatomic outcomes in patients with radiation retinopathy due to the ability of corticosteroids to restore capillary permeability and blood-retina barriers. However, research into this technique has remained limited to small case series and anecdotal reports with minimal follow-up. Two small, prospective case series investigated treatment of radiation-induced macular edema and radiation papillopathy with intravitreal injections of triamcinolone acetonide [77, 78]. Both reports demonstrated vast and meaningful improvements in visual acuity and central macular thickness (CMT) within the first weeks following treatment, though follow-up in these cases were extremely limited. A retrospective case series by Baillif et al. investigated dexamethasone implants to treat radiation-induced macular edema, with short-term improvements in visual acuity; visual improvements did not last past 5 months [2].

Multiple studies have also used intravitreal corticosteroids to treat recalcitrant radiation retinopathy, previously treated with off-label bevacizumab, a well-known anti-vascular endothelial growth factor (VEGF), and/or laser photocoagulation [5, 39, 44, 71, 81]. For the majority of patients, stabilization or improvement of visual acuity or CMT regressed within 6 months of treatment.

Intravitreal Anti-vascular Endothelial Growth Factors

In recent years, the advent of intravitreal anti-VEGF agents such as bevacizumab, ranibizumab, and aflibercept has revolutionized the treatment of exudative retinal diseases, including radiation retinopathy. Several retrospective case series and anecdotal reports have demonstrated the beneficial visual and anatomic effects of intravitreal bevacizumab and ranibizumab, and more recently, prospective studies have examined the use of aflibercept (Fig. 10.1) [23, 29, 30, 33, 37, 43, 55, 56, 61, 72, 74]. Similar to laser photocoagulation and corticosteroid treatment, discontinuation of anti-VEGF treatment appears to result in recurrence of symptoms of radiation retinopathy. Unlike other treatments, however, anti-VEGF injections can be given

frequently to maintain and improve visual and anatomic outcomes in patients affected by radiation retinopathy. Still, the challenge remains of how often to treat patients to minimize burden while maximizing the visual and anatomic gains of anti-VEGF treatment.

To date, the prospective literature in this field has been limited (Table 10.2). In 2013, Finger and Chin published a small, prospective, nonrandomized clinical trial of 10 eyes from patients with recalcitrant radiation retinopathy from external beam radiation or plaque brachytherapy [29]. Patients had previously been treated with anti-VEGF treatment with incomplete response, defined as persistent macular edema or leakage. Over the course of 1 year, subjects received 12 injections of high-dose ranibizumab (2.0 mg). Seventy percent of subjects completed the trial of which 100% had stable or improved BCVA by the year 1 endpoint. Thirty percent of subjects withdrew from the trial due to worsening retinopathy. By the end of the study, 80% of eyes also had statistically significant improvement of central foveal thickness.

Schefler et al. reported the use of ranibizumab with or without targeted retinal photocoagulation (TRP) for radiation-induced macular edema in a phase IIb, randomized, prospective study [72]. Forty eyes from 40 subjects were randomized to one of three cohorts and treated with monthly or pro re nata (as needed, PRN) ranibizumab (0.5 mg) with or without TRP through 1 year. By the year 1 endpoint, 97% of eyes had VA 20/400 or better; the monthly cohort had the largest visual gains, and there was no indication of significant benefits of TRP. CMT gains were also significant among eyes treated with monthly ranibizumab, and anatomic improvements were significant among all three cohorts. In the second year of the study, all subjects were transitioned to a treat-and-extend protocol dependent on the exudative state of their macula at each visit. Results from year 2 are ongoing.

Aflibercept has also been a promising anti-VEGF agent for exudative retinal diseases and is now FDA approved for wet AMD and all stages of diabetic retinopathy. Its efficacy in the treatment of radiation retinopathy has also been documented through two prospective studies. In a study by Fallico et al., nine eyes from nine subjects with radiation-induced macular edema were treated with 2.0 mg aflibercept every 4 weeks until maximum visual acuity was achieved or until there were no signs of edema on optical coherence tomography; PRN treatment was enacted thereafter [23]. Overall, a mere 4.4 mean injections were given per eye over the 24-month study period; visual acuity and central foveal thickness also significantly improved by the end of study. Although the study was undoubtedly limited by a small number of patients, the results of the prospective case series were promising.

A larger, prospective, randomized trial reported by Murray et al. detailed two treatment regimens for radiation maculopathy – intravitreal injections of aflibercept every 6 weeks or treat-and-extend centered around every 6 weeks [61]. Through 1 year of follow-up, significant improvements in central retinal thickness were observed and 42.5% of subjects had VA 20/50 or better. There were no significant differences in injection frequency between the two cohorts.

Table 10.2 Prospective clinical trials in anti-vascular endothelial growth factor therapy

Author (year)	Subjects	Cohorts	Length of study	Outcomes by end of study
Finger et al. (2013)	Recalcitrant radiation maculopathy with a history of failed anti-VEGF treatment	*Cohort 1 (n = 5):* high-dose (2.0 mg) ranibizumab every 30 days *Cohort 2 (n = 5):* high-dose (2.0 mg) ranibizumab every 30 days for 4 months, PRN high-dose ranibizumab thereafter	12 months	Stable/improved BCVA in 70% of eyes Significant improvement in CFT in 80% of eyes
Kim et al. (2016)	Choroidal melanoma within 2 disc diameters of the optic nerve and/or macula with no history of retinopathy or anti-VEGF treatment	*Cohort 1 (n = 20):* 0.5 mg prophylactic ranibizumab every 2 months *Cohort 2 (n = 20):* 0.5 mg or 1.0 mg prophylactic ranibizumab every 2 months	22 months	VA 20/200 or better in 97% of eyes Evidence of radiation maculopathy in 33% of eyes
Fallico et al. (2019)	Treatment-naïve radiation maculopathy secondary to Ru-106 brachytherapy	2.0 mg aflibercept monthly for all subjects ($n = 9$) until maximum VA and/or a dry macula is achieved; PRN aflibercept thereafter	24 months	Mean 4.4 injections received BCVA significantly improved CFT significantly decreased
Murray et al. (2019)	Treatment-naïve radiation maculopathy from radiation therapy	*Cohort 1 (n = 20):* 2.0 mg aflibercept every 6 weeks *Cohort 2 (n = 20):* 2.0 mg aflibercept and treat-and-adjust thereafter (1 week adjustments)	60 weeks	BCVA of 20/200 or worse in 5% of eyes CRT significantly improved No significant differences in injection frequency between cohorts
Schefler et al. (2019)	Treatment-naïve radiation-induced macular edema with a history of radiation therapy	*Cohort 1 (n = 8):* 0.5 mg ranibizumab monthly for 12 months; treat-and-extend thereafter *Cohort 2 (n = 16):* 0.5 mg ranibizumab monthly and TRP 1 week after first injection for 12 months; treat-and-extend thereafter *Cohort 3 (n = 16):* 0.5 mg ranibizumab monthly and TRP 1 week after first injection for 3 loading doses, then PRN for 12 months; treat-and-extend thereafter	24 months	BCVA 20/400 or better in 97% of patients Most significant visual gains in Cohort 1 No visual or anatomic benefit with TRP treatment

VEGF vascular endothelial growth factor, *PRN pro re nata*, *BCVA* best-corrected visual acuity, *VA* visual acuity, *CFT* central foveal thickness, *CRT* central retinal thickness, *TRP* targeted retinal photocoagulation

All four studies in ranibizumab and aflibercept demonstrated promising visual and anatomic results for the treatment of radiation retinopathy or radiation-induced macular edema, though further efficacy and safety data will need to be confirmed in larger, multicenter trials.

Prophylactic Treatment

Several possible prophylactic treatments of radiation retinopathy have also been explored in retrospective case series and prospective trials. In prospective literature for other exudative retinal diseases, early treatment and prophylactic approaches appear to be visually and anatomically beneficial for patients [89]. For patients treated with radiation therapy for ocular or head/neck cancers, prophylactic treatment may be a reasonable approach to preserve long-term vision due to the long latency period between the end of radiation treatment and the onset of visual symptoms from retinopathy. Multiple studies have investigated the use of prophylactic laser photocoagulation and/or intravitreal corticosteroids at the time of radiation therapy [31, 40, 57], which appeared to reduce the risk of developing signs of retinopathy within the first 2 years following radiation treatment. A prospective case series by McCannel and McCannel used pars plana vitrectomy and silicone oil injection at the time of radiation treatment [58]. In theory, the oil would attenuate the excess radiation exposure to adjacent ocular structures. Through a mean 2 years of follow-up, subjects had lower rates of retinal hemorrhage, intraretinal exudates, edema, and other retinal changes compared to patients not treated with a silicone oil injection.

Some ophthalmologists have also explored the use of prophylactic intravitreal anti-VEGF injections beginning at the time of plaque removal. Two small retrospective studies analyzed the use of bevacizumab given every 4 months in the 2 years following radiation treatment [75, 79]. Overall, subjects appeared to have fewer long-term retinal complications and better visual acuity at 2 years post-plaque brachytherapy compared to patients not treated with prophylactic bevacizumab. In 2016, Kim et al. reported a prospective, phase I trial studying the efficacy of prophylactic anti-VEGF in patients receiving radiation treatment for choroidal melanoma [46]. Through 24 months, 40 eyes received 0.5 mg or 1.0 mg ranibizumab every 2 months. By the end of the study, 97% of eyes had a VA of 20/200 or better compared to 45% of historical controls, and 33% of eyes with a small/medium tumor at baseline had evidence of radiation retinopathy at 24 months compared to 68% of historical controls. No serious adverse events related to the study drug were observed throughout the course of the study.

The possibility of prophylactic treatment for radiation retinopathy may be promising for many patients who may experience anxiety at the thought of constantly monitoring their vision for decreases in acuity. Additionally, it may provide a standardized treatment protocol for patients following treatment of ocular cancers. However, before these techniques may be used in routine clinical practice, larger, multicenter trials must be conducted to determine efficacy and safety among patients.

Radiation Effects on Other Ocular Structures

Although damage to the retina is one of the most common visual side effects of radiation therapy, other important ocular structures may be damaged during treatment and require attention. Risk factors and therapeutic intervention for these side effects may vary, though proximity and quantity of radiation exposure plays a role in the incidence and extent of damage to all ocular structures.

Iris

Radiation treatment can cause direct radiation damage to anterior structures of the eye, resulting in thinning and atrophy of the iris [24, 26, 35]. This direct damage is more common when treating anterior uveal melanoma affecting the iris, the ciliary body, and/or the anterior choroid. Damage to the posterior portions of the eye may also result in indirect side effects on the iris. As discussed, radiation-induced ischemia in the retina results in an upregulation of VEGF molecules in the vitreous, leading to neovascularization of the retina and to neovascularization of the iris, specifically on the angle between the iris and the cornea [8, 27, 32, 82]. Similar to neovascularization in the retina, these new blood vessels in the iris are often weaker than normal, mature blood vessels and result in fluid leakage or inflammation. Eventually, the drainage angle can close, leading to increased intraocular pressure, neovascular glaucoma, pain, and abnormal vision.

Neovascularization of the iris can sometimes be treated in combination with treatment of posterior radiation damage. Although laser photocoagulation may be beneficial in the short term of reducing neovascularization of the iris, inhibition of VEGF agents has been shown to be one of the most effective treatments. Multiple anecdotal case reports and case series have demonstrated the use of anti-VEGF agents in treating neovascularization of the iris caused by various radiation treatments [9, 20, 63, 84, 91]. A few small, prospective clinical trials have also described the use of bevacizumab and ranibizumab in treating patients with neovascular glaucoma caused by other retinal diseases [53, 88, 90]. In these cases, anti-VEGF injections were able to reduce iris neovascularization and intraocular pressure through the study period.

Lens

Radiation cataracts are especially prevalent in patients treated with radiation therapy to the orbital. In a retrospective case series by Kinyoun, 52% of patients treated with either external beam irradiation or plaque brachytherapy for various indications demonstrated development of new cataracts [47]. By the 5-year endpoint in

the COMS study, 83% of study eyes had reported some level of new cataract development, and 12% underwent cataract surgery [15]. Improvement in visual acuity was seen in the majority of patients that underwent cataract surgery, with patients that did not experience an improvement demonstrating other side effects of radiation such as radiation retinopathy.

Prevalence of cataract formation does appear to be dependent to some extent on radiation dose and tumor location. In the COMS follow-up, 18% of patients that received a radiation dose of ≥24 Gy to the lens underwent cataract surgery compared to only 4% of patients that received a radiation dose of <12 Gy to the lens [15]. Several other studies have also demonstrated increased risk of cataract surgery in patients with increased radiation dose to the lens [25, 34, 49, 67].

Sclera

Side effects to the sclera are few and uncommon following radiation treatment. In rare cases, scleral necrosis may be observed, though most mild cases can be managed minimally with observation (Fig. 10.2) [10, 16, 35, 36, 68]. Severe cases of necrosis can require grafting or, in some cases, enucleation. Scleral necrosis is most commonly seen in cases of larger, more anterior tumors that require higher doses of radiation. In a retrospective review, Correa et al. reported that 8 of 87 patients (9.2%) treated with plaque brachytherapy for posterior uveal melanoma developed scleral necrosis during follow-up [17]. Four patients were successfully treated with a scleral graft, three were managed by observation, and one required enucleation following two unsuccessful scleral grafts. In a study by Gunduz et al., scleral necrosis was observed in 15 of 136 patients (11%) treated with plaque brachytherapy for

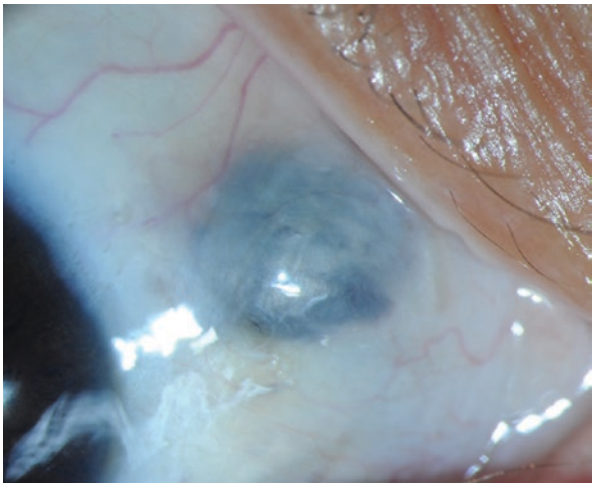

Fig. 10.2 Scleral necrosis in a patient treated with plaque brachytherapy for an anterior uveal melanoma

ciliary body melanoma [36]; tumor thickness and increased intraocular pressure were found to be risk factors for development of scleral necrosis. The authors theorized that thicker tumors may indicate increased risk of scleral invasion, which would explain consistency with previous studies which have shown that scleral necrosis generally develops in the treatment of tumors that have invaded the scleral lamellae [59].

Choroid

Following radiation treatment, the choriocapillaris may be damaged and lead to atrophy of the retinal pigment epithelium [1]. Over time, the larger choroidal vessels may become less perfused and lead to vascular closure. Choroidal neovascularization can occur due to increased VEGF levels from ischemia, but the new blood vessels are often abnormal and weak. Choroidal neovascularization can be treated as described by intravitreal anti-VEGF injections.

Optic Nerve

Radiation optic neuropathy is characterized by sudden, painless vision loss within the first several months to years after radiation therapy [18, 45, 52]. Radiation damage to the optic nerve can result in atrophy and pallor and may present as optic nerve edema. However, in some cases, the optic nerve may not appear clinically different if damage occurs in the retrobulbar space. Upon presentation with sudden vision loss, physicians must differentiate between optic neuropathy and tumor recurrence, though tumor recurrence typically presents with a slower loss of visual acuity [35]. Generally, prevalence of radiation-induced optic neuropathy is increased among patients treated with more than 50 Gy in total radiation dosage to the optic nerve head [83].

Treatment for radiation optic neuropathy is still debated with limited prospective literature. The use of intravitreal steroids may be useful in reducing edema, though visual gains may be small and transient [22, 69, 78]. Finger and Chin have also used intravitreal bevacizumab in a small case series of patients affected by radiation optic neuropathy with anatomic and visual improvements [28].

Conclusion

In the last few decades, plaque brachytherapy has become an increasingly popular treatment option for uveal melanoma and has allowed patients to keep their eye and maintain visual acuity. However, visual and anatomic side effects from radiation

damage are common in the months and years following therapy with limited options for follow-up treatment. In the COMS trial, radiation retinopathy, cataract development, and optic neuropathy were the most frequently observed side effects in patients treated with plaque brachytherapy, with 45% of patients demonstrating a visual acuity of 20/200 or worse by the 3-year time point. Thus, the benefits and drawbacks of radiation compared to enucleation must be carefully reviewed with patients prior to treatment, and risk of radiation damage must be assessed. Radiation retinopathy, one of the most common causes of visual loss following brachytherapy, currently has no FDA-approved treatment, though multiple prospective trials have demonstrated excellent visual and anatomic outcomes when patients are treated with intravitreal anti-VEGF injections. The optimal treatment regimen for radiation retinopathy is still debated, however, with no widely accepted management plan among vitreoretinal specialists. Additional trials are ongoing, which will hopefully provide more insight into the efficacy and optimal management strategy for patients with radiation retinopathy. Overall, plaque brachytherapy and other radiation therapies still provide an effective alternative to enucleation for uveal melanoma, though patients should be carefully followed by an ophthalmologist in the years after for the development of damage to any important ocular structures.

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Chapter 11
Nanoparticles for the Treatment of Uveal Melanoma

Abhijit Narvekar, Cadmus Rich, Anneli Savinainen, and Ivana K. Kim

Nanoparticles and Viruses in Oncology

Nanotechnology is a rapidly advancing technology with applications in many fields including medicine. According to the US National Nanotechnology Initiative (NNI), nanotechnology is the understanding and control of matter at the nanoscale, at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications [1]. In healthcare applications, nanoparticles can be used in imaging, to deliver small molecules, genes, and nucleic acids and as vaccines. Nanotechnology offers many possible benefits in oncology for cancer therapy, detection, and diagnosis. It offers the means to target chemotherapies directly and selectively to cancerous cells and neoplasms, guide in surgical resection of tumors, and enhance the therapeutic efficacy of radiation-based and other current treatment modalities [2]. The first nanotechnology-based cancer drugs used liposomes (Doxil®), protein nanoparticles (Abraxane®), or polymer-drug conjugate (Oncaspar®) as delivery agents and have been approved by regulatory authorities.

Genetically engineered viruses have been used in cancer therapy. Talimogene laherparepvec (T-VEC; IMLYGIC™) was the first oncolytic virus therapy approved by the US FDA and is indicated for the local treatment of unresectable cutaneous, subcutaneous, and nodal lesions in patients with melanoma recurrent after initial surgery. IMLYGIC is a live attenuated HSV-1 that has been genetically modified to

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replicate within tumors and produce the immune stimulatory protein GM-CSF. Its mechanism of action involves lysis of tumors, followed by release of tumor-derived antigens which together with virally derived GM-CSF may promote an antitumor immune response [3, 4]. Similar therapies based on other oncolytic virus types (Reovirus, Vaccinia virus) have been studied in clinical trials [5, 6]. However, the limitation of oncolytic virus-based anti-cancer therapy is that of tumor targeting, T-VEC must be administered by direct intra-lesional injection. Additionally, the tumor cytotoxicity is dependent upon the active replication of the viral agent within the tumor, which may limit its efficacy and increase the risk of toxicity after multiple administrations.

Viral capsids are considered naturally occurring nanoparticles optimized to carry and deliver a payload to specific targets. Plant viruses, bacteriophages, and mammalian viruses are being repurposed for gene delivery and to carry small molecule and protein therapeutics [7]. Within viruses, two types of agents are being increasingly studied – viral nanoparticles (VNP) and viral-like particles (VLP) which are their genome-free versions, similar to protein cages. The payload can be encapsulated or delivered through covalent attachments using the reactive amino acid side chains of viral capsid proteins. VLPs and pseudovirions (VLPs that encapsidate reporter gene plasmids of a limited size during self-assembly) are manufactured recombinantly and are comprised of only the outer protein capsid of a virus without any viral genetic material [8]. VLPs and pseudovirions can thus be used to deliver drugs efficiently to tumors without relying on viral replication and overcome the limitations of oncolytic technologies.

In this chapter we will review a novel investigational therapy that utilizes human papillomavirus (HPV) modified virus-like particles (VLPs) and is being developed for the treatment of choroidal melanoma.

HPV-Derived VLPs

The papillomavirus (PV) has a simple two-protein outer shell structure that self-assembles into a 55 nm capsid. VLPs derived from HPV L1 have been used in commercially available prophylactic HPV vaccines, GARDASIL® and CERVARIX®, for the prevention of cervical cancer and its precursor lesions. Papillomaviruses have a unique tropism toward cancer cells that is mediated by the binding of the viral capsid to specific modifications of heparan sulfate proteoglycans (HSPGs) on the surface of the cancer cell [9].

The specificity of HPV capsids is mediated through its binding to basement membrane associated with cell surface HSPGs [10, 11]. HSPGs are upregulated and exposed on the basement membrane of disrupted epithelial and mesothelial tissues and also in the tumor microenvironment, both on the tumor cell surface and within the locally secreted extracellular matrix. Figure 11.1 shows VLP interactions with HSPGs in benign but disrupted epithelium. Figure 11.2 depicts VLP binding to similarly modified HSPGs that are specifically overexpressed in malignant tumors,

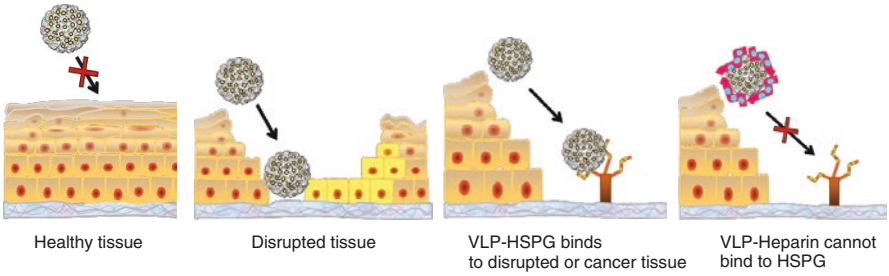
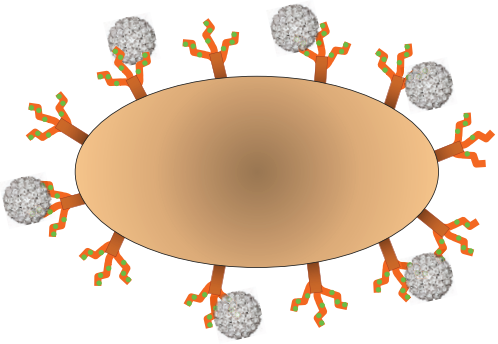


Fig. 11.1 VLP interactions with HSPGs in benign but disrupted epithelium

Fig. 11.2 VLP binding to similarly modified HSPGs



both on the cell membrane of tumor cells and in the extracellular matrix and disorganized basement membrane of the tumor.

HSPGs interact with many proteins, playing a role in cell signaling via growth factors and chemokines that modulate cell growth, motility, adhesion, and differentiation. Upregulation of HSPGs and specific modifications of their sulfation patterns are observed on tumor cells, and this leads to unregulated autocrine signaling loops promoting tumorigenesis and angiogenesis [12–15].

Exploiting this trait, HPV VLPs and pseudovirions directly bind most tumor-derived cell lines in vitro. Similarly, in vivo, binding has been noted using xenografts of human tumor cell lines and allografts of murine tumor cell lines (lung, ovarian, bladder, melanoma, colon) as well as primary human ovarian tumors [9, 16, 17]. Therefore, it appears that tumor cells consistently express modified HSPG patterns that mimic those normally found on the basement membrane but not on the apical surfaces of normal epithelial or mesothelial cells. Consequently, HPV-derived VLPs can be useful reagents to detect, target, and potentially treat a remarkably broad spectrum of cancers [9].

AU-011

AU-011 is a first-in-class investigational therapy currently being clinically evaluated for the treatment of primary uveal melanoma. AU-011 therapy involves administration of the drug (VLP conjugated to a novel phthalocyanine-based dye, IRDye®700DX, that acts as a photosensitizer) via either intravitreal (IVT) or suprachoroidal (SC) routes of administration which is followed by photoactivation of the drug with a laser device. The VLP with the conjugated drug creates a viral-like particle bioconjugate (VPB), and VPBs that combine the targeting of the VLP with the activity of the conjugated moiety are a novel platform technology for potentially treating many forms of solid cancers.

AU-011 comprises a modified human papillomavirus VLP that is recombinantly manufactured and conjugated with appr*o*ximately 200 molecules of IRDye®700DX (the "Dye"). At its core, the AU-011 VLP is comprised of two PV capsid proteins (modified L1 and wild-type L2). The native L1 protein amino acid sequence has been modified to reduce immunogenicity and the potential to cross-react with commercially available cancer vaccines such as GARDASIL®9. These two proteins create a 6 protein capsomere (approximately 5 L1 and 1 L2 proteins per capsomere), and approximately 72 of these capsomeres spontaneously assemble to create the synthetic capsid structure of the VLP. Therefore AU-011 does not contain any viral DNA and does not pose the risk of viral replication. The VLP conjugated with IRDye®700DX results in a pro-drug that is nontoxic to cells because it is not active until it is light activated. Upon light activation at 689 nm, the dye molecules preferentially transfer energy to ground-state oxygen and create singlet oxygen which is highly toxic to cells. Therefore, the treatment provides dual selectivity: (i) the photosensitizer is delivered specifically to the malignant tumor cells by the VLP and, (ii) focused light activation of the dye just over the tumor and a small margin results in the selective necrosis of the tumor cells while sparing healthy cells like benign melanocytes [8, 18].

Mechanism of Action

The dual mechanism of action of AU-011 is illustrated in Fig. 11.3. The upper portion of the figure shows the selective binding of the VPB to modified HSPGs on tumor cells and subsequent light activation causing singlet oxygen generation, cell membrane disruption, and acute tumor cellular necrosis. The lower portion shows that acute cellular necrosis is a type of immunogenic cell death that involves the release of damage-associated molecular patterns (DAMPs) and tumor neoantigens that lead to T-cell activation and an antitumor immune response. This pro-immunogenic mechanism of action has demonstrated a protective effect against tumor re-challenge in preclinical models (data on file, Aura Biosciences, Inc.). The immune response to the tumor is a critical part of the mechanism of action of

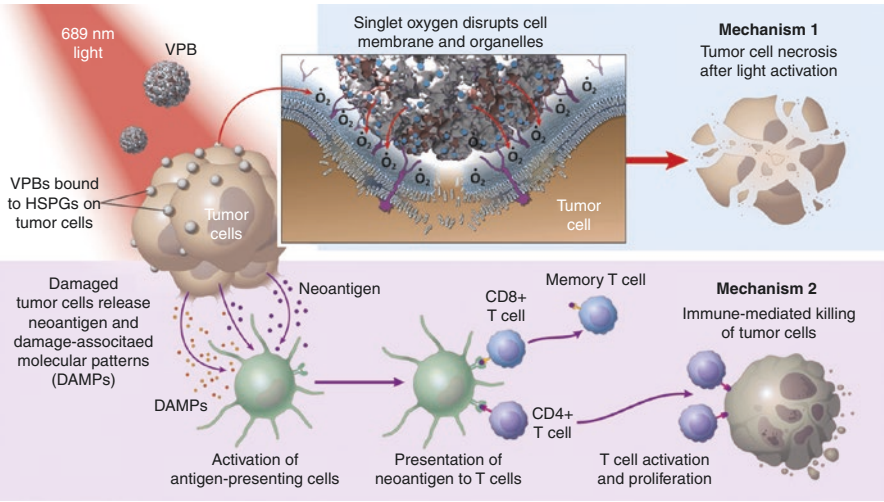


Fig. 11.3 Dual mechanism of action of AU-011

AU-011, and whether it may also be protective against micrometastases will be investigated in a long-term observational registry by assessing development of metastasis over a 5-year period.

An important part of the novel mechanism of action of AU-011 lies in the principle of multivalency. Compared to the traditional bivalency of antibody cancer treatments, VLPs, with a large and three-dimensional shape, have hundreds of sites that facilitate tumor binding which increases the affinity to the target tumor cells considerably over traditional bivalent antibody cancer treatments [19]. In comparison to antibody-drug conjugate technology, in which typically 3–5 dye molecules are delivered, each VLP delivers approximately 200 (+/−40) molecules of IRDye®700DX enabling high amounts of the light sensitizer to the tumor.

The targeted binding of the VLP on tumor tissue is a fundamental precursor to induction of the cytotoxic effect of IRDye®700DX following light activation. This direct cytotoxic effect on tumor cells is an advantage over previously investigated photodynamic treatments that had no specific tumor targeting but instead relied on the effect of systemically delivered light sensitizers to damaged blood vessels (i.e., Visudyne). It is hypothesized that since treatment with AU-011 is extremely potent and highly targeted to the tumor itself, healthy ocular tissues will be preserved.

Activation of AU-011 is performed by the Laser Photoactivation System, which consists of a laser console, a compatible slit lamp adaptor, and a compatible standard slit lamp biomicroscope that are used to aid in the visualization of the patient's eye and to focus the laser beam onto the desired ocular target. The laser delivers a light at 689 nm and at a nominal irradiance (I) of 600 W/cm^2 for 83 s. This, in turn, results in a nominal fluence (F) of 50 J/cm^2 delivered to the tumor as F (J/cm^2) = I (W/cm^2) x time (seconds). The laser light is applied to the tumor after injection (6–8 h for IVT and 4–6 h for SC) of a specified dose of AU-011.

Preclinical Data

In vitro cell binding assays show that AU-011 binds to the surface of cells through HSPGs in the same manner as wild-type HPVs [9].

In vitro studies have demonstrated the tumor-specific targeting, the role of HSPG in this targeting, and light-activated cytotoxicity of AU-011 for a panel of human tumor cell lines including the 92.1MEL uveal melanoma tumor line. The panel consisted of cutaneous melanoma and ovarian, lung, breast, cervical, head and neck, bladder, and uveal melanoma cell lines. AU-011 binding was similar among all lines tested and was blocked by preincubation of AU-011 with soluble heparin. The EC_{50} values for cell killing were similar across cell types ranging from 20 to 70 pmol/L. Light-activated cell killing by AU-011 was also inhibited in the presence of soluble heparin, confirming that tumor binding to HSPGs is necessary for the targeted cytotoxic mechanism of action [8].

Investigation of the photoactivation parameters of 689 nm light was conducted in vitro to support the parameters to be used in the clinical setting. Changes in irradiance did not influence the cytotoxicity of AU-011. However, the EC_{50} values for cytotoxicity were found to be dependent on the fluence level, and a fluence of 50 J/cm^2 was found to achieve the maximal effect, with no appreciable gain in in vitro cytotoxicity at higher fluence values. The fluence of 50 J/cm^2 at 689 nm was therefore selected for further nonclinical development and the clinical program (data on file, Aura Biosciences, Inc.).

In the rabbit orthotopic xenograft model, AU-011 penetrated and distributed throughout the tumor tissue after IVT injection (data on file, Aura Biosciences, Inc.), with high levels detected at 6–8 h post IVT injection. In naïve rabbits, AU-011 was detectable in vitreous and retina/choroid samples between 0.5 and 48 h after IVT injection. At 96 h, the levels of AU-011 were comparable to the negative control. No detectable levels of AU-011 were found in the lens. Systemic exposures to AU-011 after IVT administration were near or below the limit of detection at all time points (data on file, Aura Biosciences, Inc.).

Ocular distribution of VLPs after suprachoroidal administration was evaluated by injecting AlexaFluor488*VLP (surrogate for AU-011) into the suprachoroidal space (SCS) in New Zealand White (NZW) rabbits. Ocular distribution was evaluated with optical coherence tomography (OCT) and fundus autofluorescence (FAF) over time. Data showed that the distribution after a 100 μl injection into SCS was about 75% of the posterior globe at ≤0.5 h post-dose and remained relatively constant for at least 7–10 days [20].

After SC administration in a rabbit (NZW) orthotopic xenograft model of CM, AU-011 was able to penetrate the tumor and was distributed throughout the tumor. Tumor distribution was evaluated using immunohistochemistry (IHC) staining of AU-011. Data suggest that AU-011 is well distributed in the tumor by 3 h and supports laser treatment to activate AU-011 4–6 h post-SC injection in the clinic. Furthermore, data show that tumor and choroid/retina levels are approximately 5–6

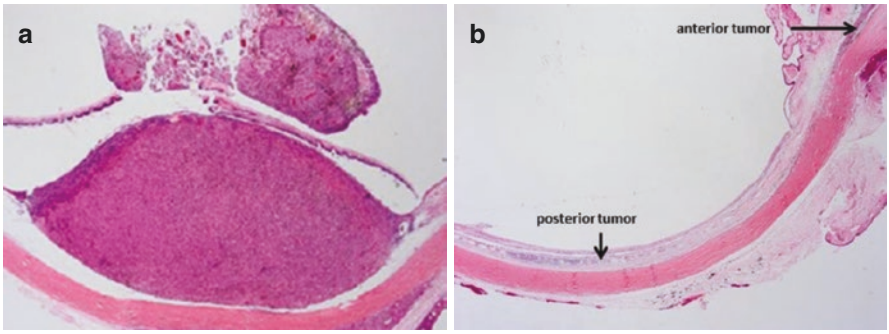


Fig. 11.4 (**a**) Rabbit orthotopic xenograft CM model: rabbit eye treated with saline control (H&E 5x). (**b**) Rabbit orthotopic xenograft CM model: rabbit eye treated with AU-011 and light activation (H&E 5x)

times higher after SC administration compared to IVT administration, while there was negligible exposure in the vitreous. Greater bioavailability of AU-011 in the tumor and less exposure in surrounding ocular tissue may allow for a greater therapeutic window (data on file, Aura Biosciences, Inc.).

Lastly, antitumor response of AU-011 administered by either intravitreal or suprachoroidal route followed by light activation has been demonstrated in in vivo studies using a rabbit orthotopic CM model. In this model, 92.1MEL human tumor cells are implanted in the choroid, and subsequent to tumor growth, treatment is administered. Tumor response is evaluated using clinical exams and histopathology. Treatment with AU-011 using either IVT or SC administration route followed by light activation resulted in a positive tumor response (e.g., complete response, necrosis of tumor, decrease in size of tumor) (Fig. 11.4a, b). Furthermore, a dose-dependent response was seen in a study which used three doses of AU-011 (5, 20, and 50 μg) administered using the IVT route [8, 20].

Clinical Data

AU-011 is being investigated in subjects with indeterminate choroidal melanocytic lesions and small choroidal melanoma (IL/ CM) using the intravitreal and suprachoroidal routes of administration. A Phase 1b/2 clinical study (NCT03052127) investigating AU-011 administered using the IVT route has completed enrollment, and follow-up is ongoing [21]. A Phase 2 clinical study (NCT04417530) of AU-011 via suprachoroidal administration has been initiated [22]. A long-term observational registry (NCT03941379) to assess long-term safety in patients who have participated in an Aura-sponsored study for the treatment of IL/CM is ongoing [23].

Phase 1b/2 Study with Intravitreal Administration

This is an open-label, ascending single- and repeat-dose and cycle, multicenter study designed to evaluate the safety, immunogenicity, and efficacy of single- and repeat-dose regimens and cycles of AU-011 and up to two laser applications in subjects with primary CM. Key eligibility criteria included participants 18 years and older with a clinical diagnosis of primary CM from 0.5 to 3.4 mm in thickness, ≤13.0 mm in largest basal diameter (LBD), either with documented growth or key risk factors associated with growth. As the study progressed, it was determined that criteria for the future Phase 3 trial would include documented growth ≥0.3 mm in tumor thickness within 2 years of enrolment, tumor thickness 0.5–3.0 mm and LBD ≤13.0 mm [24, 25].

The primary objective of this study is to evaluate the safety of IVT administration of one of three dose levels (20, 40, and 80 μg), repeat-dose regimens combined with one or two laser applications and given as 1, 2, or 3 weekly treatments. The study used a standard 3 plus 3 dose escalation design for the first eight cohorts which were completed with safety reviews to establish safety between each cohort. There were no dose-limiting toxicities (DLTs) with escalation to the maximal therapeutic regimen with 3 weekly treatments of 80 μg of AU-011 followed by two laser administrations 6–8 h later (these 3 weekly treatments are defined as 1 cycle of the maximal therapeutic regimen). Twelve additional subjects were enrolled in Expansion 1 (Cohort 9) and received one cycle of the maximal therapeutic regimen without significant safety events or DLTs. Expansion 2 (Cohorts 10, 11, and 12) was then enrolled with 21 subjects to receive two cycles of the maximal therapeutic regimen of AU-011, one cycle at entry and a second cycle at week 12 or soon thereafter any inflammation from the first cycle had resolved to at least a minimal level to allow administration of the second cycle (Fig. 11.5).

Subject enrollment of 57 subjects was completed in January 2020, and treatment completed for 56 subjects in April 2020; 1 subject in observation cohort (Cohort 10) was not treated.

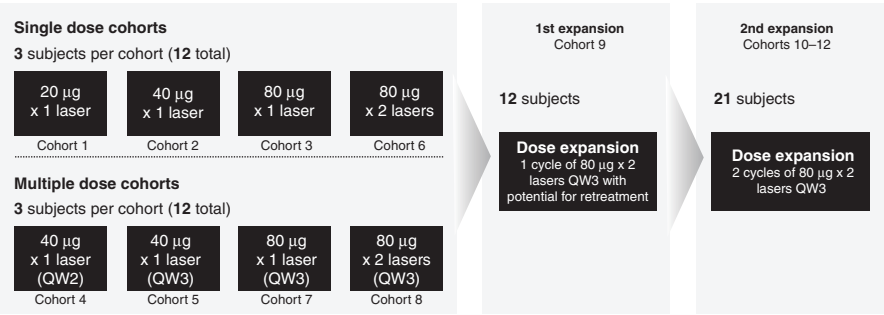


Fig. 11.5 Phase 1b/2 study design

There have been no deaths or DLTs in the study to date. Three [3] serious adverse events (SAEs) have been reported; all three were assessed as severe (severity grade 3): one event was unrelated and two were deemed related to the study treatment. The SAE unrelated to study treatment was papillary renal cell carcinoma for which subject underwent a right partial nephrectomy after which the event was considered as resolved. The two treatment-related SAEs involved vision loss in the study eye, occurred in subjects with juxtafoveal tumors with a tumor edge within 1.0 mm of the fovea and were observed after two treatments of AU-011 had been administered. The most common AEs related to AU-011 or laser of posterior inflammation/vitritis, anterior chamber inflammation/iritis, and increased intraocular pressure were manageable with steroid treatment and ocular antihypertensives (Table 11.1). One subject with severe vitreous opacities was treated with vitrectomy. Intraocular inflammation was expected based on the secondary mechanism of action of AU-011, and steroid treatment was not started with initial treatment until the inflammation was observed to increase the chance that a secondary immune response would occur.

Efficacy was evaluated overall and for subsets of the 57 enrolled subjects, including those with prior documented growth of tumor thickness (DG; $n = 32$), Phase 3 eligible based on defined Phase 3 criteria ($n = 22$), Phase 3 eligible subjects at high risk for vision loss (tumors within 3 mm of the fovea or optic disc; $n = 19$), and Phase 3 eligible subjects receiving maximum treatment exposure (two cycles of 3 weekly treatments with 80 μg/2 lasers; $n = 15$).

Interim data after all subjects completed 6-month follow-up are presented in Tables 11.2 and 11.3. Tumor control rates in all treated subjects were 73% (41/56) at 6 months and 55% (37/56) with a median follow-up of 12 months (mean follow-up of 15 months) including early dose escalation subjects with low-dose single treatments. In DG subjects, the rate was 81% (26/32) at 6 months and 66% (26/32) with a median follow-up of 12 months (mean follow-up of 14 months). In Phase 3

Table 11.1 Treatment-related adverse events that occurred in ≥15% subjects

Treatment-related adverse events	**Mild**	**Moderate**	**Severe**	**Total[a] ($n = 56$)**
Anterior chamber inflammation	42.9%	25.0%	1.8%	69.6%
Vitreous inflammation	30.4%	48.2%	7.1%	85.7%
Increase in intraocular pressure	17.9%	23.2%	0	41.1%
Floaters/vitreous opacity	10.7%	3.6%	1.8%[b]	16.1%
Treatment-related serious adverse events	**Mild**	**Moderate**	**Severe**	**Total ($n = 56$)**
Vision loss (juxtafoveal tumor)	0	0	3.6%[c]	3.6%

[a]Table presents percentage of subjects with AEs by severity and overall; subjects with more than one AE are counted in the highest severity group

[b]1 Subject with vitreous opacity treated with vitrectomy

[c]2 SAEs of visual loss in subjects with juxtafoveal tumors

Jul 22, 2020 Data cutoff

Table 11.2 Tumor control rates

Populations	Subjects (*n*)	Tumor control rate (at 6 months)	Tumor control rate[a] (mean/median follow-up in months)
All dose cohorts			
All subjects	56	73%	55% (15/12)
Documented growth subjects	32	81%	66% (14/12)
Ph3 eligible subjects	22	86%	68% (13/11)
Ph3 eligible high risk for vision loss subjects	19	89%	74% (12/9)
Therapeutic regimen			
Ph3 eligible subjects	15	100%	80% (8/9)

Tumor control – all subjects that did not meet definition of tumor progression (growth in tumor height >0.5 mm; LBD >1 mm due to definitive tumor growth) and not treated with standard of care
[a]With all available follow-up, Jul 22, 2020 Data cutoff

Table 11.3 Vision preservation rates

Populations	Subjects (*n*)	Vision preservation rate (at 6 months)	Vision preservation rate[a] (mean/median follow-up in months)
All dose cohorts			
All subjects	56	91%[b]	91%[b] (15/12)
Documented growth subjects	32	91%	91% (14/12)
Ph3 eligible subjects	22	91%	91% (13/11)
Ph3 eligible high risk for vision loss subjects	19	89%	89% (12/9)
Therapeutic regimen			
Ph3 eligible subjects	15	87%	87% (8/9)

[a]With all available follow-up, Jul 22, 2020 Data cutoff
Vision Failure: long-term decrease in vision >15 letters (>3 lines)
[b]1 subject not included as loss of vision was due to tumor progression and plaque treatment, not related to AU-011

eligible subjects treated with two cycles, the rate was 100% (15/15) at 6 months and 80% (12/15) with a median follow-up of 9 months (mean follow-up of 8 months) (Table 11.1). Analysis of tumor thickness growth rates prior to study entry compared to growth rates after treatment shows statistically significant reductions in tumor growth rates in all subsets with documented growth ($p < 0.05$) [24, 25].

Vision preservation, defined as ≤15 letter loss in ETDRS-BCVA, was achieved for >90% of all treated subjects and all subjects with documented growth. It is important to note that 43 out of 56 treated subjects had a tumor ≤3 mm from either the fovea or the optic disc. In Phase 3 eligible subjects treated with two cycles, the rate was 87% (13/15) at 6 months and also 87% (13/15) with a median follow-up of 9 months (mean follow-up of 8 months), with 14/15 subjects with tumor location ≤3 mm from either the fovea or the optic disc (Table 11.2) [24, 25]. A reduction in

tumor size is not expected in many subjects because the majority of CMs start in a preexisting nevus and AU-011 will only bind to malignant melanoma cells (not normal melanocytes within the nevus). Patients with indeterminate choroidal melanocytic lesions and small CM have a small component of melanoma cells in the overall lesion, and while these are expected to be effectively targeted and killed, the potential to see an overall reduction in tumor thickness after treatment is low. As an example, Fig. 11.6 shows a study subject with a stable tumor on fundus photos and B-scan ultrasound images 6 months after treatment compared to before treatment. In addition, the acute necrotic cell death is highly immunogenic, and these dead cells are replaced by fibrosis, so the reduction in malignant necrotic cells that would occur is at least partially replaced with fibrosis.

The interim efficacy data from this ongoing study are encouraging, and further characterization of efficacy will be feasible upon availability of additional follow-up information through study completion.

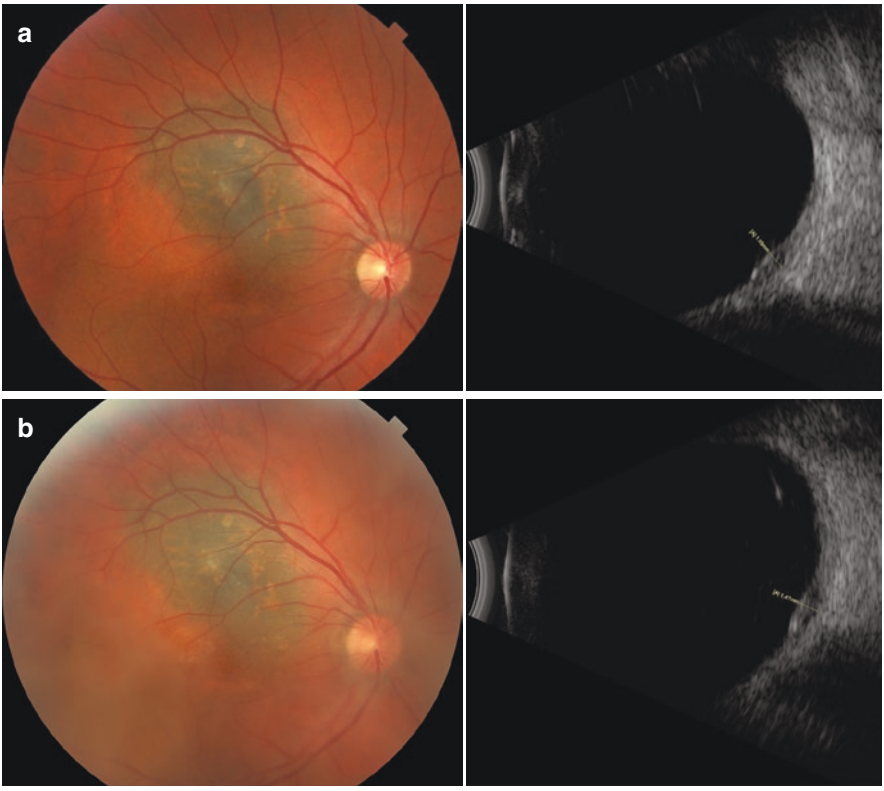


Fig. 11.6 Phase 1b/2 study case. (**a**) Fundus photo and ultrasound B-scan at day 1 before AU-011 treatment. (**b**) Fundus photo and ultrasound B-scan 6 months after treatment showing stable tumor

Phase 2 Study of Suprachoroidal Administration

AU-011-202 is a Phase 2 study of AU-011 via suprachoroidal administration with a dose escalation phase (open-label, ascending single and repeat dose) and a randomized, masked dose expansion phase designed to evaluate the safety and efficacy of AU-011 in subjects with indeterminate choroidal melanocytic lesions and small choroidal melanoma. The objectives are to assess the safety, tolerability, and immunogenicity of AU-011 and to determine the highest tolerated regimen utilizing the SC route of administration. Objectives also include establishing the initial efficacy of AU-011 with SC injection in the randomized expansion phase of the study. Approximately 31 adult subjects (approximately 11 subjects in the 5 cohorts in the dose escalation phase and up to 20 subjects in the randomized dose expansion phase) are planned for enrollment (Fig. 11.7) [24].

The safety profile in subjects enrolled in the single-dose cohorts is favorable with no DLTs or SAEs reported to date.

Registry

A long-term observational registry of patients with primary choroidal melanoma (CM) and indeterminate lesions (IL) is ongoing and is open to patients who have previously participated in an Aura Biosciences-sponsored clinical study for their IL or CM. The primary objective is to assess long-term safety following treatment with AU-011, observation, SOC, or alternative standard of care treatments. Secondary objectives include the assessment of durability of response, long-term visual outcomes, development of metastatic disease, and overall survival. All subjects will be followed for a minimum of 5 years including the time enrolled in a previous Aura-sponsored clinical study.

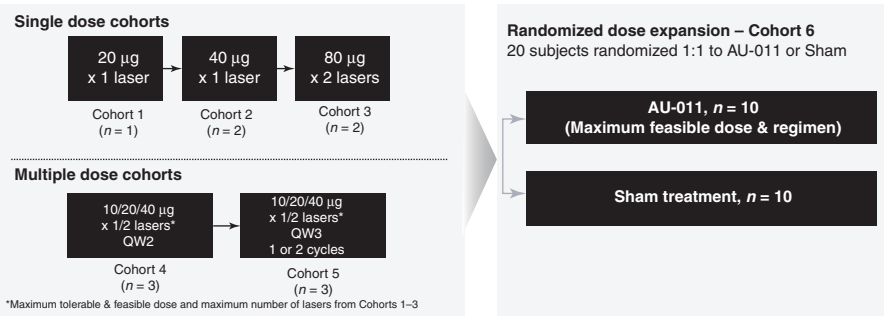


Fig. 11.7 Phase 2 study design

Summary

Uveal melanoma is the most common primary ocular malignancy in adults which has a high risk of metastasis and mortality. Current most commonly used standard of care treatment options include radiotherapy and surgery. While radiotherapy achieves excellent tumor control, surgical procedures are required for radiotherapy, for placement and removal of plaque in brachytherapy, and for placement of markers for tumor localization in proton beam therapy. Radiotherapy lacks tumor tissue specificity and is associated with long-term complications such as radiation retinopathy or maculopathy, papillopathy, and neovascular glaucoma, often leading to severe vision loss [26, 27]. Non-radiation-based treatments such as transpupillary thermotherapy (TTT) and photodynamic therapy (PDT) are less efficacious and, therefore, less frequently used modalities. Enucleation is required as primary therapy for very large tumors and may become necessary secondarily due to tumor recurrence or for blind, painful eyes resulting from radiation complications.

AU-011 has the potential to be a targeted, vision-sparing treatment for choroidal melanoma. In vitro data demonstrate AU-011 selectively binds to choroidal melanoma cells through heparan sulfate modifications on the cancer cell surface and elicits potent and selective anticancer activity upon photoactivation. In vivo studies using the orthotopic xenograft rabbit model of CM demonstrate robust tumor response when AU-011 is delivered using either IVT or SC routes. Clinical data thus far are encouraging in terms of tumor control and vision preservation with an acceptable safety profile. Follow-up data from the ongoing studies and future studies are needed to confirm these findings and the potential of this novel investigational therapy.

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Part III
Systemic Therapies

Chapter 12
Surveillance for Metastatic Disease

Caroline A. Nebhan, Douglas B. Johnson, Steven A. Deppen, and Anthony B. Daniels

Introduction

Less than 5% of patients with uveal melanoma will have metastatic disease at initial presentation, but about half of patients will ultimately develop metastatic disease [1]. Patients typically experience rapid decline upon development of metastatic disease, with median overall survival ranging from 4 to 15 months [2, 3]. Despite general advances in therapeutics for other cancer types over the past decades, there exists a paucity of effective systemic treatment options in uveal melanoma. Patients with oligometastatic disease may be candidates for local treatments. As such, a major consideration in the management of uveal melanoma is how to best surveil patients so that metastatic disease can be identified for earliest possible intervention, when the most effective treatments are available as options. This subject has many challenges, most of which stem for lack of prospective trials in this area. Consideration for the ophthalmologist, medical oncologist, and uveal melanoma patient include patient-specific risk stratification, imaging modality, and schedule for surveillance following diagnosis of primary uveal melanoma. In this chapter, various current strategies for metastatic surveillance in uveal melanoma are evaluated, along with an assessment of the evidence supporting their use.

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Initial Staging Imaging

Although it is rare that patients with a new diagnosis of uveal melanoma have metastatic disease at the time of their initial presentation, National Comprehensive Cancer Network (NCCN 2.2020) guidelines recommend baseline imaging at the time of diagnosis of a primary uveal melanoma to screen for distant metastatic disease and stage the tumor. These guidelines recommend a continuum of options with little consensus regarding an optimal approach. Evaluative choices range from dedicated liver imaging with ultrasound or MRI to more comprehensive CT imaging of the chest, abdomen, and pelvis (with intravenous contrast as permitted by the patient's renal function) [4]. One study of 1000 patients with newly diagnosed uveal melanoma found that initial imaging of the chest and abdomen identified all cases of metastatic disease at presentation. Additionally, almost all patients with metastatic disease at presentation had liver involvement, and imaging the chest only identified a single additional patient beyond what would have been identified through liver imaging alone [5].

Considerations for Surveillance Imaging

Existing Guidelines for Surveillance

Currently, little consensus exists to guide surveillance strategies in uveal melanoma. In the United States, the NCCN guidelines recommend consideration of annual surveillance imaging for low-risk disease, surveillance imaging every 6–12 months for 10 years after diagnosis for medium-risk disease, whereas for high-risk disease the NCCN recommendation is for surveillance imaging every 3–6 months for 5 years and then every 6–12 months until year 10 [4]. The preferred imaging modality for surveillance is not specified; guidelines broadly recommend that preference be given to the radiology expertise of the institution, with considerations to limit radiation exposure to the individual patient. European guidelines suggest liver ultrasound every 6 months for 10 years after diagnosis of low-risk disease. In cases of high-risk disease, MRI/ultrasound is recommended every 3 months along with annual chest CT +/− brain MRI [6].

Does Early Diagnosis Improve Patient Outcomes?

There are no recent prospective, randomized trials which evaluate surveillance imaging strategies in uveal melanoma. Such individual trials performed in more common malignancies, such as colon cancer, do not always suggest survival

benefit [7]. In the case of uveal melanoma, any benefit of early detection of metastatic disease is thought to stem from the potential for surgical resection of oligometastatic disease, as current systemic treatment options for metastatic disease remain poor [8]. A recent analysis compared two retrospective cohorts of patients: one group of patients treated and followed from 1975 to 1987 and another treated and followed from 1982 to 2011. The mean overall survival after diagnosis of metastatic disease among patients who underwent treatment was 5.2 months versus 6.3 months, respectively (no statistical difference), highlighting the paucity of systemic treatment advances made over the 30-year period [9]. Numerous retrospective studies have sought to determine the efficacy of routine surveillance in localized uveal melanoma. A 1991 study by Gragoudas et al. found that the majority of patients (65%) were diagnosed with metastatic disease upon development of symptoms, while the remaining patients were diagnosed by surveillance liver function tests (LFTs, including serum alkaline phosphatase, lactate dehydrogenase, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase), routine imaging for other reasons (primarily surgical planning for unrelated diseases), or surveillance imaging. It should be noted that very few (16%) patients in this retrospective analysis underwent any type of abdominal surveillance imaging for metastatic uveal melanoma. However, a small but statistically significant survival advantage was found in the group that was diagnosed by imaging (5.0 months, versus 3.1 months for the symptomatic group, $p = 0.004$) [10]. The authors suggested that this "survival advantage" likely represented lead time bias. In a cohort of 349 patients diagnosed with metastatic disease after either symptom development or routine surveillance imaging, Kim et al. found that routine surveillance resulted in a survival advantage only in the first year after diagnosis, again suggesting lead time bias [11]. A 2011 meta-analysis of 31 available studies performed between 1980 and 2009 found no survival benefit to routine surveillance imaging [12].

Based on the paucity of effective systemic treatment options for metastatic uveal melanoma at present, the time from diagnosis of metastatic disease to death can be short. Unfortunately, advances in cancer treatment across the spectrum of malignancy have not been reflected in the treatment of uveal melanoma. An analysis of SEER data showed no significant change in the 5-year relative survival rate from 1973 to 2008 [13]. Similarly, a meta-analysis performed in 2011 of over 30 retrospective studies of surveillance strategies in uveal melanoma failed to demonstrate a survival advantage [12]. Patients who underwent systemic treatment during this period (pre-2011) generally received traditional cytotoxic chemotherapy. Newer treatment strategies such as immune checkpoint inhibitors, which have dramatically changed survival outcomes in cutaneous melanoma, have not provided similar results in metastatic uveal melanoma. Overall response rates for PD-1 inhibitors and/or CTLA-4 inhibitors in uveal melanoma range from 5% to 17%, with PFS ranging from 2 to 3 months [14–16]. This is thought to be due to the poor immunogenicity of uveal melanoma [17, 18]. Another recent study suggested that, while

response rates to pembrolizumab were low (20%), rates were higher among the subgroup of patients without bulky hepatic metastatic disease. This suggests a potential benefit in early identification of metastases [19].

Future treatment options may include antigen-stimulating compounds, autologous tumor-infiltrating lymphocyte (TIL) infusions, and epigenetic therapies [20–22]. Tebentafusp is a novel bispecific T cell receptor redirector, which targets gp100, a surface marker expressed on uveal melanoma. A recent study demonstrated improved survival compared with investigator's choice therapy. Although this data is not yet mature at time of publication, preliminary results suggest an improved 1-year overall survival rate of 73% with tebentafusp compared to 58% with investigator's choice (94% of patients in that group had pembrolizumab or ipilimumab) [23].

Presently, localized therapies targeting limited disease seem to achieve the greatest efficacy, including metastasectomy, hepatic lobectomy, and endovascular chemoembolization [24, 25]. In addition, while immune checkpoint inhibitors tend to have low patient response rates for metastatic UM, there is some evidence that it is specifically the subset of patients with low disease burden who are most likely to respond favorably [19]. Among these, disease that is found to be surgically resectable appears to achieve the most significant improvement in overall survival; the survival benefit among these patients is almost double that of unresectable disease patients (14 months versus 8 months, $p < 0.001$). Patients who achieved R0 resection experienced the greatest survival benefit (27 months) [26]. For patients whose disease is not amenable to surgical managements, advanced percutaneous therapies may be considered. Such interventions include hepatic arterial chemo-infusion of fotemustine and percutaneous hepatic perfusion of melphalan, both of which have demonstrated progression-free survival benefits [27–29]. However, such options are dependent on the expertise of local interventional radiologists and require careful patient selection, considering disease burden, anatomic feasibility, and healthy liver status. As systemic treatment options improve, the role of surveillance in uveal melanoma will gain even greater importance.

Time to Metastasis

Of patients initially diagnosed with uveal melanoma localized to the eye, 24% will develop metastatic disease to the liver within 5 years and 40% within 10 years of initial diagnosis [30, 31]. Patients may also present with metastatic disease much later; a retrospective analysis of 463 patients found that, of those who ultimately developed metastatic disease, 65% did so within 5 years of diagnosis of the initial ocular primary tumor, an additional 21% between 5 and 10 years after initial diagnosis, and the remaining 13% of patients developed metastases beyond this 10-year mark [32]. Even 20 years after initial diagnosis, metastatic disease is the leading cause of death in a majority of patients with uveal melanoma [33].

Why Do Late Metastases Develop in Uveal Melanoma?

The concept of hepatic micrometastatic disease and tumor dormancy in uveal melanoma was first postulated in the 1970s by Zimmerman et al., known as the Zimmerman-McLean-Foster hypothesis. This group observed a high risk of death for 2 years following enucleation, arguing for hematogenous spread of disease and possible tumor dormancy [34]. Updated data from the Collaborative Ocular Melanoma Study (COMS) has disproved the original Zimmerman-McLean-Foster hypothesis that enucleation led to accelerated tumor cell dissemination. However, this hypothesis spawned the development of experimental modeling, along with advances in laboratory techniques, to better understand uveal melanoma tumor kinetics [35]. Challenges in studying kinetics of metastasis include the low frequency of uveal melanoma, prolonged window of metastatic occurrence, and relative paucity of translational model systems [36, 37]. Studies that attempted to determine the doubling rate of metastatic disease in uveal melanoma from careful analysis of surveillance imaging have demonstrated a doubling time of untreated metastases from 24 to 350 days (median 63 days) [38, 39]. This suggests that micrometastatic foci of disease are established approximately 5 years before clinical detection [40]. Once established, micrometastases may remain dormant, meaning that individual tumor cells experience arrest of the cell cycle in G0 phase [41]. Micrometastases do have the potential to grow and eventually become vascularized, but this is often delayed. Numerous studies have sought to understand the mechanisms of tumor dormancy [42]. One area of focus has been the role of angiostatin, an endogenous inhibitor of angiogenesis, which is thought to be produced by the primary tumor to suppress the growth of concurrent micrometastases. One study found that treatment with angiostatin, an endogenous inhibitor of angiogenesis, could reduce the number of micrometastases in a murine model of ocular melanoma [43]. As part of the seminal COMS report, one autopsy study evaluated specimens from 435 patients who died with metastatic uveal melanoma. The most common sites of metastatic disease were the liver (93%), lung (24%), bone (16%), skin and subcutaneous tissue (11%), and lymph nodes (10%). Among the small subpopulation of patients who underwent autopsy at time of death (n = 27), the rate of liver and lung metastasis was found to be higher than in cases without autopsy (100% versus 93% liver metastases, 50% versus 22% lung metastases), suggesting that, at the time of death, some patients have metastatic disease that is not clinically apparent [44].

Radiation Exposure

The imaging modality most frequently used for surveillance of uveal melanoma in the United States is computed tomography (CT). As with any screening or surveillance regimen that relies on X-rays for resolution, a major concern with uveal

melanoma surveillance is the risk of radiation exposure associated with CT imaging and PET-CT imaging. Typical radiation exposure for CT abdomen/pelvis ranges from 10 to 15 mGy, while PET-CT exposes patients to 25 mGy radiation. This is particularly important given the often long delay until appearance of metastatic disease (necessitating repeat imaging over many years). The estimated risk of surveillance CT chest/abdomen/pelvis performed annually over 10 years results in an attributable risk of cancer of 0.9% in male patients and 1.3% in female patients. PET-CT imaging portends an even greater attributable cancer risk of 1.6% for men and 1.9% for women [45]. While these attributable risks assume annual surveillance imaging, most uveal melanoma patients can expect to be scanned between two to four times per year, according to the current recommendations.

Psychological Effects

For patients, surveillance imaging can be a source of both reassurance and anxiety. Studies in other malignancies found a 37% rate of clinically significant anxiety surrounding the surveillance imaging event. Risk factors for anxiety included poor doctor-patient relationship and prior history of relapse [46]. It is also important to consider the negative psychological effects of false-positive imaging results; this can be a significant source of distress for patients and caregivers. While definitive diagnosis of metastatic disease requires histologic confirmation, the broad range of imaging modalities with potential application in uveal melanoma have different false-positive and false-negative rates. Studies in breast cancer evaluated the psychological effects of intensive versus limited imaging follow-up schedule over a 5-year period, finding no significant difference in health-related quality of life (defined as emotional well-being, social functioning, satisfaction with care, and overall quality-of-life perception) [47, 48]. Because uveal melanoma recurrence can occur decades after initial diagnosis, the impact of any psychological distress may be more deleterious than other cancers with more rapid development of metastatic disease.

Risk Stratification to Guide Surveillance

Well-established prognostic factors in uveal melanoma include American Joint Commission on Cancer (AJCC) traditional tumor-node-metastasis (TNM) staging system as well as cytogenetics, gene expression profiling, multiplex ligand probe amplification for chromosome 3 analysis, and mutational analysis (including next-gen sequencing). Appropriate risk stratification of an individual patient represents an essential component of determining the patient's best surveillance regimen; conventional wisdom suggests that patients with high-risk disease generally require more aggressive surveillance than those with lower-risk disease.

As discussed, American Joint Commission on Cancer (AJCC, also known as the tumor-node-metastasis [TNM]) staging as published by the NCCN staging

guidelines recommends complete clinical staging with imaging be performed at the time of diagnosis. The AJCC TNM staging system is generally considered to be the benchmark in patient stratification, separating patients into 4 tumor categories, 17 tumor subcategories, and 4 stages. Tumor staging depends on the primary tumor thickness and largest basal diameter, as well as considerations for involvement of the ciliary body and extraocular extension. With the 7th edition of the AJCC staging of uveal melanoma, a number of changes were made to better correlate with outcome. These include expanded T staging (from T1-T3 to T1-T4) and stratification based on extraocular extension. Correlation of staging to rates of metastatic recurrence demonstrated 2-, 5-, and 10-year rate of metastatic disease to be 10%, 25%, and 34%, respectively [49, 50]. More recently, the 7th edition was updated in January 2017 to the 8th edition. This update established a new N subcategory, separating patients with extrascleral extension and those with regional spread into the noncontinuous orbit.

Changes in practice patterns have led to more routine application of fine needle aspiration of primary tumor at the time of diagnosis. Coupled with advances in genetic techniques, this has allowed for deeper understanding of genetic biomarkers with predictive value in uveal melanoma [51]. This led to development of The Cancer Genome Atlas (TCGA) prognostication system, developed by Damato et al. in 2010 [52]. In this system, uveal melanomas are categorized into one of four classes (A, B, C, D) based on the presence or absence of chromosome 3 monosomy and the presence and degree of chromosome 8q gain. Class A patients bear the best prognosis, while class D patients have the greatest risk of metastasis (HR class D versus A 30.0, $p < 0.001$) [53]. A large retrospective analysis comparing the efficacy of AJCC staging to TCGA staging found that at 5 years, TCGA classification showed a higher predictive value than did AJCC classification, even after application of a multivariate model (Wald statistic for TCGA 61.5 versus AJCC, 35.5) [54].

Cytogenetics can provide further prognostic information. Reporting a patient's status can identify monosomy 3 and 8q gain; these alterations have been associated with worse prognosis. A multicenter retrospective analysis of over 500 patients found that adding data on a patient's chromosome 3 and 8q status to their AJCC staging resulted in more accurate prognostication in uveal melanoma [55]. Similarly, concurrent loss of chromosome arm 1p and 3 was found to predict worsened disease-free survival [56].

Despite the validated utility of cytogenetic stratification, such systems can be limited by sampling error due to tumor heterogeneity and lack of standardized testing platforms. In an effort to overcome this, the DecisionDx-UM™ gene expression profile (GEP) test was developed. This PCR-based system has the benefit of being a stand-alone, easy-to-interpret test that measures expression of 15 genes from primary uveal melanoma samples (including 12 differentially expressed genes and 3 control genes). Tumors are stratified as class 1 (low metastasis risk) or class 2 (high metastasis risk). Validation in a multicenter cohort of 450 patients found that >95% of class 1 patients were free of metastatic disease at 4 years, compared to less than 20% of class 2 patients [57]. As part of the Collaborative Ocular Oncology Group, the GEP was further validated as having a higher prognostic accuracy over TNM staging and chromosome 3 status at 3-year follow-up [58]. While GEP class 2

patients carry the highest risk of metastatic development (5-year risk of 72%), class 1 tumors can also metastasize; class 1 tumors are further subclassified as class 1B (5-year metastasis risk 21%) and 1A (5-year metastasis risk 2%). Attempts to understand the molecular basis for these divergent risks of metastasis led to the identification of cancer-testis antigen *PRAME*, an antigen preferentially expressed in melanoma [59]. PRAME expression in uveal melanoma has been shown to confer an additional metastatic risk beyond GEP status, which is of particular importance in the one-third of class 1A patients whose tumors are PRAME-positive, conferring increased metastatic risk [60]. Similarly, GEP and PRAME status were found to have improved prognostic accuracy over traditional TNM staging (8th edition) in a retrospective cohort study of 240 patients [61].

An emerging approach to monitor for recurrent disease in solid tumor oncology generally is harnessing circulating tumor cells in peripheral blood, where increased circulating tumor cells correspond to increased risk for metastasis. This approach has particular potential in uveal melanoma due to the primary role of hematogenous spread in this disease. Early studies have begun to evaluate the utility of circulating tumor DNA in uveal melanoma. One small study enrolled patients with early-stage or metastatic uveal melanoma and performed blood draws at time of diagnosis. Early-stage UM patients with circulating tumor cells in peripheral blood at time of diagnosis were found to have increased risk of metastatic disease and worse outcomes as compared to early-stage patients without evidence of circulating tumor cells in peripheral blood at diagnosis [62].

Screening Schedules and Patterns of Metastatic Disease

Patients with hepatic metastases have worse prognoses than patients without hepatic metastases, with overall survival of 18.4% versus 52.8% at 1 year and 2.9% versus 19.8% at 3 years [63]. Given the overwhelming propensity of uveal melanoma to metastasize to the liver and the negative association with liver metastases with survival, any surveillance protocol should evaluate the liver at a minimum. However, this strategy misses the 10% of patients who present only with extrahepatic metastases.

The chronology of systemic surveillance is impacted by numerous factors. Most important of these are underlying tumor biology and natural history of metastatic development. Also important are patient-specific factors, including performance status which may limit systemic or surgical treatment options, as well as patient preferences.

With the advent of GEP testing in 2010, clinicians have more data to develop "personalized" screening schedules for individual patients. Numerous studies have demonstrated difference in management among patients with GEP class 1 and class 2 disease; in three separate studies, lower-risk class 1 patients underwent lower-intensity screening protocols as compared to higher-risk class 2 patients [64–66]. Class 2 patients were also more likely to be referred to medical oncology and/or

clinical trials [66]. Based on these conclusions from smaller studies, a multicenter, prospective trial evaluated the clinical utility of the GEP on metastatic screening schedule based on an individual patient's risk. This study included 138 patients from nine centers across the United States, with patients diagnosed with uveal melanoma between March 2018 and February 2019. This cohort included 93 class 1 patients (67%) and 45 class 2 patients (33%). A majority of patients in both classes were referred to medical oncology (51% of class 1 patients, 93% of class 2 patients), but this was significantly more likely among class 2 patients ($p < 0.0001$). Medical oncologists were responsible for ordering surveillance imaging among 44% of class 1 patients and 76% of class 2 patients. As expected, physicians recommended more frequent surveillance testing for class 2 patients than class 1 patients. Class 2 patients were more likely to receive a recommendation for every 3–4-month chest and abdominal imaging, while class 1 patients were more likely to receive a recommendation for every 6-12-month chest and abdominal imaging. Liver function testing was infrequently recommended for both class 1 and class 2 patients. Significant differences among imaging modality were also identified; ultrasound was more likely to be recommended for class 1 patients, while MRI was more likely to be recommended for class 2 patients.

Surveillance Modalities

Peripheral Blood

Due to the relative ease and low cost with which routine laboratory tests can be performed on peripheral blood, there has been much effort to determine whether there is any role for routine blood testing either independently or in combination with imaging studies in monitoring for metastatic disease. The COMS Report 23 explored the role of diagnostic testing to surveil for development of metastatic disease. This trial represents the only prospective, randomized trial of surveillance imaging in uveal melanoma. 2320 enrolled patients with localized uveal melanoma were enrolled and stratified based on tumor size, using tumor size to guide an examination schedule. In the context of this part of the COMS, "examination" included physical examination and liver function tests (LFTs) performed at described time points and chest X-ray performed annually. LFTs in this context referred to AST, ALT, GGT, and AlkPhos. Patients in the large tumor trial underwent examination at 6 and 12 months after diagnosis and then annually thereafter. Patients in the medium tumor trial were examined every 6 months for the first 5 years after diagnosis and then annually thereafter. When a patient developed clinical symptoms and/or LFTs elevation (defined as 2x ULN for AST, ALT, >1.5x ULN for AlkPhos, and >2.0 mg/mL for total bilirubin), they were referred for further imaging (CT, MRI, or other imaging). The COMS Report 23 found that routine screening using LFTs was of low sensitivity (14.7%) but high specificity (92.3%) [31].

LFTs in the absence of imaging have been shown to be a poor surveillance mechanism, though the overall specificity and negative predictive value was above 90% [67]. However, a larger retrospective study of 307 uveal melanoma patients followed over a 10-year period found that LFT abnormalities could be detected in 50% of patients during the 6 months prior to detection of hepatic metastasis on imaging. This study found that LDH and AST were predictive upon reaching 80% of the upper limit of normal, while AlkPhos and GGT were most predictive above the upper limit of normal [68]. These values were in contrast to the very high thresholds used in the COMS study (which required two times the upper limit of normal to be considered "abnormal," thus explaining the reduced sensitivity in that study).

Chest X-Ray

Multiple studies have demonstrated low yield of CXR in identifying metastatic disease, largely due to the high propensity for metastatic disease to the liver (rather than the lungs) [69]. Routine CXR has identified rare instance of cardiac metastasis [70]. The COMS reported the sensitivity of chest X-ray as only 1.8% [31]. Therefore, CXR should be avoided for routine surveillance for metastatic disease.

Computed Tomography

In the United States, CT is the most common imaging modality used for surveillance of uveal melanoma. CT is widely available and is not organ-specific, providing information on all the structures of the regions imaged (Fig. 12.1). Image

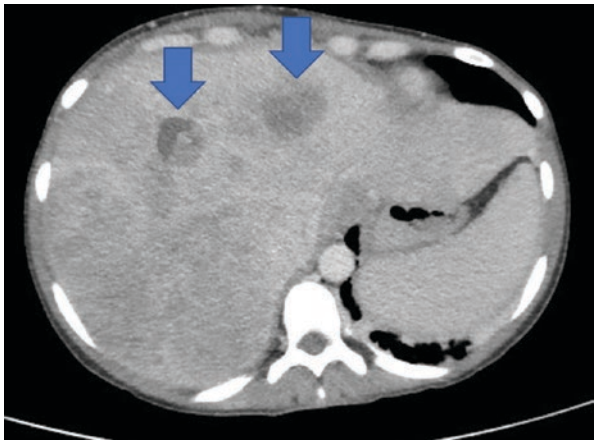

Fig. 12.1 CT imaging showing hepatic, lymph node, and pulmonary metastases of uveal melanoma

Table 12.1 Imaging modalities for surveillance of metastatic development in uveal melanoma

Modality	Advantages	Disadvantages
CXR	Inexpensive Rapid exam time Widely accessible	Low sensitivity and specificity Ionizing radiation exposure (0.1 mSv)
CT	Provides information on all organs of scanned region Preferred modality for lung imaging Rapid exam time	Ionizing radiation exposure (10–26 cGy) Less sensitivity for small liver lesions (<1 cm) Requires contrast for higher sensitivity; limited utility in patients with renal impairment
Ultrasound	No ionizing radiation exposure Good sensitivity for hepatic lesions	Liver-only, will not evaluate extrahepatic metastases
MRI	Excellent hepatic imaging No ionizing radiation exposure	Expensive, Many pateints have contraindications Lengthy exam time
PET	Full-body imaging	Ionizing radiation exposure (25 Gy) Disease may not be FDG-avid Low sensitivity in smaller (<15 mm) lesions Expensive

acquisition is relatively rapid and inexpensive. However, CT requires IV contrast for optimal imaging of the liver, and this may be contraindicated in patients with poor renal function. Even with contrast, it is less sensitive for identifying liver lesions than other modalities (Table 12.1). By the time a liver lesion is visible on CT, up to 90% of patients had multiple hepatic lesions. A 2011 retrospective analysis of 76 patients with biopsy-proven liver metastases from uveal melanoma demonstrated the typical appearance of multiple, hypodense, heterogenous, and enhancing lesions, with a mean dominant lesions size of 46.9cm^2. Among this cohort, 69% of patients had at least one abnormality on LFTs [71].

MRI

MRI is arguably the best imaging modality for evaluation of the liver (Fig. 12.2), but it is costly and time-consuming and may be contraindicated in many patients (e.g., metallic implants, cochlear implants, pacemakers, and claustrophobia). A retrospective analysis of locally controlled choroidal melanoma patients in Japan found that patients undergoing annual surveillance MRI resulted in diagnosis of hepatic metastases in 9% of the cohort. Importantly, only 1 of these patients was symptomatic at the time of imaging, and none had LFT derangements on routine blood draw. Patients were diagnosed 63.0 ± 47.1 (range: 4.0–145.4) months after local treatment [72]. Because of the high sensitivity of MRI for smaller lesions (>1 cm), it

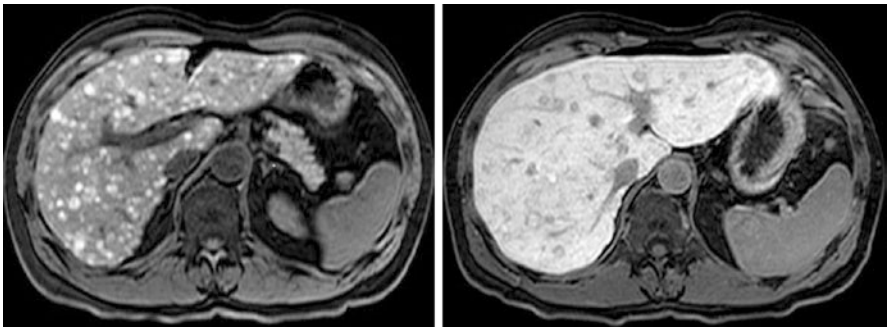

Fig. 12.2 MRI of hepatic metastases of uveal melanoma

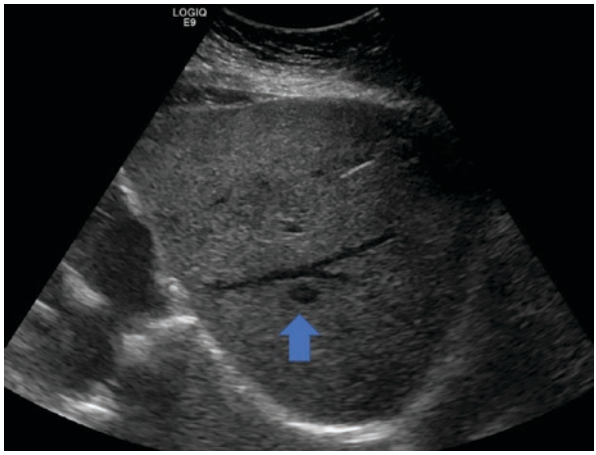

Fig. 12.3 Abdominal ultrasound of hepatic metastases of uveal melanoma

theoretically may allow earlier diagnosis of smaller lesions more amenable to curative surgical intervention, though this has not been proven [73].

Ultrasound

Ultrasound has the distinct advantage of no radiation exposure and comparatively low cost, making it an attractive option for surveillance (Fig. 12.3). However, the dynamic nature of ultrasound image generation does make the quality of imaging performed operator-dependent. A 2016 retrospective analysis evaluated patients with localized uveal melanoma who underwent staging with CT chest/abdomen/pelvis at time of diagnosis and then were followed with liver ultrasound and concurrent LFTs every 6 months for 5 years after diagnosis and annually thereafter. The sensitivity of a combined ultrasound surveillance strategy was found to be 96%, with 88% specificity and a 45% positive predictive value. Only 13% of those with metastatic disease had concurrent LFT abnormalities at the time of diagnosis [74].

In a retrospective study of metastatic uveal melanoma patients, Eskelin et al. found that ultrasound was diagnostic in 87% of patients, and LFT abnormalities were seen in 70% of patients. They therefore calculated that annual screening with abdominal ultrasound and LFTs can identify 59% of metastatic disease prior to onset of symptoms [69].

PET-CT

PET is not a standard component of the initial evaluation in the uveal melanoma primary tumor. Primary (intraocular) uveal melanoma is often not ^{18}F-FDG-PET avid, both because of small tumor size (T1–T2 tumors) and tumor-intrinsic properties [75, 76]. Despite this, PET-CT modality is thought to have some utility in the setting of metastatic surveillance (Fig. 12.4). In a small retrospective study, PET-CT has demonstrated high sensitivity (100%) and specificity (100%) for detection of metastatic disease to the liver [77]. However, even metastatic uveal melanoma is not always FDG-avid; some reports have found false-negative rates as high as 59% [78]. Also worth considering is the lower limit of resolution for PET; extrapolating from other malignancies, the lower limit of resolution on PET is about 10–12 mm for hepatic and pulmonary lesions [79, 80]. PET is generally a good modality for the identification of lymph node, pulmonary, and bony metastatic diseases but less ideal than other modalities for the identification of hepatic metastases due to the increased background uptake of the liver that can obscure smaller FDG-avid lesions [73]. A prospective study of ten patients with biopsy-proven metastatic uveal melanoma

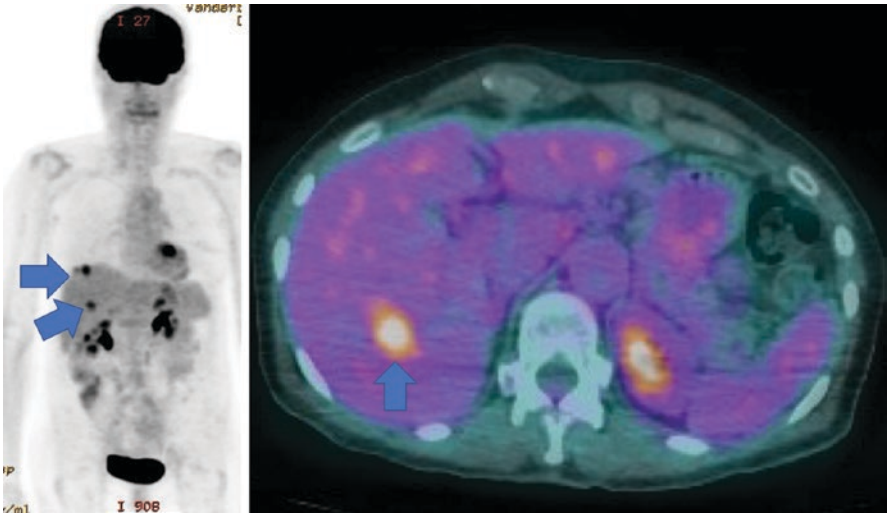

Fig. 12.4 PET-CT of hepatic metastases from uveal melanoma

compared the sensitivity and specificity of FDG-PET and MRI in the same patient, finding that only 4% of hepatic lesions were detected by PET-CT alone, compared to 65% by gadolinium-enhanced MRI alone [81]. Similarly, a European study found that MRI was superior to PET-CT in identifying smaller (more resectable lesions), finding MRI to have sensitivity and positive predictive value of 67% and 95%, respectively, compared to 41% and 100% for PET-CT [82].

An advantage of PET imaging is a theoretical increased likelihood of identification of extrahepatic malignant disease as opposed to non-malignant processes. This is of particular interest in specific practice regions in which endemic infections lead to high frequency of chronic abnormalities, such as the "histoplasmosis belt" of the Southeastern United States. Up to 70% of pulmonary nodules less than 2 cm seen on surveillance CT imaging have been found to be benign, suggesting that PET-CT may improve specificity [83]. However, the relatively low rates of FDG avidity in UM leave these questions unlikely to be answered in this setting.

One study demonstrated efficacy of paired modality imaging. For example, one found that PET-CT and abdominal ultrasound performed at the time of diagnosis were complementary in the staging of melanoma, though interestingly, this approach was three times more likely to identify second primary tumors than metastatic uveal melanoma, reflecting the low underlying incidence of metastatic disease at the time of presentation [84].

Conclusions and Expert Opinion

There is presently no consensus on the optimal surveillance for metastatic disease in uveal melanoma. Most physicians pursue an approach of initial staging imaging and risk stratification of primary tumor and then imaging based on schedule as dictated by the individual patient's risk for metastasis. Imaging modality is chosen based on access and local expertise as well as patient-specific limitations. The majority of physicians use CT for surveillance due to its high accessibility, moderate cost, and good sensitivity, although MRI likely has better sensitivity and specificity for the liver which is the most common metastatic site. In contrast, PET-CT is probably less useful as a single surveillance modality due to its insensitivity for the liver. While the survival benefit of routine surveillance for a tumor that has few good treatment options has not been established, the advent of new local (hepatic) treatments with better outcomes, and the future development of better systemic therapies, will likely tilt the balance in favor of a benefit to surveillance. Future prospective comparative studies are needed to determine the ideal testing strategy. Our suggested algorithm for metastatic surveillance based on gene expression profile risk stratification is presented in Fig. 12.5.

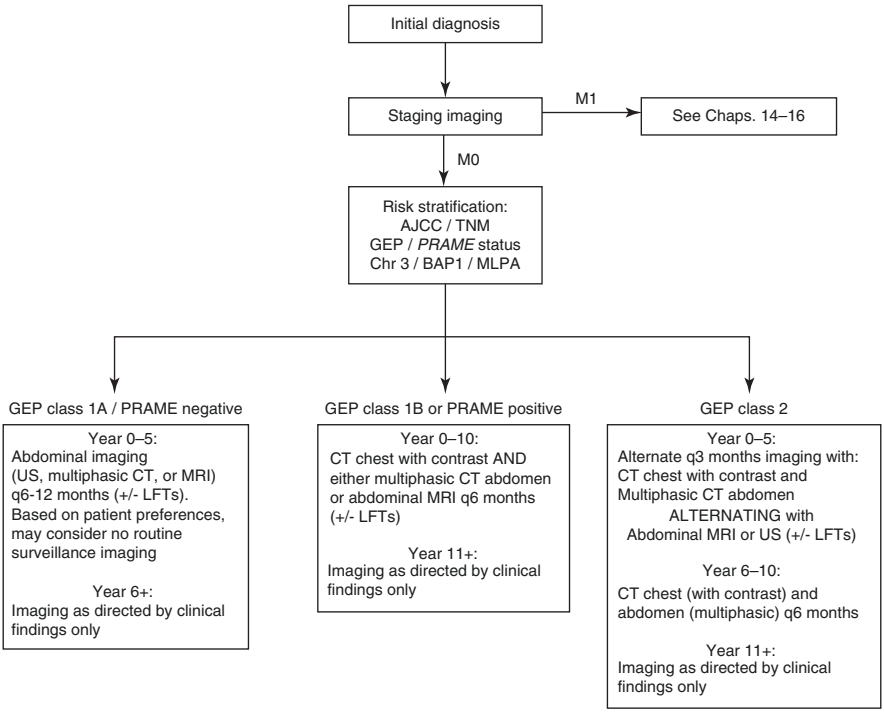

Fig. 12.5 Suggested algorithm/decision tree for surveillance of metastatic disease in uveal melanoma

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Chapter 13
Adjuvant Therapy of Uveal Melanoma

Leonel Hernandez-Aya and Jose Lutzky

Background

Uveal melanoma (UM) is a rare malignancy that arises from melanocytes within the uveal tract of the eye. The overall mean age-adjusted incidence of primary uveal melanoma in the US is 5.2 per million population (95% CI 5.0–5.4). It has not changed significantly between 1973 and 2013 and neither has the 80.9% 5-year relative survival [1]. Long term follow up of patients diagnosed with uveal melanoma suggests that the 15-year survival rate is around 50% [2].Treatment of the primary uveal melanoma with brachytherapy, proton beam radiation, or enucleation is often very effective, with local recurrence rates of less than 5% [3, 4]. In contrast, therapeutic approaches for advanced uveal melanoma have been historically characterized by low response rates and poor outcomes. When compared to its cutaneous counterpart, clinical outcomes in uveal melanoma are invariably poorer. Although UM is often diagnosed at an early stage, 50% of patients will develop metastatic disease primarily to the liver as a first site [5, 6]. A recent meta-analysis of 912 patients treated for metastatic uveal melanoma with various forms of therapy reported a median PFS of 3.3 months (95% CI 2.9–3.6) and a 6-month PFS rate of

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27% (95% CI 24–30). The median OS was 10.2 months (95% CI 9.5–11.0) and the 1-year OS was 43% (95% CI 40–47) [7]. Other large series have yielded similar results [8].

Treatment of Advanced Disease

Patients treated with liver directed treatments had statistically significant longer PFS and OS in the larger meta-analyses of patients with advanced uveal melanoma [8, 9]. A number of different approaches have been employed with varying degrees of local disease control [10–29].

As more research on the biology and molecular makeup of uveal melanoma has emerged, it has become clear that simply mimicking the treatment approaches used in cutaneous melanoma is an inappropriate strategy destined to fail. As such, systemic chemotherapy has been found to be largely ineffective [30]. Uveal melanoma is clearly a distinct entity requiring interventions directed at its tumor-specific biology. Accordingly, recent clinical trials for advanced uveal melanoma have focused on targeted inhibition of pathways important in the proliferative and anti-apoptotic signals for the malignant cells. These have included trials targeting the MAPK, AKT/MTOR, PKC and Hippo pathways but results so far have been less than encouraging [31–40].

The loss of BAP1 expression is associated with an immunosuppressive microenvironment in uveal melanoma [41] which might explain at least in part the inferior outcomes of patients with UM when treated with immunotherapy. A bioinformatics analysis of The Cancer Genome Atlas revealed that UM have a low tumor mutation burden and low rates of T-cell inflammation [42]. A number of phase II trials with the anti-CTLA-4 blocking antibodies ipilimumab and tremelimumab have reported generally low response rates. Outcomes appear to be improved with the combination of ipilimumab and nivolumab [43–46].

Interestingly, there has been a resurgence of interest in the use of adoptive cell therapy in uveal melanoma, triggered by early positive results and ongoing trials are evaluating the role of unmodified as well as engineered tumor infiltrating lymphocytes (TIL) in advanced uveal melanoma [47].

Still in the immunotherapy arena there are signals of significant clinical activity for the engineered T-cell redirector tebentafusp (IMCgp100) with reports of prolonged overall survival in previously treated patients. OS rates at 1 year in the IMCgp100-01 and IMCgp100-102 (phase I arm) studies were 73% and 74%, respectively [48–52].

Other systemic approaches have focused on new targets such inhibition of the newly described FAK/YAP pathway [40] and PARP inhibition in tumors with deficient DNA repair mechanisms such as UM with BAP1 loss [53]. Inhibition of certain histone deacetylases (HDAC) has been found to induce differentiation and a

low-grade phenotype in UM and have been tested in the clinical setting [54–56]. Concomitant treatment with MEK inhibitors or checkpoint inhibitors might be synergistic [57, 58].

Rationale for the Adjuvant Treatment in Patients with High-Risk Uveal Melanoma

The high risk of distant metastasis is suggestive of the presence of subclinical micrometastases at the time of primary tumor treatment [2] as demonstrated by the ability to detect circulating ocular melanoma tumor cells in the peripheral blood of patients who were documented to be clinically free of metastasis, even at the time of diagnosis [59]. Systemic therapy may be more effective treating microscopic rather than clinically detectable disease in the adjuvant setting, before clonal evolution and multiple mechanisms of resistance emerge. In addition, patients are in better performance status before they develop metastatic disease and there is general consensus that performance status is an important predictor of response in multiple oncologic settings. Other facts that support the rationale for adjuvant therapy uveal melanoma include the ability to select for high-risk groups by a variety of validated clinical, pathological and molecular criteria [60–70].

Currently, there are no standard adjuvant therapies to reduce risk of distant metastasis in uveal melanoma. UM has not been included in phase III clinical trials with immunotherapy for melanoma because of the known differences in biological behavior and clinical outcomes. Nevertheless, despite these many obstacles, several adjuvant studies have been conducted in uveal melanoma over the years.

In selecting patients with uveal melanoma for adjuvant clinical trials a number of considerations are necessary. Enlisting experienced investigators and centers that see large numbers of these patients is essential. No less important is the careful definition of the inclusion/exclusion criteria, such as selecting patients at high risk of recurrence, in order to maximize statistical power by enriching the relevant patient population. This could be achieved by a variety of clinical, molecular and hybrid prognostic tools and staging systems [71–81].

Adjuvant Systemic Therapies for Uveal Melanoma

Chemotherapy

The historical use of alkylating agents such as dacarbazine and temozolomide for advanced cutaneous melanoma led to the study of these chemotherapies in uveal melanoma. In metastatic UM, outcomes with chemotherapy have been

disappointing with response rates ranging from 0% to 15%. For instance, a prospective single arm phase II study of the combination of dacarbazine and treosulfan in metastatic UM showed no responses and the median PFS was only 12 weeks [82]. The SUMIT study was a phase III clinical trial that investigated dacarbazine plus the MEK1/2 inhibitor selumetinib versus dacarbazine plus placebo. Objective response rates were only 3% with the combination and 0% with dacarbazine alone [31]. Similarly, temozolomide did not show objective responses in patients with metastastic uveal melanoma [32].

In the adjuvant setting, dacarbazine also failed to demonstrate overall survival benefit in a randomized clinical trial that enrolled 348 patients with choroidal melanomas measuring more than 10 mm in diameter and/or 5 mm in tumor height. Patients were treated with dacarbazine for 6 months versus observation. With a median follow-up of 39 months, dacarbazine did not improve survival compared to observation (5-year survival rates 72% with chemotherapy vs. 68% without chemotherapy) [83].

Adjuvant intra-arterial hepatic infusion of the alkylating agent, fotemustine, was studied to reduce the incidence of liver metastasis [84]. The study enrolled 22 uveal melanoma patients with choroidal involvement, and longest basal dimension more than 20 mm, extrascleral extension or apical height more than 15 mm. The patients were treated with fotemustine for 6 months. Fotemustine was not shown to improve survival compared with matched historical controls (75% vs. 56% respectively; $p = 0.5$). The multicenter, randomized phase III trial (FOTEADJ) with adjuvant intravenous fotemustine for 6 cycles also enrolled patient with high risk of recurrence defined by clinical criteria (diameter >15 mm with extra scleral extension and/or retinal detachment or diameter >18 mm) or genomic high risk signature by array-CGH (monosomy 3 or deletion of 3p associated with gain of chromosome 8). The trial was stopped for futility after 244 patients were enrolled. With a median follow-up of 3 years, the 3-year metastasis free survival (MFS) was 60.3% in the chemo group and 60.7% in the surveillance group (HR 0.97 [0.6.4–1.47]). The 3-year OS was 79.4% [73.2–85.7], with no difference between the 2 groups of patients [85].

Ongoing studies are investigating targeted therapies and immunotherapies in combination with chemotherapy (Table 13.1).

Targeted Therapy

Recent advances and better understanding of the genetics and molecular pathways involved in the pathogenesis of uveal melanoma have led to the investigation of targeted therapeutic strategies in UM. Mutations in *BRAF* are rarely found in uveal melanoma (0–1%). However, approximately 90% of uveal melanomas harbor mutually exclusive activating mutations that involve the GNAQ or GNA11 genes, which encode for G alpha subunits of G-proteins and drive oncogenesis through the mitogen-activated protein kinase (MAPK) and PI3K/AKT pathways [86, 87]. In

Table 13.1 Adjuvant Studies in Uveal Melanoma

Treatment	Mechanism	Result	Type of Study	*n*	Reference
Chemotherapy					
Dacarbazine	Alkylating agent	No difference in 5-year survival rate (71% with dacarbazine vs 68% observation)	Randomized	348	Desjardins et al. 1998
Fotemustine Intravenous	Alkylating agent	No OS difference	Randomized	244	Piperno-Neumann et al. 2017
Dacarbazine and Interferon alfa-2b	Combination therapy	No difference in 5-year OS rate was 66% for treated vs 37% for observed patients ($p = 0.92$)	Phase II	38	Binkley et al. 2019
Targeted Therapy					
Sunitinib or valproic acid	Tyrosine Kinase inhibitor HDAC inhibitor	2-year OS rate 95.6% for sunitinib; 2-year OS rate 90.7% for valproic acid	Phase II	90	Sato et al. 2020 (NCT02068586)
Crizotinib	c-MET inhibitor	No difference in 32 month RFS; Median RFS 30.6 months	Phase II	34	Khan et al. 2020 (NCT02223819)
Immunotherapy					
BCG	Immune modulator	No difference in survival rate (59% with BCG vs. 70% observation; $p = 0.60$	Randomized	113	McLean et al. 1990
Interferon Alfa-2a	Immune modulator	No difference in 5-year survival rate (76% in treated vs 83% historical controls; $p = 0.91$)	Phase II	121	Lane et al. 2009
Interferon Alfa-2b	Immune modulator	No difference in 3-year survival rate (82% in treated vs 90% historic controls; $p = 0.27$)	Phase II	39	Richtig et al. 2006
Dendritic Cell Therapy	Vaccine	Stopped due to poor accrual	Phase II	23	Bol et al. 2016
ipilimumab	Anti CTLA-4	Stopped due to poor accrual	Phase I/II	10	Fountain et al. 2019
Ipilimumab + nivolumab	Anti CTLA-4 + Anti PD-1	Recruiting	Phase II	50	NCT03528408
Liver-directed therapy					
Prophylactic Liver radiation therapy	Radiation	Terminated due to lack of accrual	Phase II	2	NCT02336763
Fotemustine Intra-arterial hepatic	Alkylating agent	No difference in 5-year survival rate (75% in treated vs 56% historical controls; $p = 0.5$)	Phase II	22	Voelter et al. 2008

addition to driver mutations, monosomy of chromosome 3, loss of chromosome 3 heterozygosity [88], and inactivating mutations of the BRCA1-associated protein 1 (BAP1) oncosuppressor gene are highly associated with the metastatic risk of UM [71, 72]. Furthermore, epigenetic events mediated by DNA methylation and histone modification that lead to changes in gene expression promoting differentiation, are also being investigated as potential targets.

c-Kit Inhibitors

The KIT gene encodes c-kit, a transmembrane receptor that has tyrosine kinase activity with growth-promoting properties. Expression of c-Kit has been described in up to 75% or choroidal melanomas with a positive association between c-kit staining and mitotic activity in UM [73]. Blocking c-Kit in vitro leads to tumor cell death [73, 74]. Sunitinib inhibits multiple tyrosine kinases that signal through several pathways, including the MAPK pathway and has activity against c-Kit and vascular endothelial growth factor receptor (VEGFR) with antiproliferative and anti-angiogenic effects. Sunitinib demonstrated activity in a pilot study of 20 patients with c-Kit positive metastatic UM [75]. A partial response was achieved in 1 patient and stable disease in 12 patients. In the adjuvant setting, a retrospective cohort study demonstrated improved OS compared to historical controls. There were 54 patients in the sunitinib cohort and 74 patients in the control group. The 5-year OS rate was higher in the adjuvant sunitinib group (75% vs. 55%, HR 0.53, $p = 0.041$) [76, 77].

The role of sunitinib in the adjuvant setting is under investigation. Sato and colleagues recently presented preliminary data of an ongoing randomized phase II study (NCT02068586) of adjuvant sunitinib or valproic acid in patients with high risk UM defined by (a) M3 + 8q amp; (b) Class 2 [78]. Patients were enrolled within 6 months of initial treatment of primary UM and were randomized to receive either sunitinib 25 mg daily or valproic acid (VPA) 750 mg daily as adjuvant treatment for 6 consecutive months. The primary endpoint was 2-year overall survival (OS) rate compared to historical controls (70%). The study was not powered to compare the efficacy between each arm. A total of 90 patients were enrolled. With a median follow-up of 40.2 months, the 2-year OS rates of the sunitinib and VPA group were 95.6% (90% CI 86.5–98.6) and 90.7% (90% CI 80.1–95.8), respectively. The 18-month RFS rates of the sunitinib and VPA group were 75.6% (90% CI 63.1–84.3) and 62.8% (90% CI 49.4–73.5), respectively. Nine of 45 patients in the sunitinib arm and 4 of 43 patients in the VPA arm could not complete the 6-month treatment due to toxicity (sunitinib $n = 6$, VPA $n = 2$) or systemic progression (sunitinib $n = 3$, VPA $n = 2$). These preliminary results suggest that adjuvant sunitinib and VPA are tolerable treatments for high-risk uveal melanoma. Since the data with sunitinib appeared more promising, a Cohort 2 was created to investigate the efficacy of sunitinib (NCT02068586) [78].

c-MET Inhibitors

c-MET, or hepatocyte growth factor (HGF) receptor, is encoded by the MET gene and normally expressed in cells of epithelial origin. HGF, the ligand for c-MET, is typically produced in mesenchymal cells including hepatic stellate cells [79]. High expression of c-MET is observed in 60–86% of UM cases [80]. c-MET overexpression is associated with greater cell migration capacity and inferior clinical outcomes [81, 89]. Binding of HGF to c-Met triggers a signaling cascade that activates the MAPK, STAT, and PI3K-AKT pathways. In a series of 60 patients with resected UM, higher levels of c-MET expression were found to be associated with a significantly higher risk of death from metastatic disease [90]. Crizotinib is a selective small molecule tyrosine kinase inhibitor (TKI) of c-MET. Preclinical studies showed that downregulation of cMet resulted in decreased cell proliferation and migration [91, 92]. Orthotopic xenograft mouse models transplanted with UM cells treated with crizotinib demonstrated significant suppression of metastatic spread with treatment compared to control mice [91]. Crizotinib was evaluated in the adjuvant setting based on preclinical data suggesting that Met is highly expressed in UM [91].

Results from a multi-center single arm phase II clinical trial of crizotinib in adjuvant uveal melanoma were recently presented [93]. This trial enrolled high-risk UM patients within 90 days of completing primary therapy. High risk was defined by presence of one of the following criteria: (1) primary lesion ≥12 mm in base diameter; (2) class 2 by DecisionDx-UM testing. A total of 34 patients were enrolled and received at least one dose of the 12 four-week cycles of crizotinib (250 mg twice daily). The primary endpoint was distant RFS. With a median duration of follow up of 28.7 months, 15/32 evaluable patients developed distant disease relapse, with 14 relapsing within 32 months. The median RFS was 30.6 months (95% CI: 27.8–58.5%). The study did not meet its primary endpoint of increasing the 32 month RFS from 50 to 75% with crizotinib. Of note, 9/32 (28%) patients required dose modification or discontinuation due to AE which may have limited efficacy [93].

Other TKIs have shown activity in metastatic uveal melanoma including cabozantinib and sorafenib and may be considered for studies in the adjuvant setting [30, 33, 34].

Epigenetic Therapies

Epigenetic processes, including DNA promoter methylation and histone modifications, are important cellular events during tumorigenesis [94]. DNA expression is regulated by histone acetylation and deacetylation. Thus, inhibition of histone deacetylase (HDAC) regulates DNA expression. Due to the relative genetic simplicity of uveal melanoma, epigenetic dysregulation plays a crucial role in UM

pathogenesis [95]. HDAC inhibitors were identified in screening studies of compounds that could shift uveal melanoma cells from the class 2 to the class 1 signature. HDAC inhibitors have been shown to induce differentiation of uveal melanoma cells and dormancy of micrometastatic disease [54].

The high-risk subtype of UM characterized by M3 and BAP1 mutations, has a distinct global DNA methylation state [96]. Several drugs with epigenetic effects have been used in clinical trials for UM [97]. Decitabine, an inhibitor of DNA methylation, was studied in patients with unresectable liver metastases including a few patients with UM ($n = 4$). Decitabine was administered by hepatic arterial infusion every 4 weeks. There were no limiting toxicities. However, during study treatment or post-study exposure to immune checkpoint therapy, no objective tumor responses were observed in the four patients with UM liver metastases [98]. Valproic acid is a potent class I and II HDAC inhibitor and is being investigated in an adjuvant trial in patients with high-risk uveal melanoma (NCT02068586).

Panobinostat has been shown to induce G1 cell-cycle arrest, and a shift to a differentiated melanocytic gene-expression profile in cultured UM cells inhibiting the proliferation of UM cells [54]. Other HDACs such as trichostatin A (TSA), tenovin-6, entinostat, depsipeptide, vorinostat, quisinostat, MC1568, and MC1575 have shown promising results in preclinical UM models [99, 100]. New approaches to restore the epigenetic modifications in high risk UM are emerging to decrease the risk of metastatic disease in UM. Bromodomain and extra-terminal (BET) protein inhibition is being investigated. The BET family of proteins, including BRD2, BRD3, BRD4, and BRDT, are epigenetic regulators hypothesized to control expression of oncogenic drivers including *MYC*. JQ1, a first generation BET inhibitor, showed potent cytotoxic activity in *GNAQ/11*- mutant cell lines and in a uveal melanoma mouse model [56]. PLX51107 is an oral small molecule BET inhibitor currently being assessed in a multicenter phase Ib dose escalation study in various solid and hematologic malignancies (NCT02683395) including uveal melanoma. Pending results from the studies in the advanced setting, BET inhibition may be considered in the adjuvant setting.

Immunotherapy as Adjuvant Therapy for Uveal Melanoma

Immunotherapy with checkpoint inhibitors has revolutionized the treatment of cutaneous melanoma. However, unlike advanced cutaneous melanoma where immune checkpoint blockade has demonstrated durable clinical benefit and superior overall survival [101–105], systemic checkpoint inhibitor therapies studied in patients with advanced uveal melanoma have produced unsatisfactory results. Potential explanations for the limited efficacy of immunotherapy in uveal melanoma include the major differences of biology between uveal melanoma and its cutaneous counterpart. For instance, the low mutational burden of UM is considered a relevant factor for the limited efficacy of immunotherapy [106]. Among 80 cases of ocular melanoma in the NCI's Genomic Data Commons (GDC) dataset, the median rate of

somatic mutation is 15 mutations per tumor far lower than the median mutational rate in cutaneous melanoma [96]. Also, 80% of ocular melanomas contain mutually exclusive mutations in either GNAQ or GNA11 signaling proteins, which have not been successfully targeted with therapeutics. Nevertheless, some patients treated with immunotherapy achieve a clinical benefit [107]. Accumulating evidence suggest that uveal melanoma cells use distinct mechanisms to elude the immune system [107, 108]. The eye is considered an immune-privileged site with multiple immunosuppressive mechanisms including the blood-ocular barrier and soluble immunosuppressive factors in the aqueous humor such as transforming growth factor (TGF)-B, alpha-melanocyte-stimulating hormone (alpha-MSH), calcitonin gene-related peptide (CGRP), vasoactive intestinal protein (VIP), and indoleamine 2,3 dioxygenase (IDO) [109, 110]. Also, the immune-modulatory microenvironment of the liver, which is the typical site of metastasis for UM, could further protect metastatic UM cells from immune surveillance [111].

The initial clinical trials with immunotherapeutic approaches in the adjuvant setting of UM were reported in the 1990s. Most of these trials had limited sample sizes and did not apply the current stratification factors to define high-risk population. For instance, a small-randomized clinical trial of the immune modulator bacille Calmette-Guerin (BCG) enrolled 113 patients with posterior uveal melanoma. There was no difference in survival between the patients treated with adjuvant BCG and the control group (59% vs 70%, respectively; $p = 0.60$) [112]. A study of low-dose interferon-alfa-2a enrolled 121 patients from 1995 to 1999 and resulted in no meaningful improvement in 5-year survival rates for interferon alfa-2a compared to historical controls (76% vs 83%, respectively; $p = 0.91$) [113]. Richtig et al. investigated interferon alfa-2b in 39 patients and found no difference in the 3-year survival rate of treated (82%) versus historic controls (90%) ($p = 0.27$) [114].

Novel immunotherapeutic approaches are under investigation in uveal melanoma. Dendritic cell vaccines, immune checkpoint inhibitors, and adoptive T-cell therapies are undergoing active investigation in uveal melanoma.

Dendritic Cell Vaccines

Dendritic cells are antigen-presenting cells that activate naïve antigen-specific T cells inducing immunologic antitumor responses. Despite the immunoprivileged microenvironment of the eye, immune cells have been found within uveal melanomas [115–117]. Dendritic cell-based immunotherapy has been studied in cutaneous melanoma. Since cutaneous and uveal melanoma share some tumor antigens such as gp100 and tyrosinase, dendritic cell-based therapies have also been tested in UM as an attempt to enhance the host's antitumor immunity. In a study of 14 patients with advanced UM and HLA-A*02:01 phenotype that were vaccinated with autologous dendritic cells loaded with tumor-associated antigens gp100 and tyrosinase, the vaccine induced de novo immune responses in all patients and tumor-specific CD*+ T cells were detected in 29% of patients [118]. In this trial, the median OS of

the vaccinated patients was 19 months, which exceeded the OS of other systemic therapies. Additionally, the toxicity with dendritic cell vaccination is minimal and compares favorably with other treatments.

In the adjuvant setting, an open-label phase II study enrolled patients with high-risk UM (monosomy 3) and HLA-A02:01 phenotype. Patients received an autologous, monocyte-derived dendritic cells transfected with mRNA encoding the tumor-antigens gp100 and tyrosinase. This study was stopped prematurely due to poor accrual (NCT00929019) [119]. From 2009 to 2016, a total of 23 patients enrolled into this study, 61% (14 patients) developed metastatic disease, of which 52% (12 patients) had died. Dendritic cell vaccinations were well tolerated. Tumor-specific T cells induced by the vaccine were present in 17 patients (74%). Interestingly, patients with tumor-specific T cells had better OS. Median OS was 45 months with a 3-year OS rate of 60% for patients without detectable tumor-specific T cells and 58.0 months and 87% for patients in whom tumor-specific T cells were found ($P = 0.016$) [119]. A phase III multicenter clinical trial of a dendritic cell vaccine is currently ongoing (NCT01983748).

CTLA-4 Inhibition in Uveal Melanoma

Ipilimumab is a fully humanized IgG1 monoclonal antibody (mAb) binding to the anti-cytotoxic T-cell lymphoma-4 antigen (CTLA-4). Ipilimumab is an approved therapy for patients with metastatic cutaneous melanoma (Yervoy® Prescribing Information, 2011) as a single agent and in combination with nivolumab [101, 103, 105]. Ipilimumab has also been studied in uveal melanoma.

In advanced uveal melanoma, the data for CTLA-4 inhibition is limited. Phase II trials with the anti-CTLA-4 blocking antibodies ipilimumab and tremelimumab have reported ORR between 0–7.7% and median OS of 5.2–10.3 months [120]. A multi-institutional retrospective review of 39 patients with advanced uveal melanoma who received ipilimumab at either 3 mg/kg (35 patients) or 10 mg/kg (4 patients) showed an objective response rate of 2.6% (1 responder) at 12 weeks and 23 weeks. Among responders, one had complete response (CR) and one had a late partial response (PR) at 100 weeks after initiating therapy [121]. Another 13 patients who received ipilimumab at 10 mg/kg as part of the ipilimumab ocular melanoma expanded access program (I-OMEAP) study group, demonstrated no objective responses with median survival of 36 weeks [122]. A similar study using tremelimumab at 15 mg/kg every 90 days in 11 patients with advanced uveal melanoma, demonstrated no objective responses with a median progression-free survival (PFS) of 2.9 months, and median overall survival (OS) of 12.8 months [123].

In the adjuvant setting, the data for CTLA-4 inhibition is restricted to a small single-institution phase I/II trial of adjuvant ipilimumab for high-risk uveal

melanoma [124]. This study enrolled patients with definitively treated uveal melanoma within 12 months of enrollment. High-risk of recurrence was defined as (1) Class 2 high-risk molecular gene signature per DecisionDx-UM or (2) monosomy 3 or apical thickness >8 mm on baseline echography. Ten patients with high-risk DecisionDx-UM® Class 2 gene expression profile were enrolled and treated with ipilimumab. The primary objective of this study was distant metastasis-free survival (DMFS) at 36 months from the time of study enrollment. At a median follow-up of 4.5 years, DMFS at 36 months was estimated at 80% (95% CI, 58.7–100). At the time of data cutoff, 4 patients had developed distant metastatic disease with a median time to progression of 3.25 years, and there was one death unrelated to melanoma or treatment. Median DMFS for the total study population was 4.6 years. Median OS was not reached for the total enrolled population. For the patients who developed metastatic disease, median OS was 20.8 months (1.7 years). Despite small sample size and inherent limitations of this study, the distant metastatic rate is lower than expected in high-risk ocular melanoma from historical control [124]. DecisionDx-UM® Class 2, carries a historical DMFS of 50% at 32–36 months. Further validation in subsequent studies will be required to establish a definitive clinical benefit for ipilimumab in the adjuvant setting.

PD-1 Inhibition in Uveal Melanoma

Nivolumab (BMS-936558) and pembrolizumab (MK-3475) are monoclonal antibodies that bind to the PD-1 cell surface membrane receptor, a negative regulatory molecule expressed by activated T and B lymphocytes. Inhibition of the interaction between PD-1 and its ligands, PDL1 and PDL2, promote immune responses and antigen-specific T cell responses to both foreign and self-antigens. PD-1 receptor blockade is an effective approach for immunotherapy of a variety of solid tumors.

In a retrospective review of 56 patients with stage IV uveal melanoma who received an antibody to either PD1 or PD-L1 between 2009 and 2015, the objective response rate (ORR) was 3.6%. Stable disease (SD) more than 6 months was reported in 5 patients (9%) with a median survival of 7.6 months [120]. Normal serum LDH, no previous ipilimumab, and presence of lung metastasis correlated with better response to pembrolizumab in advanced UM, whereas the presence of liver metastasis correlated with lower response to pembrolizumab. These results sharply contrast the clinical activity of anti-PD1/PD-L1 in cutaneous melanoma. Given these results, it is not surprising that recent studies in uveal melanoma have focused on combination therapies rather than single agent anti-PD-1 therapy. Therefore, the role of PD-1 inhibition in the adjuvant setting for uveal melanoma remains unclear.

Anti PD-1 and Anti CTLA-4 Combination Therapy in Uveal Melanoma

Ongoing avenues of research are focusing on the role of combined checkpoint inhibition with both PD-1 inhibitors and CTLA-4 inhibitors. These combinations have shown a synergistic effect on outcome in metastatic cutaneous melanoma. The combination of these immune checkpoint inhibitors was approved for the treatment of unresectable or metastatic melanoma in the USA in January 2016 and in the European Union in May 2016. In advanced or metastatic uveal melanoma, outcomes appear to be improved when the combination of ipilimumab (3 mg/kg) and nivolumab (1 mg/kg) has been used [46, 125]. Information from these studies could inform future patient selection. The activity of this combination in the adjuvant setting has been studied in cutaneous melanoma (CheckMate-915, NCT03068455) and is being explored in high-risk uveal melanoma patients as well (NCT03528408). This is an open-label, multi-site, single-arm phase 2 study of adjuvant nivolumab combined with ipilimumab for the treatment of patients with completely treated high-risk ocular melanoma. The primary endpoint is 3-year relapse-free survival. This study is recruiting and results are eagerly awaited.

Future Directions

Current areas of clinical research in uveal melanoma include novel HDAC inhibitors, BET protein inhibitors and novel immunotherapy combinations. High-risk tumors with class 2 gene expression profile are characterized by biallelic mutational inactivation of the tumor suppressor BAP1, which functions in normal vertebrate development to regulate an epigenetic switch from proliferation to differentiation, in large part by inhibiting HDAC4. New HDAC inhibitors to reverse the phenotypic effects of BAP1 loss are being investigated. For instance, the HDAC inhibitor quisinostat, has a distinctive activity spectrum that includes high potency against HDAC4 [100]. Investigating a new generation of epigenetic compounds with increased potency in BAP1-mutant cancers and combining these agents with other anticancer therapies in clinical trials is a promising strategy in uveal melanoma.

LAG-3 is an immune checkpoint receptor with a biological role in T cell regulation. Analysis of immune-cell infiltrates from human tumors show that a subset of CD4+ and/or CD8+ cells co express LAG-3 and PD-1 and may be associated with decreased T-cell effector function and tumor escape [126]. Preclinical models have provided evidence that dual inhibition of LAG-3 and PD-1 blockade offers synergistic anti-tumor activity [127].

A recent publication has reported that clonally expanded T cells were found to be present in the UM microenvironment that were shown to recognize melanoma-specific antigens [128, 129]. In addition, using single-cell RNA-sequencing (scRNA-seq) of UM samples these studies have demonstrated that the predominant

checkpoint exhaustion marker on CD8+ T cells is lymphocyte activation gene-3 (LAG3), rather than CTLA4 or PD1 [128]. Analysis of immune-cell infiltrates from human tumors show that a subset of CD4+ and/or CD8+ cells co-express LAG-3 and PD-1 and may be associated with decreased T-cell effector function and tumor escape. These findings coupled with the absence of PD-L1 and PD-L2 expression in tumor cells may explain the ineffectiveness of CTLA and PD1 blockade in metastatic UM. Preclinical models have provided evidence that dual inhibition of LAG-3 and PD-1 blockade offers synergistic anti-tumor activity [130–133]. A clinical trial evaluating the combination of PD-1 and LAG-3 blockade in metastatic UM is ongoing [134]. Depending on these results, targeting LAG3 with checkpoint inhibitors in the adjuvant UM setting may become an attractive approach.

Finally, an update on the outcomes of the ongoing tebentafusp T-cell redirector trials is due and encouraging data will likely result in the adjuvant testing of this agent in high risk UM [48].

Conclusions

The risk of recurrence and mortality of uveal melanoma remain high despite highly effective treatment of the primary tumor. Therefore, novel strategies to target micro metastatic disease in the adjuvant setting are urgently needed. Adjuvant trials results have been disappointing mainly because of the lack of active agents but also because of low accrual rates and limited funding to investigate this rare disease. This is an area of unmet need and carefully designed clinical trials are necessary given sample size limitations and the dismal prognosis of patients that develop metastatic disease after treating the primary tumor. Defining the patient population best suited for these trials is of paramount importance to balance risk of recurrence and toxicity of therapeutics in the adjuvant setting. Several novel targeted compounds and immunotherapy combinations are being investigated and results are eagerly awaited. Other immune checkpoint modulators are in development, such as those targeting LAG3 and clinical testing of these is anticipated. Inhibitors of the MAPK, AKT and Hippo pathways as well as new epigenetic modifiers may potentially be investigated in the adjuvant setting in uveal melanoma. Enrolling patients in clinical trials is essential to develop effective adjuvant therapies in uveal melanoma.

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Chapter 14
Liver-Directed Therapies for Hepatic Metastases

Flavio E. Baio, Barrett C. Riddle, Mark A. Sultenfuss, and Nestor F. Esnaola

General Principles

The liver is the most common site of metastases from uveal melanoma, affecting 70–90% of cases, and is the only site of metastases on 50% of cases [1–6]. There is no clear consensus of management of metastatic uveal melanoma.

Among patients with hepatic metastases, therapy directed specifically toward the liver metastases has been associated with responses that may have clinical utility.

Surgical approaches that have been studied include metastasectomy, segmentectomy, and hepatectomy that can be carried out laparoscopically or open.

Oncological surgical outcomes in these studies have been described as R0, R1, or R2 allowing often, although not always, for direct comparisons among studies. R0 resection indicates microscopically margin-negative resection, in which no gross nor microscopic tumor remains in the primary tumor bed. R1 resection indicates the removal of all macroscopic disease, but microscopic margins are positive for tumor. Lastly, R2 indicates gross residual disease with gross residual tumor that was not resected.

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Surgical Management of Liver Metastasis

Resection Versus No Resection

In 2009, Mariani et al. gathered information about 255 patients that underwent surgical resection of patients with metastatic uveal melanoma. The resection was carried out with the aid of intraoperative ultrasound and frozen section if they were not able to obtain preoperative biopsy. Miliary metastases, defined as multiple, diffuse millimeter-size, dark-punctate lesions, during laparotomy, were a contraindication to continue the operation. If R0 resection was not achievable, cannulation of the proper hepatic artery was performed to continue with chemotherapy. The median overall survival following surgical resection was 14 months compared to 8 months in those who had no surgery. In patient where R0 resection was achieved, an overall survival of 27 months was recorded versus 17 months in the R1 group and 11 months in the R2 group. R0 resection seems to confer an overall survival at 2 years of 54.4%; R1 resection, 30.5%; and R2 resection, 14.4% ($p < 0.001$). Additionally, having more than four lesions was a negative predictive factor for overall survival. A diagnostic laparoscopy could stratify patients for possible miliary disease and avoid unnecessary laparotomy [7] (Fig. 14.1).

Frenkel et al. in 2009 analyzed the outcome of 74 patients who developed liver metastasis from uveal melanoma. Thirty-five of them underwent hepatectomy. The group who underwent resection had a median survival time 3.7-fold higher compared to the non-operated cohort. Achieving an R0 resection was noticed again to be a positive prognostic factor: these patients survived 1.9 times longer than those with residual disease (R1/R2) [8] (Figs. 14.2, 14.3, 14.4).

In 2014, Gomez et al. reported a statistically significant difference in patients with metastatic uveal melanoma to the liver treated with surgical resection and

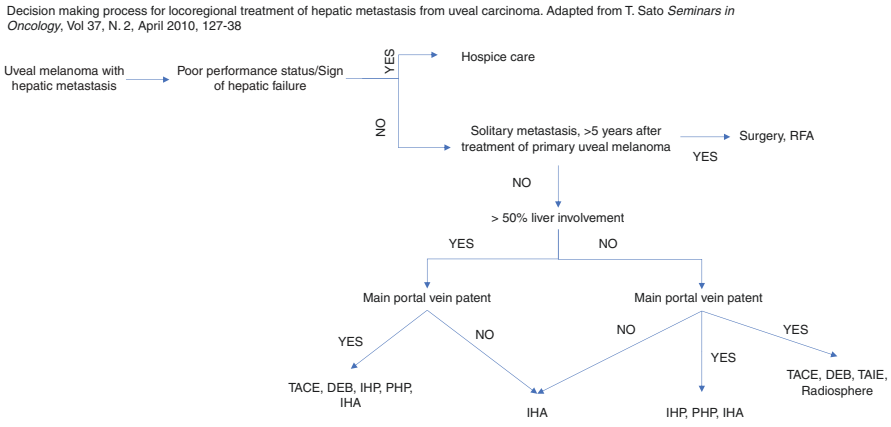

Fig. 14.1 Decision-making process for locoregional treatment of hepatic metastasis from uveal carcinoma (Adapted from Sato [18])

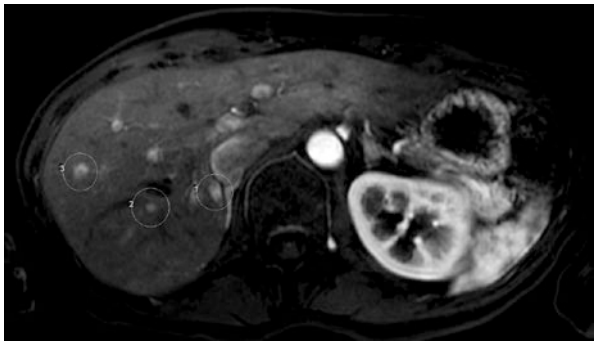

Fig. 14.2 Metastatic liver disease of a patient with uveal melanoma

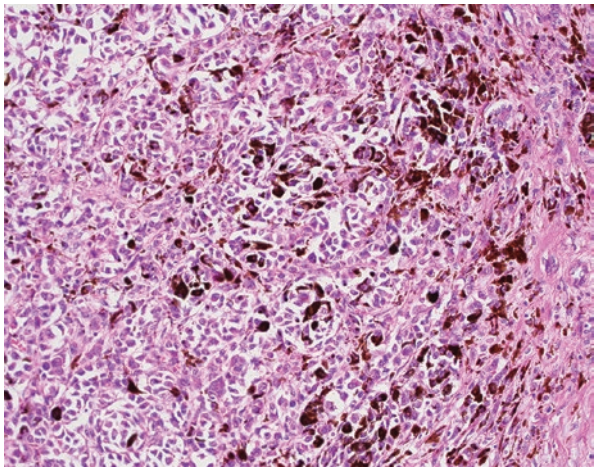

Fig. 14.3 Tumor cells have a high nuclear to cytoplasmic ratio, with minimal eosinophilic cytoplasm. There are some visible nucleoli present (low-power magnification)

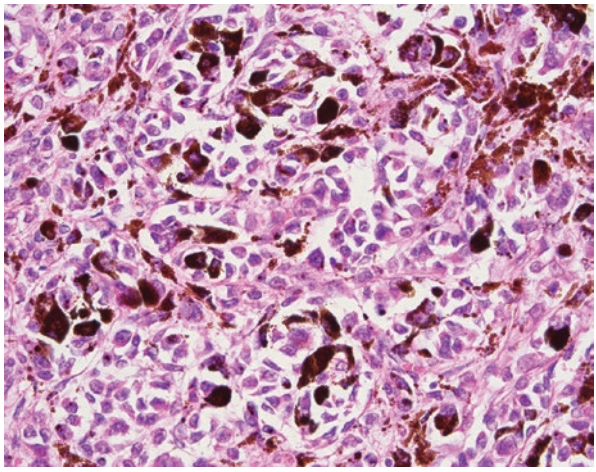

Fig. 14.4 High magnification showing atypical cells with high nuclear to cytoplasmic ratio, arranged in nests. Within the nests, the tumor cells are discohesive. In addition, we can see associated melanophages with melanin pigment

radiofrequency ablation (RFA). In their cohort of patients with metastatic disease ($n = 155$), the subpopulation who underwent curative-intent resection ($n = 17$) had a median overall survival of 27 months compared to the best supportive care of 8 months ($p < 0.001$). Eleven patients underwent metastasectomy or segmental resection [9].

Resection Versus Resection and RFA

Mariani et al. in 2016 compared surgical resection only with radiofrequency ablation and surgical resection. In their cohort of 792 patients, 72 patients developed liver metastases that were resectable on imaging. Fifty-seven patients underwent surgical resection and the remaining 15 underwent radiofrequency ablation followed by surgical resection. All surgical procedures were performed by laparotomy. Both groups had similar pathological features. There was no statistically significant difference in overall survival and disease-free survival in the two groups. In patients undergoing surgical resection only, the overall survival was 27 months. The group that underwent radiofrequency ablation followed by surgery had an overall survival of 28 months [10].

Metastasis in >50% of the Liver

Rivoire et al. in 2005 in a retrospective study analyzed a cohort of 602 patients with UM. Sixty-three of those patients developed liver metastases as first extraocular manifestation. They were all evaluated by a multidisciplinary team; before undergoing surgical resection, a residual liver function (RLF) of 30–40% was calculated via imaging. These patients showed liver lesions not bigger than 30 mm (median 12 mm [5–75 mm]; 75 mm in one patient). Of the 63 patients with metastatic burden, 28 patients underwent surgical resection. Fourteen of them achieved R0 resection, 6 of them a major resection, and the other 8 a minor resection [11]. In patients with more than 50% of the liver involved (Fig. 14.1), surgical resection confers a better prognosis. According to this study, the median survival for patients undergoing R0 resection was 25 months (11–110 months) compared to 16 months (3–35 months) of the R2 group.

Surgery and Hepatic Artery Infusion Versus Systemic Chemotherapy

Salmon et al. in 1998 had the objective to achieve macroscopic curative resection of liver metastasis in patient with UM. Their prospective clinical trial enrolled 75 patients with metastatic uveal melanoma (UM), exhibiting large (>5 cm), intermediate-sized (0.5–5 cm), or miliary liver metastases, with authors reporting combination of the three in some patients. Of all patients, 7 underwent right

hepatectomy (1 of them needed resection of segment VI), 11 patients received a left hepatectomy, 24 patients had various atypical multiple metastasectomy. Patients with a curative-intent resection had a better overall survival than patients who underwent tumor reduction or simple biopsy of the lesions (22 months versus 10 months, $p < 0.001$). In their cohort, the type of adjuvant chemotherapy did not influence the overall survival [12].

Rivoire et al. in the remaining 14 patients that received R2 surgical resection (1 patient underwent major resection and 13 a minor resection) they all received postoperative chemotherapy via an intra-arterial catheter placed during the surgical procedure. The median survival for patients undergoing R0 resection was 25 months (11–110 months) compared to 16 months (3–35 months) of the R2 group. The remaining 35 patients who developed liver metastasis had a median overall survival of 11 months (3–52). This last cohort received chemotherapy or best supportive care [11].

Rietschel et al. in 2005 at Memorial Sloan Kettering Cancer Center reported their 10-years' experience treating metastatic UM patients. The authors examined the chart of 119 patients with metastatic UM. They were able to extract meaningful data for 88 patients; 19 of them underwent surgical resection. Ten of them achieved complete surgical resection (R0). The authors compared the overall survival (OS) of patients who received local treatment (either surgical resection or intrahepatic chemotherapy) versus systemic chemotherapy. The first group had a median OS of 32.4 months versus 9.5 months [1].

Akyuz et al. in 2016 described a laparoscopic approach for the treatment of liver metastases. Of their 44 patients, 16 underwent surgical treatment, 2 of them underwent laparoscopic surgical resection, and 14 patients underwent laparoscopic radiofrequency ablation. Twenty-eight patients received systemic therapy only. The OS for the surgical group was 35 months compared to the 15 months of systemic therapy only ($p < 0.0001$). The 5-year survival was 0% for the systemic therapy and was 22% in the surgical group. In their multivariate analysis, the surgical resection was the only independent predictor of overall survival [13].

Treatment of liver metastases secondary to uveal melanoma remains an area of great debate. The current literature describes R0 surgical resection and radiofrequency ablation in selected patients as a meaningful tool to improve overall survival compared to chemotherapy and/or best supportive treatment.

Overall, when technically feasible, metastatic tumor burden reduction has been associated with significant increase in overall survival.

Nonsurgical Options

Ablative Therapies

Various ablative techniques are available for the treatment of hepatic metastases from uveal melanoma. Radiofrequency ablation (RFA) generates heat via alternating current resulting in coagulation necrosis of surrounding tissues. Microwave ablation is similar to radiofrequency ablation in that it uses heat but achieves tissue

heating faster and with larger ablation zones. Microwave and radiofrequency ablative therapies yield similar results in clinical studies, and the choice between modalities is usually due to operator preference. Cryoablation uses the Joule-Thomson effect to rapidly freeze tissue. The ice-ball formed during cryoablation provides the benefit of direct visualization of the ablation zone during the procedure; however, some studies have shown higher rates of adverse events with cryoablation when compared to heat-based ablative therapies. Irreversible electroporation (IRE) is a predominantly nonthermal ablative technique which causes cell membrane disruption through the creation of holes in the cell membrane. The downside to IRE is that it requires the patient to be under general anesthesia, while the other types of ablative therapies can be performed under conscious sedation [14].

Ethanol ablation has been well studied in hepatocellular carcinoma and is well known for its ability to cause cytoplasmic dehydration, denaturation of cellular proteins, and thrombosis of small vessels. These effects lead to coagulation necrosis similar to that achieved by thermal ablation. However, the distribution of ethanol throughout a liver mass is more uniform when injected into a relatively "soft" tumor within a background of "hard" cirrhosis as opposed to a tumor in normal hepatic parenchyma, which is more oftentimes the case with most metastatic liver lesions [15]. This explains why better outcomes have been seen with ethanol ablation in hepatocellular carcinoma rather than liver metastases.

All previously mentioned ablative techniques are minimally invasive and allow access to tumors that are not surgically resectable due to their location or extent of disease. In regard to hepatic metastasis of uveal melanoma, there is a paucity of literature in all ablative therapies except radiofrequency ablation. Mariani et al. (2009) showed that the median overall survival was greatest when patients were able to have R0 resection which is defined as margin-negative resection with no gross or microscopic tumor left in the primary tumor bed. However, this is usually not feasible since patients generally present with more extensive disease. Mariani et al. in 2016 used radiofrequency ablation in patients with uveal melanoma hepatic metastases either alone or with surgery to increase the number of patients with R0 resection. They found that there was no statistically significant difference in the overall median survival between the group that received surgery alone (27 months) and the group that received radiofrequency ablation with or without surgery (28 months) [10].

Intra-arterial Therapies

In addition to ablative therapies, there are several intra-arterial therapies available and utilized in the treatment of metastatic uveal melanoma. From a physiologic perspective, the liver is a prime candidate for intra-arterial intervention due to its dual blood supply. The liver receives approximately half of its oxygen supply from the portal system and the other half from the hepatic artery. However, both primary and secondary liver tumors receive most of their blood supply from the hepatic artery [16]. This allows for embolization of the tumor blood supply while sparing

adjacent normal liver parenchyma. These techniques also provide the ability to deliver very high dose of a chemotherapeutic agent while limiting systemic toxicity.

One of the most widely utilized intra-arterial liver-directed therapies is transarterial chemoembolization (TACE). This technique involves hepatic artery embolization and infusion of concentrated chemotherapeutic drugs. There are several considerations when selecting a patient for TACE, including patency of the main portal vein, adequate liver function, and the extent of disease which must be limited to or dominant in the liver [17].

There is debate over which chemotherapeutic agents yield the best results, but most studies utilized either fotemustine or 1,3-bis(2-chloro-ethyl)-1-nitrosourea and cisplatin followed by an embolic agent such as ethiodized oil and gelatin sponge or polyvinyl alcohol particles. However, some studies suggest that the embolization process rather than the chemotherapeutic agent provides the most impact on disease [18]. Bedikian et al. (1995) were the first to utilize TACE in the treatment of hepatic metastasis from uveal melanoma. They used cisplatin and noted that the addition of other drugs did not have a significant impact on outcome. The median survival time was 12 months but was 14.5 months among patients who responded to chemoembolization compared with 5 months among nonresponders [19]. As previously mentioned, the extent of liver involvement is an important factor to consider. Gupta et al. (2010) observed that patients with less than or equal to 25% liver involvement had an OS of 14 months, patients with >25–50% involvement had an OS of 5.1 months, patients with >50–75% involvement had an OS of 5.5 months, and patients with >75% involvement had an OS of 2.4 months [20]. Sharma et al. (2008) demonstrated that another important prognostic factor might be related to tumor pattern seen on angiography. They found that patients who had a nodular pattern on angiogram survived significantly longer than patients who had an infiltrative angiographic pattern (621 days versus 114 days) [21]. These studies illustrate the utility of TACE as an effective regional treatment strategy in patients with hepatic metastases from uveal melanoma.

There has been some evidence supporting the use of drug-eluting bead TACE (DEB-TACE), which involves infusion of beads which are preloaded with chemotherapy drugs, which administer the chemotherapy into the tumor over a short period of time. Fiorentini et al. (2009) selected ten patients with liver metastases from uveal melanoma and treated them with DEB-TACE beads preloaded with irinotecan (DEBIRI). They found that all patients had an objective response to DEBIRI, with 3 patients having a reduction of disease burden by 90%, 3 patients having a reduction of 80%, and 4 patients having a reduction between 60% and 70% [22]. These results showed that DEBIRI is a highly effective treatment for liver metastases from uveal melanoma.

Hepatic Artery Immunoembolization

A similar intra-arterial therapy for metastatic uveal melanoma is hepatic artery immunoembolization. The premise of this treatment is to allow for easier tumor antigen detection by stimulating antigen-presenting cells in the liver metastases

with simultaneous tumor embolization. Sato et al. (2001) utilized granulocyte-macrophage colony-stimulating factor emulsified in ethiodized oil instilled in the liver and found that this may induce an inflammatory response in the tumor and that patients may develop a systemic immune response outside the tumor which could prevent against further spread. They treated 13 patients with hepatic metastatic uveal melanoma and found the median survival to be 14.5 months with a 1-year survival rate of 61%. Immune responses were seen in tumors outside the liver in a few patients (abscopal effect) [23].

Radioembolization

Radioembolization is another intra-arterial modality utilized in the treatment of hepatic metastases from uveal melanoma. The basis of this therapy utilizes radioactive microspheres to deliver high amounts of radiation to tumor while minimizing damage to normal hepatic parenchyma. The most common microsphere isotope used is yttrium-90 which is selectively lodged within the vasculature of the tumors where they deliver an average energy of 0.94 MeV, with a maximum range of 1.1 cm. This technique was first reported by Kennedy et al. (2009), who treated 11 ocular melanoma patients with hepatic metastases, and reported a 1-year survival rate of 80%, with no treatment-related deaths or radiation-induced liver disease [24]. Radioembolization with yttrium-90 was further evaluated by Klingenstein et al. (2013) who looked at 13 uveal melanoma patients with hepatic metastases, who showed a median overall survival of 7 months after treatment. They attributed this difference in survival to several factors: the amount of tumor burden, the median duration between diagnosis of liver metastases and treatment was 5 months, and that Radioembolization was utilized as salvage therapy in their study on patients, of which 77% had previously undergone chemotherapy. Taking these factors into account, the median overall survival for their study was 19 months [25]. These studies illustrate the effectiveness of radioembolization as both a treatment and salvage therapy for patients with hepatic metastases from uveal melanoma.

Hepatic Intra-arterial Chemotherapy

Direct delivery of hepatic intra-arterial (HIA) chemotherapy is another technique for treatment of metastatic uveal melanoma. The premise of this therapy is to deliver the maximal strength of chemotherapy intra-arterially to the tumor, limiting systemic toxicity. In the literature, intra-arterial delivery has been delivered through catheters placed in a variety of ways, including surgically and percutaneously [26]. This technique was evaluated against systemic intravenous chemotherapy by Leyvraz et al. in 2014. They randomized 171 patients to receive either hepatic

intra-arterial therapy or systemic intravenous therapy with fotemustine. They found that there was no significant difference in the median overall survival between the two groups (14.6 months for HIA chemotherapy versus 13.8 months for systemic therapy). However, despite this, they did show that the patients who received HIA chemotherapy had a significantly longer progression-free survival, when compared to systemic therapy (4.5 months versus 3.5 months), and a greater response rate in the HIA chemotherapy group (10.5% versus 2.4%) [27]. Agarwala et al. (2004) compared HIA chemotherapy to TACE and found no significant difference in response rate or toxicity between the HIA chemotherapy group and the TACE group [16].

Many different liver-directed therapies are available for hepatic metastases in the setting of uveal melanoma, and there is much debate over which method yields the best results. When possible, surgery with R0 resection yields the best results. However, not all patients are amenable to surgical resection at the time of presentation [10]. This highlights the importance of liver-directed therapies in the treatment algorithm for patients with metastatic uveal melanoma.

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Chapter 15
Systemic Therapy for Metastatic Uveal Melanoma

Eric H. Bernicker

Metastatic uveal melanoma is a lethal tumor with a resistance to therapeutic intervention that has frustrated clinicians for years. While effective therapies exist for the primary tumor in the eye, once distant disease has developed, therapeutic options remain limited [1]. Multiple chemotherapeutic agents have been studied and utilized to little avail. Intrahepatic therapies, detailed in a different chapter in this volume, can often achieve responses that buy time, but ultimately the disease progresses. Cellular therapies such as TILs remain under study and also are discussed in a yet another chapter. The small numbers of patients afflicted with metastatic UM pose a problem in terms of performing trials with adequate power to assess activity of the agent under study. Finally, the twin advances of targeted therapies against specific driver mutations and immunotherapy approaches that have revolutionized oncologic therapeutics over the past decade have largely passed by patients with metastatic UM. This chapter will review the history of attempted treatment interventions and the reasons for the low response rate to various agents and offer some possible glimmer of hope for patients suffering with advanced disease.

Chemotherapy

Patients with metastatic uveal melanoma overwhelmingly present with liver involvement; once metastatic disease develops, patients on average survive for 6–12 months despite treatment [2].

Multiple trials using chemotherapy have been performed in patients with advanced disease; almost all were negative and showed a consistent failure of

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cytotoxic agents to cause meaningful responses. Reviewing all of the negative trials would be time-consuming and ultimately not helpful, so a focus on a few of the negative trials will highlight the decades of frustration with this disease.

Early reports in the 1990s suggested that a combination of multi-agent chemotherapy and the biological agent LPH interferon-2b – called BOLD plus interferon – had activity in patients with metastatic uveal melanoma [3]. Among 20 evaluable patients, 4 had responses for a response rate of 20%. Pulmonary toxicity was seen in three patients from the bleomycin, a well-known side effect that can be clinically serious. Because of the efficacy of this regimen in a heretofore untreatable disease, the EORTC performed a trial to look at efficacy and tolerability [4]. Twenty-four patients with a median age of 60.5 years were treated with the same protocol. There were zero objective responses, 2 patients remained stable and 20 exhibited progressive disease. The lack of any meaningful signal of response, coupled with an intensive schedule of administration and significant toxicity, led to the demise of this regimen for patients with metastatic uveal melanoma.

Temozolomide is an oral alkylating agent analogous to the intravenous drug dacarbazine, often used in cutaneous melanoma and brain tumors such as glioblastoma multiforme. Because of its ease of administration and relatively predictable side effects, it was tested by Bedikian and colleagues in 14 patients with metastatic uveal melanoma as a single agent in a phase II trial [5]. While the medication was well tolerated, of the 14 patients, none responded and only 2 patients had stable disease.

The antiangiogenic agent bevacizumab has been combined with temozolomide, there are some preclinical rationales for a synergistic combination between these agents, and activity has been seen clinically in other tumor types [6]. The combination was studied as a first-line treatment in patients with metastatic uveal melanoma. Temozolomide was given daily for a week every 2 weeks, and bevacizumab was administered bi-monthly to 36 patients with metastatic uveal melanoma [7]. At 6 months, only 8 of 35 (23%) patients had not progressed; however 5 patients had long-lasting stable disease. Gene-expression classification, such as is often acquired at diagnosis to help risk stratify individual patients, was not reported. While non-progression can certainly be a meaningful parameter for patients as long as the therapy is fairly well tolerated, ultimately it is often actual response as demonstrated on imaging in clinical trials that ultimately leads to overall survival benefit in advanced malignancies.

Fotemustine is a nitrosourea alkylating agent used in the treatment of metastatic melanoma in Europe but not approved for use in the United States. Like many agents with some activity in metastatic cutaneous melanoma, it has been tested in patients with metastatic UM. Leyvraz and colleagues developed a trial to randomize patients with metastatic uveal melanoma to either intravenous or hepatic intra-arterial fotemustine [8]. Patients received the drug weekly for the first cycle and then after 5 weeks off, every 3 weeks until progression. After 171 patients were randomized, the trial was halted for futility. After a median follow-up of 1.6 years, 155 patients had died and only 16 were still alive. While there was a better radiographic response rate for the intrahepatic group and better progression-free survival,

the overall survival was not improved, and there were two toxic deaths in the intra-arterial arm.

Because of the early hope that there was a signal of efficacy using this drug, a randomized multicenter study was opened comparing 100 mg/m^2 of fotemustine to surveillance in high-risk patients after initial therapy of the eye. The high-risk classification was determined by either clinical criteria of a tumor diameter greater than 15 mm with extra-scleral extension or retinal detachment or a genomic high-risk signature. The primary objective was an increase in metastases-free survival from 50% to 70%, perhaps in retrospect an overly optimistic goal in an essentially chemo-resistant disease. The study proved that a multicenter study performed in a rare tumor subtype was feasible, enrolling 244 patients in 7 years. However, the study was stopped for futility; after a median follow-up of 3 years, there was essentially no difference in metastases-free survival between the two arms (60.7% in the surveillance arm versus 60.3 in the fotemustine group).

Treosulfan is an alkylating agent that was found to be synergistic with gemcitabine in animal studies. The combination of treosulfan and gemcitabine in metastatic UM was explored in a study performed at multiple centers in Europe [9]. Thirteen of the patients were treatment naïve, and one had previously received chemoimmunotherapy. There was one complete response, three partial responses, and stable disease in eight cases. The median overall survival was 61 weeks although the median PFS was 28 weeks and the survival rate at 1 year was 80%. Schmittel et al. in a second study added cisplatin to this promising combination, and as is often seen as more patients go onto a particular regimen under study, the results were quite different [10]. Seventeen of 19 patients were evaluable for response; there were zero objective responses, 59% progressed, and the median PFS of all patients was 3 months. Finally, treosulfan was combined with dacarbazine, which at least unlike gemcitabine had a track record with being used in the clinic against melanoma [11]. The results here were equally disappointing; of the 14 patients treated, 4 had stable disease and the other 10 progressed; the median survival was 30 weeks. These small exploratory combination trials vividly demonstrate that small numbers of patients on trials coupled with known or unrecognized differences in patient selection can lead to markedly different outcomes in early trials and have plagued many rare tumor types when it comes to identifying active agents or combinations.

Targeted Therapies

Many of the biggest advances in treatments for advanced malignancies center around the dual accomplishments of fast and accurate testing for genomic mutations and then identifying active small molecule therapies. In cancers such as metastatic cutaneous melanoma and adenocarcinoma of the lung, these oral thymidine kinase inhibitors (TKI) have shown remarkable clinical activity coupled with fairly good tolerance and preserved quality of life for patients [12]. It is now

unthinkable to treat patients with lung or melanoma prior to ascertaining the genomic profile, and many patients experience prolonged disease control and response [13, 14].

The genetic landscape of uveal melanomas is detailed in a number of other chapters in this volume, but there are not common driver mutations in UM, such as GNAQ/11, that are currently druggable, as have been seen in a number of other malignancies. While BRAF mutations were not found, some studies found that there was constitutive activation of the ERK pathway and downstream MAPK activation [15]. It was hoped that drugs targeting these downstream pathways would yield responses.

Selumetinib is an orally available small molecule inhibitor of MEK 1/2. In a small phase II study, selumetinib was compared with the oral agent temozolomide (which we have previously seen has virtually no activity in this disease) and doubled the progression-free survival time. Based on that, a randomized phase II multicenter study was launched comparing selumetinib in treatment-naïve patients with metastatic uveal melanoma against either temozolomide or intravenous dacarbazine [16]. The primary endpoint was chosen to be progression-free survival. In 98 patients randomized, the median PFS was 7.3 weeks in the chemo group and 16 weeks in the selumetinib group. No patients randomized to chemotherapy achieved evidence of radiographic response, whereas 49% of the selumetinib achieved at least a 30% regression. The 4-month progression-free survival improved from 8.5% with chemotherapy to 43.1% with selumetinib.

These encouraging results led to the selumetinib in metastatic uveal melanoma (SUMIT) study [17]. This was a phase III, double-blinded trial in which treatment-naïve patients with metastatic UM were randomized (in a 3:1 design) to selumetinib plus dacarbazine versus selumetinib and placebo. As in the earlier trial, the primary endpoint was PFS. No significant improvement in PFS was seen with the study combination.

Other attempts have been made to use different agents to target MEK. Trametinib is an orally available small molecule MEK inhibitor and has become a central agent in the treatment of BRAF-mutated cutaneous melanoma. Falchook et al. included 16 UM patients in a phase 1 study looking at trametinib dosing in advanced disease [18]. Two of the 16 had tumor reduction of 24%, but the median PFS was only 1.8 months. A recent systemic review of six studies looking at various MEK inhibitor trials in advanced UM found little clinical efficacy and suggests that MEK monotherapy is not active in this disease [19].

Other targets, such as MET overexpression or c-kit, have been explored in an attempt to develop personalized approaches to treating advanced UM. Luke et al. treated patients with the oral MET inhibitor cabozantinib (versus temozolomide or dacarbazine) [20]. Crossover to the oral TKI was allowed if patients progressed on the chemotherapy arm. There was no improvement in progression-free survival nor median overall survival, but the experimental arm had an increase in toxicity. Whole exome sequencing was performed; the mutational patterns seen were consistent to previously described landscapes of mutations, and a very low mutational burden was demonstrated.

Imatinib is an oral small molecule inhibitor of c-kit that has shown dramatic activity against GI stromal tumors, a chemo-resistant sarcoma. High expression of KIT (>90% of cells staining positive) is seen in 55% of primary UM and 76% of UM liver metastases [21]. However in a trial looking at imatinib in metastatic UM, there was zero responses. Mutation analysis of the KIT gene did not show mutations in the exons found in GIST – another example that often activity of targeted therapies correlates with gene mutations and not with protein expression.

Immunotherapy

The immunotherapy revolution has made a major impact in the management of metastatic cutaneous melanoma. Single-agent or combination immune checkpoint inhibitor therapy achieves frequent responses to patients with metastatic disease, and the clinical benefits are often very durable. In Keynote 006, patients with advanced cutaneous melanoma were randomized to either pembrolizumab on an every 2- or every 3-week schedule or ipilimumab [22]. An analysis of the 5-year results showed that the median overall survival rate was 32.7 months in the pembrolizumab groups and 15.9 months in the ipilimumab arm. In the CheckMate 067 trial, patients with metastatic cutaneous melanoma were randomized to receive nivolumab with ipilimumab for four cycles followed by nivolumab alone, nivolumab plus placebo, or ipilimumab alone. AT 5-year follow-up, the median overall survival was 60 months (median not reached) in the combination arm, 36.9 months in the nivolumab arm, and 19.9 months in the ipilimumab arm [23]. These studies confirmed significant activity, often durable, in a disease that had previously had minimal benefit of cytotoxic chemotherapy when added to toxic cytokine therapy. Two decades earlier, adding high-dose interleukin-2 and interferon-alpha 2b to a three-drug regimen of cisplatin, vindesine, and dacarbazine had seemed in phase II trials to give meaningful responses, albeit with a high cost of toxicity [24]. However, like many studies in difficult to treat disseminated malignancies, subsequent and analyses and well-designed phase III trials later showed little benefit from the cytokine-chemotherapy combinations over chemotherapy alone [25, 26]. Thus, the excitement for the new generation of immunotherapy approaches to melanoma was understandable and justified.

Unfortunately, the hope that the significant activity of immune checkpoint inhibitors would translate into activity in metastatic uveal melanoma was soon dashed. Kottschade and colleagues presented their findings on ten patients with metastatic uveal melanoma treated with pembrolizumab [27]. The median age of the patients was 65 years and 70% were female. Of the eight evaluable patients, there was one complete response, two partial responses, and one stable disease; four patients had rapid progression of their disease. Similarly, Algazi et al. detailed their experience with patients with metastatic uveal melanoma treated with anti-PD-1 or anti PD-L1 [28]. Fifty-eight patients treated over a 6-year period from nine academic centers received either pembrolizumab (68%), nivolumab (29%), or atezolizumab (4%).

Eighty-six percent of the patients had received some type of prior systemic therapy, including ipilimumab in 63%. The overall response rate was 3.6%, stable disease was noted in five patients (with stable being defined as more than 6 months), and the median PFS was 2.6 months. While well tolerated, the low response was disappointing.

The poor responses of metastatic uveal melanoma to immunotherapeutic approaches perhaps should not be surprising given what is known about the tumor microenvironment and immune infiltrates. It has been noted for a number of years that as tumor mutational burden – defined as an increasing number of non-synonymous mutations – increases, clinical response rates to immunotherapy drugs improves. Snyder et al. showed with whole-exome sequencing in patients treated with CTLA-4 inhibitors that tumor mutational load was associated with the degree of clinical benefit and improved overall survival [29]. Other studies across a variety of tumors also suggested that TMB often correlated with sensitivity to treatment with PD-1/PD-L1 blockade, for example, in non-small cell lung cancer [30, 31]. Uveal melanoma has one of the lowest tumor mutational burden signatures, thus not surprisingly reflected in its poor responsiveness to immune checkpoint therapy [32]. While the TMB story is unlikely to be the sole explanation of the poor responsiveness of these tumors to immunotherapy, it is also reasonable to expect that a median somatic mutation density of 1.1 per megabase in uveal melanoma versus 18 in cutaneous melanoma would have an impact on neoantigen formation and cloaking from the patient's immune system.

The poor responsiveness of metastatic uveal melanoma to single-agent drugs led to studies of combinations of immune checkpoint drugs, such as CTLA-4 and PD-1/PD-L1 inhibitors. Heppt et al. treated 64 patients with ipilimumab and either pembrolizumab or nivolumab [33]. Approximately 22% of the patients had previously had single-agent treatment with either ipilimumab or pembrolizumab; 78% were treatment naïve. The best overall response rate was 15.6% with a complete response rate of 3.1% and partial response of 12.5%. The median duration of response was 22.5 months. As would be expected from combination therapy, the tolerability was different than with single agent: 39.1% of treated patients had either grade III or IV immune-related adverse events. Nevertheless, in a disease state with essentially no viable treatment options, the response rate and disease control rate were encouraging.

Recently, the MD Anderson group detailed their experience with the combination [34]. The patients received an induction phase with ipilimumab 3 mg/kg IV and nivolumab 1 mg/kg every 3 weeks for four doses and then maintenance nivolumab for up to 2 years, as long as the treatment was well tolerated and there was no evidence of disease progression. The primary endpoint of the study was overall response rate (ORR), and the secondary endpoints were median PFS and 1-year OS. Thirty-three patients were evaluable; 43% had received prior lines of therapy. The ORR was 18%. Six patients achieved a response: one had a Cr and five had a PR. Two patients had had prior single-agent immunotherapy with pembrolizumab and progressed; one of those patients responded to the combination. (This effect has also been noted in metastatic renal cell carcinoma [35].) In responders, the median duration of response was 12.1 months, and the median OS was 19.1 months.

However the combination was toxic; grade 3–4 treatment-related events (which are serious) were seen in 40% of patients, and seven patients required systemic steroids to manage immune-related complications.

Work is of course being done to try to identify biomarkers that could predict which patients will be responsive to and which will be resistant to immunotherapy. In the meantime, off of a research protocol, the combination should be considered the frontline systemic therapy for patients fit enough. Close monitoring of patients for immune complications is important.

Possible Future Approaches

It is certainly theoretically possible that the development of a better MEK inhibitor could cause investigators to re-look at a targeted therapy approach to treating advanced uveal melanoma, much in the same way that the development of selpercatinib and larotrectinib completely changed the clinical management of patients whose tumors harbor RET or NTRK translocations [36, 37]. In addition, cellular therapies such as TILs and CAR-Ts might hold promise; those efforts are detailed in a different chapter. Short of that, however, most likely approaches that are a capable of inflaming the tumor micro-environment and turning a cold UM metastases into a "hot" one could potentially have promise.

There is strong emerging data that approaches that target the tumor microenvironment, when given in conjunction with systemic checkpoint inhibitors, can lead to improved response rates. One example of this approach was seen in treating patients with unresectable cutaneous melanoma with either Ipilimumab alone or with talimogene laherparepvec (TVEC) [38]. TVEC is a genetically modified herpes simplex virus type 1 that expresses GM-CSF, an immunostimulatory cytokine. One hundred ninety-eight patients were randomized with either TVEC/ipilimumab or ipilimumab alone. Thirty-nine percent of patients in the combination group responded versus 18% in the ipilimumab alone arm. Importantly, responses were seen away from the injected sites, suggesting that the TVEC's local effects helped stimulate a more robust systemic response at distant sites.

Interleukin 12 (IL-12) is a potent cytokine with many potential actions in the tumor microenvironment that make it an attractive agent to study in combination with immune checkpoint inhibitors, such as reducing regulatory T cells, promoting NK cell activation, and increasing interferon gamma [39, 40]. Algazi et al. looked at administering intratumoral plasmid Il-12 through electroporation along with intravenous pembrolizumab in patients with cutaneous melanomas and "cold" tumors with low amounts of TILs (a CD8 percentage of less than 25% in tumor biopsy specimens) [41]. Twenty-three patients were treated, ten of whom had had prior exposure to anti-PD-1 antibodies and seven had seen anti-CTLA4 antibodies. The ORR was 48%, the median progression-free survival was 5.6 months, and the median overall survival was not reached at a follow-up of 19.6 months. Granted, this patient population was not composed of patients with uveal melanomas, but

these were patients with metastatic cutaneous melanoma who had immune signatures suggestive of low chance of response to IO approaches, many who had already progressed on prior immunotherapy treatments. Perhaps therapies that help inflame the tumor microenvironment coupled with systemic immune checkpoint drugs can build on this data in cold tumors such as uveal melanomas.

Tebentafusp (IMCgp100) is a novel BITE that is in development and showing promise in the treatment of advanced UM. It is a bispecific fusion protein that targets the melanoma-associated antigen gp100 and anti-CD3, which draws T cells to the tumor micro-environment [42]. It was studied in both patients with metastatic UM as well as cutaneous melanoma. The 1-year overall survival rate was 65% in both cohorts and was generally well tolerated. Increases in serum CXCL10, a T cell chemokine, were seen, as well as an increase of cytotoxic T cells in the TME. Confirmatory studies are underway and combination trials with other immune system stimulators.

Conclusion

Uveal melanoma has been the paradigm of both the difficulty of conducting clinical trials in a rare tumor type and a highly resistant tumor. Still, the emerging molecular classifications has at least allowed better identification of patients at higher risk for relapse and thus closer screening – so that patients will be able to be enrolled on trials with hopefully lower tumor burdens. The combination of nivolumab and ipilimumab shows unmistakable albeit low activity, but some patients experience significant benefit. The question now is what future combinations of immunotherapy drugs with either radiation or oncolytic viruses will allow greater numbers of patients to benefit and have more prolonged responses.

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Chapter 16
Cell Therapy for Uveal Melanoma

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Introduction

Recent advances in therapies to target the tumor's immune system escape mechanisms have improved outcomes in patients with cancer. However, responses to these immunotherapies vary depending on the immunobiological profile of different cancers [1]. Current approaches using checkpoint inhibitors in uveal melanoma (UM) have had limited success with responses ranging from 5% to 17% [2, 3]. Another immunotherapeutic approach is adoptive cell therapy (ACT) — a strategy that takes into account the biology of specific tumors and has the potential to improve outcomes in solid malignancies such as UM. ACT involves the infusion of ex vivo expanded or modified tumor-specific cytotoxic cells, usually T lymphocytes, into a patient. These immune cells then trigger an immune response that leads to destruction of malignant cells in the patient [4]. Despite its promise and resounding success against hematological malignancies, to date ACT has had limited efficacy in solid tumors due to the immune-suppressive tumor microenvironment. Thus,

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understanding the tumor microenvironment in solid tumors is vital for ACT to successfully overcome its current limitations.

Adoptive Cell Therapy in Uveal Melanoma

To date, multiple types of ACTs have been developed for solid malignancies like UM, including ex vivo expanded tumor-infiltrating lymphocytes (TILs), T cells with engineered T cell receptors (TCR) for increased specificity to tumor-specific antigens, and T cells modified with chimeric antigen receptors (CAR) [5]. The following paragraphs discuss these three distinct types of T cell therapies.

TILS TILs are lymphocytes that infiltrate tumor tissue, allowing them to target tumor-associated antigens (TAAs) or neoantigens to initiate immune attack against cancer cells. TILs and TCR T cells recognize malignant cells through the interaction of their TCRs and the antigens presented by the major histocompatibility complex (MHC) [4, 6]. The potential of ex vivo expanded TILs for solid tumors was first demonstrated in trials for metastatic melanoma [7]. To develop TILs as ACT, T cells are first harvested from blood or tumor samples and then stimulated with high doses of IL-2 for expansion ex vivo. Once sufficiently expanded, T cells are reinfused into the patient in order to initiate a heightened immune response [5]. Notably, lymphodepletion with chemotherapy (or radiation) is given before infusion to enhance the efficacy of adoptively transferred T cells [8]. Preclinical and clinical studies have shown enhanced expansion and persistence of T cells after lymphodepletion through a variety of mechanisms, including removal of T regulatory cells, increased production of homeostatic cytokines, and depletion of cellular elements that act as cytokine sinks, thus increasing the availability of interleukins for adoptively transferred T cells [8, 9]. However, potential issues regarding use of TILs include immune tolerance due to expression of TAAs on self-tissue, limited efficacy of non-modified TILs due to lack of co-stimulation and cytokine signaling, and challenges with their isolation and expansion [5, 6].

Research using ACT in UM is ongoing; however, only one clinical trial using TILs in metastatic uveal melanoma has been published so far. This phase II study by Chandran et al. procured tissue from metastasectomies to generate autologous TIL cultures [10]. The liver was the most commonly resected metastatic site; 90% had GNAQ or GNA11 mutations and 38% BAP-1 mutations. T cells were expanded ex vivo with high-dose IL-2 (6000 IU/ml). Patients received a non-myeloablative lymphodepleting conditioning chemotherapy consisting of cyclophosphamide (60 mg/kg) daily for 2 days followed by fludarabine (25 mg/m^2) daily for 5 days. A single infusion of TILs was administered a day after completion of this regimen followed by high-dose IL-2 (720,000 IU/kg) every 8 h. Patients were evaluated by CT or MRI for tumor regression monthly for 3 months and every 2–3 months thereafter. Out of 27 eligible patients, 21 were treated with TILs. Seven out of 20 evaluable patients showed tumor regression (35%). Most of these responses (6/7) were

considered to be partial although one patient with numerous liver metastasis achieved a complete response. Two patients with stable disease required an additional TIL infusion for progressive disease. Of note, three patients had been refractory to immune checkpoint blockade before receiving TILs. Toxicities were mostly hematological and thought to be associated with the conditioning regimen; no autoimmune adverse events were seen. Further analysis revealed that the patients who responded had a higher TIL autologous tumor reactivity as determined by flow cytometry and ELISA assays. A greater absolute number of tumor-reactive T cells and higher concentrations of interferon-γ induced by autologous tumor were also associated with a clinical response. Even though this was a highly selected population (good performance status, median age of 54 years old, slow tumor growth rate, and close to normal hepatic reserve), the results of this trial support a potential role for TILs in metastatic UM including patients that are refractory to other therapies such as immune checkpoint blockade.

Engineered TCR T Cells T cells can be engineered to express modified T cell receptors (TCRs) or chimeric antigen receptors (CARs). Both strategies involve the ex vivo modification of the patient's T lymphocytes. One of the key differences between these therapeutic options is that TCR T cells depend on antigen presentation through MHC, while CAR T cells are MHC independent [4].

T cells with antitumor activity through their native receptors can be expanded ex vivo by antigen-specific stimulation of autologous or donor-derived T cells in vitro. Antigen-specific TCR sequences are identified through isolation of T cell clones followed by analysis of TCR chains [11]. Once a TCR is identified, it can be modified to enhance its affinity and specificity and engineered into T cells. Viral transfection is needed to transfer the specific TCR gene to the collected T cells [5, 6, 12]. This approach was first reported by Morgan et al. in patients with metastatic melanoma treated with MART-1-specific TCR T cells in 2006; its use has expanded progressively to other cancers such as neuroblastoma, colorectal cancer, multiple myeloma, esophageal cancer, and synovial cell sarcoma [4, 13]. While no data have yet been reported for clinical trials using engineered TCR T cells in UM, trials are ongoing (Table 16.1).

Preliminary translational studies targeting the preferentially expressed antigen on melanomas (PRAME) in UM have shown promise. PRAME is associated with higher risk of metastasis in UM and has also been identified in acute myeloid leukemia (AML), cutaneous melanoma, synovial sarcoma, neuroblastoma, and myeloma [14]. Isolated PRAME-specific T cells from healthy individuals and patients with cutaneous melanoma have demonstrated the ability to recognize and destroy cells expressing high levels of HLA class I and PRAME, although their avidity was noted to be low overall [15, 16]. However, the presence of specific and highly avid PRAME-specific T cells was seen in a patient with AML post-stem cell transplant from a single HLA-A2-mismatched donor. Cells were isolated after presenting with acute GVHD following a donor lymphocyte infusion. The T cells were highly avid to PRAME-expressing malignant cells including melanomas in the breast, colon, and cervix and renal cell carcinoma cells in vitro. This was also the case with acute

Table 16.1 T cell therapy studies for uveal melanoma

Author/Clinical trials.gov identifier	Year	Treatment	Study type	Number of patients	Cancers other than UM included in trial
Chandran	2017	Tumor-infiltrating lymphocytes	Phase II	21	None
Ongoing clinical trials					
NCT02743611		BPX-701 – HLA-A2 restricted PRAME TCR	Part 1 (Phase I) Part 2 and 3 (Phase II)	Actual enrollment: 28 subjects Active, not recruiting	Acute myeloid leukemia, myelodysplastic syndrome
NCT03635632		C7R-GD2 CAR T cells	Phase I	Actual enrollment: 7 subjects Recruiting	Other GD2 expressing cancers
NCT03068624		SLC45A2-specific cytotoxic T lymphocytes	Phase IB	Actual enrollment: 19 subjects	None

lymphocytic and myeloid leukemia; however T cells required activation with CD40L and CpG [17]. An experimental study using two previously identified high avidity PRAME-specific T cell clones demonstrated effective recognition of UM cell lines. In addition, the authors retrospectively examined 16 samples from UM metastatic sites which showed expression of PRAME and HLA class I in at least 50% of the cases [16]. Additional translational studies have shown effective recognition of neuroblastoma and synovial sarcoma cell lines by specific PRAME TCR T cells [18, 19]. In a xenograft mouse model, PRAME TCR T cells showed the capacity to control tumor growth rate with reduction in tumor volume and resolution in mice treated with T cell infusion [18].

Even though PRAME is scarcely expressed in healthy tissues except for a few sites such as the testis, endometrium, and ovaries [14], TCR T cells may have a low reactivity against proximal tubular epithelial cells and mature dendritic cells which raises concerns regarding nephrotoxicity and dendritic cell depletion [17], particularly with the use of engineered high avidity T cells against PRAME. A current phase I–II study is evaluating the role of PRAME-specific T cells as a potential treatment for UM, AML, and myelodysplastic syndrome; given its expression in other types of cancer, its applicability may be expanded further in the future.

Another antigen targeted in preclinical studies is solute carrier family 45, member 2 (SLC45A2). SLC45A2 gene plays a role in melanogenesis and produces a protein known as membrane-associated transporter protein (MATP) that is present in the melanosomes of human melanocytes and melanoma cells [20]. It is considered a melanocyte differentiation antigen (MDA) and is associated with type IV oculocutaneous albinism when mutated [21]. Along other MDAs it has been evaluated as a potential target for treatment in melanoma. An analysis of 55 melanoma patient-derived cell lines (including cutaneous, uveal, and mucosal) using tandem

mass spectrometry revealed shared HLA class I bound peptides derived from SLC45A2 [22]. Further evaluation with RT-PCR and RNA sequencing found that 72–75% of melanoma tumoral cell lines expressed SLC45A2; notably 100% of uveal melanomas tested expressed this antigen. SLC45A2 was also detected in healthy tissue with the highest expression in the testis and skin, although at a lower proportion in comparison to other MDAs such as MART1. Peptide-specific cytotoxic T lymphocytes for HLA A*0201- and HLA A*2402-restricted SLC45A2 were generated and tested against melanoma cells in vitro and in a xenograft model with evidence of specific killing of HLA-restricted SLC45A2+ cells and tumor growth control, respectively. The antitumor findings of SLC45A2 + T cells was consistent in vitro when tested in UM and mucosal melanoma cell lines. The advantage of this approach is not only the specificity of tumor recognition but also the avoidance of the "off-tumor" effect seen when other MDAs have been targeted. Primary melanocyte destruction can lead to vitiligo, vestibular toxicity, and hearing impairment, among others [23]. A phase IB study using cytotoxic SLC45A2+ T lymphocytes in metastatic UM is ongoing (NCT03068624). The T cells are infused via the hepatic artery, and a CTLA-4 inhibitor will be given to prevent T cell inhibition. The primary objective is safety, but antitumor efficacy and in vivo persistence of T cells will also be evaluated.

CAR T Cells CAR T cells are genetically engineered cells that have a chimeric receptor composed of single-chain variable fragments of antibodies that identify a cell surface antigen and an intracellular signal transduction region composed of the CD3ζ TCR chain that leads to activation of the immune response [4, 6]. Currently, most CARs include additional co-stimulatory endodomains that increase the intensity of the signal cascade for a more effective response [24]. CARs can target protein and nonprotein glycolipid antigens and, given their configuration, are MHC-independent [4, 6]. CAR T cells have proven highly effective for some hematological cancers including relapsed/refractory acute lymphoblastic leukemia and diffuse large B-cell lymphoma, which led to FDA approval for these indications [24, 25]. GD2 and HER2, discussed in more detail below, are two antigens expressed by some UM, thus representing promising targets for existing CAR therapies.

Disialoganglioside GD2 is present in the central nervous system, peripheral neurons, and melanocytes at low levels [26, 27] and has been recognized as a TAA given its high expression in several malignancies including UM, neuroblastoma, breast cancer, sarcoma, glioma, and cutaneous melanoma [28, 29]. Therapies against GD2 have been extensively studied for neuroblastoma and led to the development of anti-GD2 monoclonal antibodies (murine and chimeric) with good clinical effect through activation of the antibody-dependent cell-mediated cytotoxicity and complement-mediated cytotoxicity [29].

Research using GD2 as a target for adoptive cell therapy has been ongoing. Initial studies in CAR T cells presented issues with proliferation during co-incubation with neuroblastoma cells; the addition of co-stimulatory signals with CD28 and 4-1 BB allowed proliferation and effective cytotoxicity in a xenograft model [26, 30]. In recent trials signal modulators have been added to increase

persistence of GD2 CAR T cells. The incorporation of IL-15 within the CAR cassette resulted in increased frequency of central memory/stem cell-like phenotype T cells with reduced expression of PD-1 and LAG3. This translated to superior antitumor efficacy in vitro and in vivo [31]. The addition of a constitutively active cytokine receptor (C7R) triggering the IL-7 signaling cascade also resulted in improved serial killing capability of CAR T cells. There was an increase in proliferation, survival, and antitumor activity in vitro (particularly seen after antigen re-challenge) and in vivo in a xenograft mouse model [32]. These strategies can potentially avoid exogenous administration of TILs that usually have systemic toxicities [31, 32].

Other approaches have shown benefit in the preclinical setting including the addition of bevacizumab in a neuroblastoma xenograft model as well as chemotherapy (doxorubicin) in vitro. Checkpoint inhibitors have been administered in conjunction with CAR T cells to patients with neuroblastoma with no apparent change in expansion, persistence, or antitumor effect [33]; however, its role needs further study considering the small cohort sample (three patients) and the previously reported increased survival and CAR T cell effectiveness in vitro after checkpoint blockade [34].

Variable GD2 expression has been noted in UM cell lines [35]; early preclinical studies postulated changes in GD2 positivity when UM metastasizes and suggested it as a potential target for treatment; however further research into differential GD2 expression has been scant [35, 36]. Despite the preclinical research using GD2 for adoptive cell therapy in neuroblastoma, osteosarcoma, and breast cancer [37–39], no published studies have explored its efficacy in UM. A phase I study using C7R GD2 CAR T cells to determine maximum tolerated dose and antitumor responses in patients with cancers expressing GD2 including UM is ongoing (NCT03635632; Table 16.1).

In addition to GD2, human epidermal growth factor 2 (HER2) is overexpressed in UM. HER2 is a transmembrane glycoprotein and member of the epidermal growth factor receptor (EGFR) family. Extensive research in the breast cancer field led to development of anti-HER2 agents such as trastuzumab [40]. Adoptive cell therapies are currently of interest in anti-HER2-resistant advanced breast cancers with preclinical studies showing effective recognition and tumor cell destruction by CAR T cells [41, 42]. Other solid tumors including sarcomas, glioblastoma multiforme, pancreatic and ovarian cancer, as well as UM also overexpress HER2 [43, 44]; studies using CAR T cells in these cases have also shown promising results with tumor growth control and regression in preclinical models [43–46]. Furthermore, phase I studies using CAR T cells in glioblastoma, sarcomas, and biliary tract and pancreatic cancer have shown a response rate of 20–50%, although the best response in these cases was partial remission [47–49]. Notably, HER2 expression in UM has been reported to be similar to sarcoma; however preclinical data is scarce [50]. Forsberg et al. tested HER2 CAR T cells against cutaneous and UM cell lines and demonstrated antitumor effect in vivo and in vitro. Tumors with high HER2 expression were more sensitive to tumor-killing effect; CAR T cells were also active against malignant cells that did not respond to their autologous TILs. The main limitation noted in the study was that in vivo CAR T cells were only effective

in human transgenic NOD/SCID IL2 receptor gamma knockout mice. These mice are immunosuppressed but significantly express IL-2 which enhances persistence and tumor-killing effect of CAR T cells [50]. Currently, no clinical trial using HER2 CAR T cells in UM is available.

Other Immunotherapeutic Approaches for UM

Aside from ex vivo expanded and modified T cells, alternative cell sources for ACT have been explored. Specifically, antigen-presenting cells such as dendritic cells (DC) represent an alternative therapeutic approach for UM. DCs are normally found in the periphery, where they process antigens and undergo maturation upon encounter with tumor cells. This process leads to production of inflammatory cytokines and chemokines and migration to the lymph node for antigen presentation and activation to the T cells [51].

Clinical trials have used dendritic cells as vaccines in multiple solid tumors including metastatic melanoma, prostate cancer, renal cell carcinoma, and glioblastoma multiforme; at this time there are FDA approvals for three cancer vaccines including intravesical BCG live, sipuleucel-T, and Talimogene laherparepvec (T-VEC) [52, 53]. These vaccines use autologous DCs loaded with tumor antigens that are matured ex vivo which are then administered to the patient with the intention to initiate a protective immune response. Two clinical studies with DCs in UM have been published: one in the metastatic setting (14 patients) and the other one as adjuvant treatment in high-risk UM (monosomy 3). Ten patients (71.4%) with metastasis had stable disease as best response after one cycle of DCs loaded with melanoma antigens gp100 and tyrosinase, but seven progressed before the second cycle. When given as adjuvant, 39% of patients were free of relapse by last follow-up, while 61% of patients developed metastatic disease after DC vaccination. Notably, both groups of patients receiving DC vaccination had improved survival compared to the literature [54–57]. Clinical trials with vaccines to further delineate its benefits in UM are ongoing (NCT00334776, NCT01983748, NCT04335890, NCT00313508).

Potential Limitations of ACT for UM

Despite recent FDA approval of CAR-T cells for hematological malignancies, the FDA has not yet approved an ACT for solid tumors. Clinical trials have shown ACT has limited efficacy against solid tumors, suggesting these types of cancers are not as susceptible to immunotherapies [58]. As mentioned above, preclinical studies have encountered issues with low affinity of tumor-directed TCRs and poor T cell persistence when targeting antigens in solid tumors. Potential explanations for this phenomenon involve inefficient trafficking or homing toward the tumor, T cell

exhaustion due to immunosuppressive factors (IL-10, PDL-1, IDO1, TGF-beta), poor recruitment of immune cells, and difficulties in activation due to poor recognition of the antigen secondary to the immunosuppressive tumor microenvironment (TME) [25, 26, 59].

UM cells modify the tumor microenvironment, promoting immune escape mechanisms that prevent effective immune responses [60]. Studies have shown differences between the immune biology of the eye and the liver, which are two of the most common metastatic sites for UM [61]. The eye is an immune privileged site, through different mechanisms including the blood-ocular barrier and the presence of immunosuppressive factors such as transforming growth factor beta, vasoactive intestinal protein, calcitonin gene-related peptide, and alpha melanocyte-stimulating hormone [60, 61]. The liver can be conditionally immune privileged, albeit less than the eye, although immune cell responses can be both up- or downregulated. Reduction in MHC class I expression, production of proangiogenic factors (interleukin-8), and expression of program death ligand (PDL)-1 may help protect tumor cells once they metastasize to the liver [61].

M2 macrophages in particular are thought to play a key role in the promotion of tumor growth and development of metastases in UM [56]. M2 macrophages are characterized by an anti-inflammatory profile, promoting release of anti-inflammatory mediators, phagocytosis of apoptotic cells, collagen deposition, and angiogenesis [57]. TILs are also part of the immune microenvironment in metastatic UM tumors. CD8+ TILs are mostly localized in the peri-tumoral region between tumor and normal liver interface (few are noted within the metastatic lesions), while CD4+ TILs are found predominantly in the perivascular area within the metastasis [61, 62]. Even though TILs and natural killer (NK) cells can lyse cancer cells, their overall activity is downregulated in UM tumors, likely due to exhaustion or anergy induced by the TME [60, 63]. Multiple immune escape mechanisms have been reported in hepatic UM tumors, including production of interleukin (IL)-2 and interferon-γ and induction of indoleamine 2,3-dioxygenase (IDO), which depletes tryptophan to inhibit T and NK cell activity [56, 61].

ACT has the capacity to overcome some of these barriers, through increased specificity to tumor antigens by cell receptor modification, ex vivo expansion using IL-2, and genetic addition of cytokine modulators and costimulatory molecules to enhance proliferation and persistence after infusion, among others [4, 6, 11]. Ex vivo expansion of T cells in favorable cell culture conditions can reverse TME-mediated suppression of their effector functions by the UM TME.

The use of checkpoint inhibitors to further modify the immune microenvironment is also being explored in ACT. As previously stated, T cells can express immune-inhibitory molecules such as CTLA4 and PD1; both function as immune checkpoints that decrease T cell response when the receptors are activated [58]. These molecules maintain tolerance but can adversely affect tumor recognition and surveillance when upregulated [59]. The checkpoint inhibitors allow blockade of CTLA-4, PD-1 receptors, or PD-1 ligands, thereby activating the immune system and leading to increased recognition of malignant cells by T cells [1]. Preclinical

studies have shown that using checkpoint inhibitors enhances T cell activity in vitro. Results have been conflicting in other small clinical studies; their role in ACT needs to be studied further [4, 33, 34].

Concerns about "on-target, off-tumor" effects have limited the number of UM antigens suitable for targeting via ACT. Identifying an antigen that is mostly present in tumor cells but has very limited expression in healthy tissues is crucial; however, off-tumor toxicities can still be seen even in cases of very limited antigen expression in normal cells. Autoimmunity due to recognition of self-antigens in the setting of high-affinity TCR T cells and graft vs host disease given a mismatch in the exogenous and endogenous TCR chains also occurs [4, 64]. Preemptive strategies include introduction of a suicide gene (e.g., caspase-9), increasing specificity for tumor (using dual CAR T cells), or elimination of endogenous TCRs [6, 65, 66].

Cytokine release syndrome is a potentially serious adverse event and frequently complicates CD19 directed CAR T cell therapy. It is not as commonly encountered in CAR T cell therapies against solid tumor. Cytokine release syndrome is triggered by the cytokine cascade (IL-6, IL-10, IFN-gamma, TNF-alpha, etc.) generated by the immune system activation. Symptoms include fever, hypotension, shortness of breath, and organ failure. Severity can vary and it can be correlated with tumor burden, prior comorbidities, and onset of CRS symptoms. CRS can be treated by employing antibodies against IL-6Ra, tocilizumab, which is FDA approved as well as corticosteroids [67].

Despite the encouraging results of these novel cell therapies, significant financial and logistical challenges remain. Considering the average cost of the currently FDA-approved products of close to $500,000, it is difficult to afford for most patients [68]. Hopefully, improvements in cell manufacture and the potential use of allogeneic T cell products generated from healthy donors will help reduce the cost of these therapies.

Summary

The use of adoptive T cell therapy is emerging as a promising treatment for patients with advanced metastatic UM. While data from clinical ACT trials remain lacking for UM, research has identified specific targets (PRAME, GD2, SLC45A2, HER2). Preclinical studies using TCR-T and CAR-T cells have shown some promise with ex vivo killing of malignant cells and successful control of tumor growth in xenograft models. Indeed, engineered T cells, including CAR T cells, are undergoing evaluation in phase I–II trials in UM. Further modification of the TME using checkpoint inhibitors or other combinatorial strategies may improve the efficacy of T cell therapies for solid tumors like UM. Other strategies such as modification of the T cell product itself (e.g., with signal modulators) that can help overcome the immunosuppressive barriers are also under study.

Overall, ACT is an exciting and evolving potential treatment option for UM. Research is ongoing to identify ways to improve responses, identify neoantigens for ACT, and overcome the immunosuppressive effect of the TME. Results from these ongoing studies will elucidate the feasibility of this approach in UM.

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