Genomic, analytical, and sequencing technologies are a critical step toward use of targeted therapies for select patients with high-risk uveal melanoma.

Assessing Prognosis in Uveal Melanoma
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Background: Because uveal melanoma is the most common primary malignant intraocular tumor in adults and carries a significant risk of metastases, which are mostly unresponsive to available systemic therapy, researchers have been searching for prognostic indicators to identify patients at increased risk for developing such metastasis.

Methods: The purpose of this study is to describe recent advances in prognostic testing of patients with uveal melanoma and the impact of these advances on the management of uveal melanoma. The relevant, peer-reviewed literature as extracted and then further reviewed for scientific content.

Results: Demographic characteristics, clinical, and histopathological features alone are inadequate for predicting metastatic risk in individual patients with uveal melanoma. Some research has shown that cytogenetic abnormalities and principally transcriptomic features of tumor cells can independently predict high risk for uveal melanoma metastatic spread. Gene expression profiling of uveal melanoma cells may be accurate and biologically informative for molecular prognostication. Methods for detecting chromosomal gains and losses have predictive value but require additional clinical and cytological information. The latest step in the evolution of molecular testing has been the discovery of major driver mutations for possible use in targeted therapy.

Conclusions: Assay validation, quality control, and interpretation of results are essential for the reliability and reproducibility of these tests. Although these prognostic tests have improved the ability to identify patients at increased risk for developing metastasis, their use has not changed the management of uveal melanoma. However, genomic, analytical, and sequencing technologies will provide a critical step toward useful targeted therapies for patients with high-risk uveal melanoma.

Introduction
Uveal melanoma is the most common primary, malignant intraocular tumor in adults and carries a significant risk for metastasis that is typically unresponsive to available systemic therapy, so researchers have been searching for prognostic indicators to identify patients with an increased risk for developing such metastasis.1,2 Previously identified factors indicative of poor prognosis include older age at presentation, male sex, large tumor size (basal diameter and thickness), scleral invasion and/or extraocular extension, presence of ocular or oculodermal melanocytosis, ciliary body involvement, epithelioid cells, high values of the mean diameter of the 10 largest nucleoli, higher microvascular density, vascular mimicry patterns, tumor-infil-
trating lymphocytes and macrophages, high mitotic activity, high expression of insulin-like growth factor 1 receptor, and high expression of class 1/2 HLA.\(^{2,9}\) However, none of these factors are accurate enough to be used across the entire spectrum of uveal melanoma.

The challenge clinicians and researchers face is the ability to accurately identify high-risk patients so as to further detect the (micro) metastasizing cells at an early phase — possibly a prerequisite for proper patient selection in future therapeutic interventions.

**Methods**

The purpose of this study is to describe the recent advances in prognostic testing of patients with uveal melanoma and how these advances are being applied in clinical practice. A search of the peer-reviewed literature was performed, and relevant articles were identified and carefully reviewed for scientific content and relevance. The author critically reviewed these publications and summarized the most up-to-date information related to prognostic testing for uveal melanoma.

Several reports have been published about the clinical and histological factors associated with the development of metastasis in patients with uveal melanoma, and peer-reviewed publications discussing the prognosis of patients with uveal melanoma date as early as 1948.\(^{10}\) This review article will focus on publications since 1990, for which recent advances in prognostic testing of patients with uveal melanoma are reported when the implications of a gain or loss of specific chromosomes in these tumors were first realized.

**Results**

After relying for several decades on demographical, clinical, and histopathological features to provide a prognostic estimate for uveal melanoma, and after realizing our limited rate of accuracy for predicting outcomes in individual patients, the research focus shifted toward chromosomal and cytogenetic indicators of metastatic risk in uveal melanoma, following the trend of other cancers.\(^{11,12}\)

Early studies of karyotype analysis used fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH). Through karyotype analysis, Prescher et al\(^{13}\) discovered that monosomy 3 and increased copies of chromosome 8q were commonly found in uveal melanoma. Thus, the association between uveal melanoma with monosomy 3 and chromosome 8q gain and the development of metastasis has been known for a long time.\(^{14}\)

The loss of 1 chromosome 3 was also proven to be part of a 2-step mutation mechanism for the inactivation of \textit{BAP1}, a tumor suppressor gene.\(^{15}\) Concomitant loss of chromosomes 1p and 3 has a stronger correlation with metastasis.\(^{16}\) The association with chromosome 8q gain was also shown to be less significant than for monosomy of chromosome 3 and than either one of them separately.\(^{17}\) Gain of chromosome 8 or acquisition of an isochromosome 8q may be a later event in the setting of uveal melanoma and is seen in both low- and high-risk uveal melanoma.\(^{17}\) Gain of isochromosome 8q is more frequently associated with metastasis when it is accompanied by chromosome 8p loss (so-called isochromosome 8q).\(^{18}\) This frequently occurs in tumors that have lost a copy of chromosome 3, and it is considered an independent prognostic factor of progressive disease.\(^{17}\) Chromosome 6p abnormality was predictive of a more favorable outcome.\(^{18}\) Uveal melanomas with gain of chromosome 6p may represent a separate group of tumors with an alternative genetic pathway in carcinogenesis, because gain of chromosome 6p is frequently found in tumors with disomy 3.\(^{19,20}\) Other chromosomal abnormalities have also been detected in uveal melanoma; however, they often lead to contradictory results regarding the prognostic impact.\(^{21}\)

Although FISH has been used to detect these abnormal chromosomal gains and losses, it is a low-resolution technique associated with many false-negative and false-positive results, so its prognostic utility has been limited.\(^{22,23}\) Schouten et al\(^{24}\) described the multiplex ligation–dependent probe amplification (MLPA) that can be used for detecting the relative quantities of as many as 40 different DNA sequences. Array CGH and MLPA have higher rates of accuracy than FISH but are limited by intratumoral genetic heterogeneity and an inability to detect isodisomy 3 (tumor cells are functionally monosomy 3 but have duplicated the diseased copy of the chromosome).\(^{25}\) Molecular classification based on gene-expression profiling (GEP) of posterior uveal melanoma has also been shown to be superior to FISH and array CGH for detecting monosomy 3 and other chromosomal abnormalities, as well as the clinicopathological prognostic factors for predicting metastasis in uveal melanoma.\(^{25,26}\)

**Multiplex Ligation–Dependent Probe Amplification**

MLPA is an assay that analyzes the gain and loss of chromosomal material.\(^{24}\) Through the reaction, denatured genomic DNA is mixed with probes for the specific target genes of interest. MLPA is sensitive and sequence specific for detecting changes in DNA copy numbers, detecting deletions and amplifications of single exons. MLPA can be performed on fresh, snap-frozen, formalin-fixed, paraffin-embedded tissue samples, although fresh and snap-frozen samples have been reported to yield more reliable results.\(^{27}\)

Use of MLPA for posterior uveal melanoma tissue was described by a group of researchers led by Damato et al\(^{3}\) and Coupland et al.\(^{21}\) Their initial re-
search analyzed uveal melanoma tissue samples from 75 patients.\textsuperscript{3,21} MLPA can detect chromosomal abnormalities that correlated with metastatic death, most importantly loss of chromosome 3 and gain of chromosomal material on 8q.\textsuperscript{28} A larger subsequent study showed that losses of chromosomes 1p and 3 and gain of chromosome 8q correlated with increased rates of mortality, whereas gain in chromosome 6p correlated with improved rates of survival.\textsuperscript{29} MLPA also provided prognostic information related to chromosomal aberrations in patients with uveal melanomas, and the authors reported that their results correlated with the clinicopathological features of the tumors.\textsuperscript{29} However, although the results of MLPA provided accurate prognoses in this study, the modality did not offer discriminatory stratification of cases as robust as that seen with GEP testing.\textsuperscript{29} In addition, the estimation of metastatic risk using MLPA requires the clinician to order the test, carefully interpret its complex report, and add clinicopathological features to improve its rate of accuracy.\textsuperscript{30} The intratumor genetic heterogeneity of posterior uveal melanoma has also been studied with MLPA using formalin-fixed, paraffin-embedded tumor tissues.\textsuperscript{31} Researchers found that 25% of the studied tumors were homogeneous; consequently, they found that heterogeneity causes equivocal results on MLPA and may impair the rate of accuracy of their results.\textsuperscript{31}

In retrospective studies, MLPA results reportedly correlate well with the development of metastasis and the survival rate of patients with posterior uveal melanoma; however, the test has not been prospectively validated in a multicenter clinical trial.\textsuperscript{4,28,30} This test is now commercially available.

**Gene Expression Profile**

GEP can be used for the rapid detection of the up-regulation or down-regulation of select genes in a tissue sample.\textsuperscript{26} The technique involves isolating RNA from a tissue sample followed by its conversion to complementary DNA, whose targets are subsequently hybridized to gene chips, and microarray analysis is performed. Onken et al\textsuperscript{26} demonstrated that uveal melanomas could be divided into 2 distinct prognostic classes that predict a person's death from metastasis. The authors initially identified 62 genes that showed distinct aberrant expression patterns in the studied samples.\textsuperscript{26} When the authors combined their findings with the clinical outcome of patients, the up-regulation or down-regulation of specific gene clusters identified by the GEP assay enabled this stratification scheme to predict metastatic risk.\textsuperscript{29} Class 1 tumors generally have the clinical and pathological features known to be associated with decreased metastatic risk, such as the presence of spindle cells, whereas class 2 tumors generally have more aggressive clinical and pathological features such as epithelioid cells.\textsuperscript{26}

Subsequently, a multicenter prospective study was conducted to develop and later validate a 15-gene polymerase chain reaction–based assay that could discern between class 1 and class 2 tumors.\textsuperscript{32,33} This multicenter validation ensured that this test would yield equally reliable results in different settings and that the results would strongly correlate with rates of survival and metastasis development.\textsuperscript{33} Since then, this test has become commercially available for routine use in clinical practice and has been adopted by several national and international centers.\textsuperscript{34}

The assay examines the expression patterns of 12 class-discriminating genes identified by the previous analysis and 3 control genes shown to be unchanged in uveal melanomas.\textsuperscript{20} When compared with monosomy 3 and the clinicopathological features of the tumor, GEP demonstrated superior rates of accuracy at predicting the risk of metastatic disease in patients with uveal melanoma.\textsuperscript{25} Since the development of the assay, class 1 tumors have been further subdivided in classes 1A and B.\textsuperscript{34} The 5-year rates of metastatic risk have been estimated to be approximately 2% for class 1A tumors, 21% for class 1B tumors, and 72% for class 2 tumors.\textsuperscript{35} CGH, FISH, and MLPA provide static measurements of structural chromosomal changes, whereas GEP detects a dynamic RNA signature of the tumor microenvironment.\textsuperscript{16} This fact may explain why this test has been shown to be the most robust prognostic test for uveal melanoma available.\textsuperscript{32-34,36-38}

Although subdividing class 1 tumors has been helpful in stratifying the very-low-risk patients (class 1A tumors) from the medium-risk patients (class 1B tumors), this system lacks discrimination compared with the prior system of 2 classes.\textsuperscript{39} \textit{PRAME} was recently identified as the most significant predictor of metastasis in patients with class 1 tumors.\textsuperscript{40} Incorporating this biomarker should further improve the accuracy rate of the GEP test.

However, several points must be considered regarding the GEP test, as well as any others looking at genetic and transcriptomic features of tumors. Most importantly, any tissue sample can be tested and will originate a result (even if with a low-confidence rate). Some authors have misinterpreted the purpose of GEP, erroneously employing it in nonmelanoma specimens.\textsuperscript{41,42} It is important to emphasize that MLPA and GEP are prognostic in nature and not diagnostic. Although some have claimed that use of \textit{GNAQ/GNAI11} mutations ensures that a tumor is uveal melanoma, at least 15% of uveal melanomas do not have this mutation.\textsuperscript{43} Rather, the proper diagnostic approach of any tumor (including uveal melanoma) is by histopathology or cytopathology.\textsuperscript{56}

Another important fact is that the GEP assay can be performed in paraffin-fixed tissue, but tumor-cell procurement for this test is best obtained by fine nee-
dle aspiration biopsy at the time of plaque implanta-
tion or immediately following enucleation of the eye
containing the tumor.35 The tumor aspirate is flushed
in an RNA-stabilizing buffer solution and then frozen
until the test is performed.35

An advantage of this test is that it requires a mini-
mal amount of cells to generate a result. A single-cen-
ter study of 159 tumoral samples obtained via biopsy
showed that the obtained tumor aspirate was insuffi-
cient for GEP in a single case (0.6%) compared with
34 cases (21.9%) that had an insufficient aspirate for cy-
tology diagnosis.36 In cases of very small uveal mel-
noma, a heterogeneous genetic make-up may limit this
result (and likely any other test result) in samples yield-
ed by needle aspiration in select patients.31,44

A study performed during the early development
of the current 15-gene test — before it was commerci-
cially available — showed discordance of GEP clas-
sification in a few cases of very small uveal melano-
mas sampled at 2 distinct sites.44 Overall, discordant
results were seen in 7.5% of the tested cases compared
with up to 75% of tested cases for which MLPA find-
ings showed tumor heterogeneity.31 That early study of
GEP concluded that the tumor samples obtained
via biopsy from a single site have a small likelihood of
prognostic misclassification (and more likely in thinner
tumors < 3.5 mm in thickness); therefore, the
authors recommended taking this information into
account when advising patients with smaller tumors
about their prognosis.44

Newer genomic sequencing technologies have also
allowed us a more rapid understanding of the molec-
ular landscape of posterior uveal melanoma.45 In ad-
dition, the GEP assay (and other prognostic tests) for
uveal melanoma have provided opportunities for early
detection in patients at high risk of metastasis, thus sig-
ificantly changing how patients with uveal melanoma
are clinically monitored.35

Mutational Profiling

Although it is not as useful in the prognostication of
patients, detection of specific mutations in uveal mel-
noma has the potential to improve therapeutic options
for metastatic disease in the future. Through a search
for mutations in the oncogenic mitogen-activated pro-
tein kinase (MAPK)/extracellular signal-regulated ki-
nase pathway (ERK), Onken et al46 first identified the
GNAQ mutation in approximately 50% of uveal melano-
ma samples. A mutation in GNAQ was detected in
samples of posterior uveal melanoma at all of the
stages of malignant progression, indicating that such a
mutation may play a role in the initial development of
the tumor.46 Mutations in GNA11 were also sufficient
to induce metastases in a mouse model, so research-
ers concluded that mutations in GNAQ and GNA11 af-
fect a critical oncogenic signaling cascade to affect the
metastatic potential of tumors.43 However, this find-
ing has been questioned because GNAQ and GNA11
mutations in humans result in a benign nevus unless
accompanied by further genetic events such as BAP1
mutation.15 GNA11 mutations were initially thought to
be more aggressive than GNAQ due to their incidence
being slightly higher in metastatic tumors.43 We now
know this is because GNA11 mutations are slightly
more common in ciliary body melanomas, which have
a higher metastatic rate.43 Thus, it is more likely that
GNA11 is not any more aggressive than GNAQ.

Mutations in GNAQ and GNA11 are mutually exclu-
sive and represent early or initiating events that consti-
tutively activate the MAPK pathway.45 Although these
mutations are sensitive to MAPK, protein kinase C, and
Akt inhibitors, GNAQ and GNA11 remain difficult ther-
apic targets.47 Researchers have demonstrated that
the oncogenic activity of mutant GNAQ/GNA11 is me-
diated at least in part through YAP, which could have
therapeutic potential.47,48

Furthermore, mutations in BAP1, SF3B1, and an-
other driver mutation, EIF1AX, may be largely mutu-
ally exclusive, and they occur later in tumor progress-
ion.49 BAP1 mutations have been reported to be
strongly associated with metastasis, class 2 GEP, and
older age.50,51 Using multiple regression analysis, BAP1
mutations associated with EIF1AX mutations were also
related to class 1 GEP and the absence of ciliary body
involvement, whereas SF3B1 mutations are associated
with a more favorable outcome and younger age.48,51
BAP1 mutations can arise in the germ line, leading to
a newly described BAP1 familial cancer syndrome.49,52
Researchers and clinicians are realizing that, similar to
other cancers, uveal melanomas comprise a heteroge-
neous group of diseases, each with distinct molecular
features that might respond best to a specific therapeu-
tic strategy.55

All of these recent discoveries have led to new tri-
als to assess several classes of compounds, including
MAPK/ERK inhibitors,54 protein kinase C,55 histone
deacetylase inhibitors,56 and combination therapy,57 in
the adjuvant setting for high-risk patients and in the
setting of advanced disseminated disease. The next
generation of targeted therapy agents is also being in-
trduced into the clinical setting to evaluate the effi-
cacy of these molecular agents in patients with uveal
melanoma.58

Conclusions

Prognostic testing has become an important compo-
nent in the evaluation of patients with uveal melano-
ma to assess metastatic risk. Although gene expression
profiling (GEP) and chromosomal counting meth-
ods, such as multiplex ligation–dependent probe am-
plification, each provide different information about
metastatic risk stratification, clinicians can now offer
patients important insight about their prognosis for metastases. Although the potential for tumor heterogeneity and biopsy sampling error must be further investigated, new developments such as the identification of PRAME expression via GEP may increase the rate of accuracy in small tumors tested by GEP. Although the therapeutic options available for the treatment of metastatic posterior uveal melanoma are in their infancy, patients continue to express their desire to have accurate prognostic information about their tumors in order to plan their lives and to consider enrollment in clinical trials for adjuvant therapy, several of which are now available (NCT02601378, NCT01551459, NCT01377025, NCT01413191). Many centers are also revising their surveillance protocols to better suit the needs of patients. Recent advances in genomic sequencing technologies have also increased our knowledge of the molecular landscape of uveal melanoma, thus leading to clinical trials using targeted molecules to treat metastatic uveal melanoma. Patients whose increased risk for developing metastasis is detected by genomic testing may wish to enroll in adjuvant treatment trials or they may wish to choose periodic surveillance testing for the earlier detection of asymptomatic metastatic disease. In addition, clinicians can now offer treatments that have the potential to extend the lives of patients with uveal melanoma.

References