**'UNPACKING THE GENETIC ETIOLOGY OF UVEAL MELANOMA’**

**ABSTRACT**

**Introduction:** Uveal melanoma (UM) is the most common primary intraocular tumor affecting adults. ~50% of patients will develop metastatic disease, primarily in the liver, for which there is currently no standard of care. The multi-faceted genetic etiology of UM informs prognosis and advises the clinical management of patients.

**Areas covered:** This review highlights the multifarious nature of the genetics that differentiate uveal nevi from UM, that initiate tumorigenesis and ultimately drive metastasis, and how these can be incorporated to multivariate models to predict whether and approximately when metastatic disease will occur.

**Expert opinion:** Despite not being able to utilize current genomic biomarkers as therapeutically actionable targets in UM at present, advances in our understanding of this cancer, may lead to their inclusion to predict response to emerging therapies. Prognostication for individual patients is likely to progress from binary classifications of high- and low- metastatic risk, utilizing a multi-factorial approach to design molecular assays. Improvement in our comprehension of the genetic etiology of UM will bring us closer to the development of effective treatments.

**Keywords:** BAP1, EIF1AX, Chromosome, Copy Number, Monosomy 3, Gene-Expression Profiling, Methylation, MicroRNA, Mutation, SF3B1, Uveal Melanoma, choroidal melanoma

**ARTICLE HIGHLIGHTS**

* Uveal melanoma (UM) is a rare intraocular tumour whereby approximately 50% of patients develop metastatic disease for which there are no effective systemic chemotherapy or biological therapies at present.
* UM lacks a UV radiation mutational signature, and has a low mutational burden but has well-characterised somatic copy number alterations in chromosome 1, 3, 6 and 8, unique mRNA signatures (Class Ia, Ib and II), in addition to mutations that initiate tumorigenesis and drive metastasis.
* Predicting disease progression is most accurate when it is multifaceted, incorporating genetic, clinical and histopathological factors.
* Recent research exploring epigenetics, methylation and the consequences of miRNA dysregulation in UM provide promising avenues to explore in terms of developing targeted therapeutics.
* The rapidly development of genomic testing in UM tissue samples and liquid biopsies will bring us closer to providing effective treatments, improving survivorship and quality of life for patients.

**INTRODUCTION**

Uveal melanoma (UM) is the most common primary intraocular cancer in adults, with a mean age-adjusted incidence of 4.3 per million per year worldwide (1-3). It is assumed that UM arises from malignantly transformed melanocytes located in the uvea. Around 90% of UM arise in the choroid, the vascular layer located between the retina and sclera (4). An additional 3-4% UM arise in the iris, the colored part of the eye that controls pupil dilation; the remainder of UM occur in the ciliary body, a ring-shaped structure consisting of ciliary muscles, ciliary processes and fibrous connective tissues (4).

Primary UM are successfully treated by application of several treatment modalities, such as radiotherapy, surgical resection and enucleation (5-10). Relapse occurs when UM cells spread hematogenously outside the ocular area; with local recurrence being relatively rare (11). Lymph vessels are not present in the eye, and hence lymphatic spread is only possible when anterior extraocular tumor spread occurs, enabling tumor cell access to the conjunctival lymphatics (12). The most common site of metastatic spread is the liver, although UM cells also spread to the lungs, bones and rarely the skin (13). Up to 50% of UM patients will die of metastatic disease with 90% of those developing hepatic metastases initially (14). For these patients, the prognosis is dismal due to a lack of effective systemic treatments (14-17).

Metastatic UM spread is associated with many clinical and histological factors, such as tumor size, ciliary body involvement, extraocular extension, mitotic count, presence of epithelioid cells, closed connective tissue loops, tumor infiltrating lymphocytes and tumor associated macrophages (18-24). UM also display a range of genetic aberrations that drive tumor progression (25). In this review, the genomic landscape of UM will be explored in detail, from gross structural chromosomal abnormalities and mutations to the more recent microRNA discoveries. Also discussed are the ways in which we can utilize this information, to develop effective surveillance strategies and targeted-treatments for metastatic UM.

1. **Somatic Copy Number Alterations (SCNA)**

The most widely reported gross chromosomal abnormality in UM is a complete loss of one copy of chromosome 3, known as monosomy 3 (M3). This was initially reported by Prescher et al. in 1990 after an examination of UM using conventional karyotyping showed 43% of the 14 cases analyzed were M3 (26). A subsequent study of 54 cases showed that chromosome 3 loss was strongly associated with UM patients who have a poor prognosis (27). Since the study in 1996, several other groups have confirmed the prognostic significance of M3, and it is now widely regarded as one of the most useful prognostic parameters when considering UM patient survival (28-34). Abnormalities in chromosome 8 are also well described in UM, presenting as a gain of 8q or isochromosome 8q, which are often associated with M3-UM, and therefore with poor prognosis (29, 31, 35, 36). The region of 8q, which was found to be most commonly amplified during a comparative genomic hybridization study, was 8q24-->qter, an area that encompasses the *c-myc* oncogene (37, 38). The gain of 8q, especially *c-myc*, is thought to occur later in the genetic progression of UM, and generally follows chromosome 3 loss with increasing copies of 8q significantly correlating with reduced survival, in a dose‐dependent fashion (36, 39). Aberrations in chromosome 6, frequently found in cutaneous melanoma, have also been linked to UM prognosis. Whilst gains in 6p have been associated with a more favorable prognosis, losses in 6q, in particular the tumor suppressor *PHF10* on 6q27, have been reported to worsen survival in UM (34, 40-42). Alterations in chromosome 1p are also routinely found in UM: 1p loss occurs in metastasizing UM concurrent with M3, suggesting a role in the promotion of tumorigenesis and tumor dissemination (40, 43). Amplifications of 1q have been described to occur in as many as 24% UM; however, the prognostic significance of this is not yet fully understood (44).

1. **Mutations in UM**

UM lack the UV radiation mutational signature often found in cutaneous melanoma (45). The mutational burden in UM is low and The Cancer Genome Atlas Study (TCGA) demonstrated that UM had a somatic mutation density of only 1.1 per Mb (39). In addition to well-characterized SCNA, UM has two sets of driver mutations: one which initiates tumorigenesis in the form of mutually exclusive gain-of-function mutations in *GNAQ*, *GNA11*, *CYSLTR2*, or *PLCB4*, all of which are major players in the Gαq signaling pathway also present in uveal nevi. The other set of mutations in *BAP1*, *SF3B1/SRSF2* and *EIF1AX* influence the metastatic potential of UM.

* 1. **Initiating mutations**

**2.1.1 *GNAQ* and *GNA11***

*GNAQ* and *GNA11* are heterotrimeric proteins, which function as G protein alpha subunits; activating phospholipase C and involved in the transduction and modulation of several cellular signaling pathways. Located on 9q21.2 and 19p13.3, respectively, mutations of these GTPases *GNAQ* and *GNA11* occur in UM in either exon 4 (R183) or exon 5 (Q209), with the latter being the most frequently observed alteration (46-48). *GNAQ* and *GNA11* mutations are found in ~84% UM and are mutually exclusive to each other (46). The mutations inhibit the GTPase function of the gene, which results in an inability to hydrolyze GTP to GDP leaving the protein in a constitutively active state (46). There is little evidence to suggest that *GNAQ* or *GNA11* mutations negatively influence patient survival (49-51), and as such, are thought to be an early event in UM development (52).

**2.1.2 *CYSLTR2***

Mutations in cysteinyl leukotriene receptor 2 (*CYSLTR2*) were first described by Moore et al. in 2016, who identified a recurrent hotspot mutation encoding a p.Leu129Gln substitution in four UM lacking mutations in either *GNAQ, GNA11* or *PLCB4* (53). Further analyses by the TCGA-UM study confirmed the presence of these mutations in 4% of UM (39). Similar to *GNAQ/11* mutations, *CYSLTR2* mutations have also been observed in the same location in blue nevi. Leu129 is situated in a functional hub of the receptor, transmembrane helix 3, and mutations at this site lead to constitutively active Gαq, leaving the cell unresponsive to leukotriene stimulation.

**2.1.3 *PLCB4***

Recurrent, gain-of-function, missense hotspot mutations in codon 20 (p.D630Y) of *PLCB4* have been identified in sequencing studies in ~1–7% of UM in a mutually exclusive manner to *GNAQ*, *GNA11* and *CYSLTR2* (39, 54). *PLCB4* acts downstream of *GNAQ* and *GNA11* and plays a crucial role in intracellular signal transduction. *PLCB4* is activated upon binding of a G protein subunit, causing the cleavage of phosphatidylinositol 4,5 bisphosphate (PIP2) to produce diacylglycerol (DAG) and inositol triphosphate (IP3), utilizing calcium as a cofactor (55). The presence of a *PLCB4* mutation in a case of ocular melanocytosis with concomitant UM, suggests that alterations in this gene may play a role in initiating melanocytic proliferation (56). However, other studies have identified *PLCB4* mutations that coincide with *GNAQ* or *GNA11* mutations, which could also suggest the *PLCB4* mutations occur as secondary alterations in G-protein-related pathways (57, 58).

Mutations in *GNAQ,* *GNA11, CYSLTR2* and *PLCB4* all lead to the activation of several downstream signaling pathways, including mitogen-activated protein kinase (MAPK), phosphatidylinositol 3 kinase/AKT/mechanistic target of rapamycin kinase and Trio-Rho/Rac/YAP (59-63), highlighting the importance of these molecules for targeted therapies. *In vitro* studies examining the effects of MAPK/ERK Kinase (MEK) and protein kinase C (PKC) inhibitors on *GNAQ* and *GNA11* mutant cell lines reported a significant reduction in proliferation and induction of apoptosis (59, 64). siRNA mediated knockdown of *CYSLTR2* reduced the growth of UM cells in vitro (53). Despite promising *in vitro* results, clinical studies have shown that MEK inhibitors either alone or in combination with other agents have shown no significant improvement in either progression free or overall survival (OS) of patients with metastatic UM when compared with standard chemotherapy (14, 65). Targeting other downstream activators such as PKC, AKT, MTOR, YAP and Focal Adhesion Kinase have similarly been successful in reducing UM cell growth in *in vitro* and *in vivo* models of UM but have failed to improve OS when used in various regimens clinically (60, 66-69). Further studies are necessary to fully understand and develop these pathways as actionable targets for the treatment of patients with metastatic UM.

**2.2 Metastasis driving mutations**

**2.2.1 *BAP1***

*BAP1*, or “BRCA associated protein 1”, is a gene encoding for a deubiquitinating enzyme and is found on the short arm of chromosome 3. *BAP1* mutations are associated with cancers, such as clear cell renal carcinoma, mesothelioma and non-small cell lung cancer as well as UM (70, 71). Somatic mutations in *BAP1* have been reported in 18–48% of all UM and in around 84% of M3-UM (39, 72). The bi-allelic inactivation of *BAP1* frequently leads to a loss of nuclear BAP1 protein expression on immunohistochemistry, and this is strongly associated with metastasis and poor patient prognosis (33, 39, 51, 73-78).BAP1 is involved in numerous cellular processes, such as DNA damage response, regulation of transcription, cell cycling and cell growth (79-82). Germline mutations in *BAP1* have also been reported to be associated with a tumor-predisposition syndrome increasing susceptibility to UM, cutaneous melanoma, mesothelioma, renal cell carcinoma and other cancers (70, 83-85). In UM, the frequency of germline BAP1 mutations is around 19% increasing to over 20% in rare familial UM cases (85).

**2.2.2 *SF3B1***

Splicing factor 3B subunit 1 *(SF3B1)* is located on 2q33.1 and encodes subunit 1 of the splicing factor 3b protein complex. Recurrent mutations in *SF3B1* in codon 625 are associated with differential alternative splicing of coding and non-coding genes, resulting in abnormally spliced transcripts that are either degraded by nonsense-mediated decay or translated into atypical proteins (86). Mutations in *SF3B1* occur at a frequency of 11-34% (39, 45, 87-89) predominantly in UM with disomy 3 (D3) (25, 76) and as such were initially thought to have a protective function as they occurred almost exclusively in D3-UM with good prognostic features. However, recent data demonstrated that D3-UM with *SF3B1* mutations have a 7-fold increased risk of metastatic disease occurring at a longer follow-up time when compared to D3 tumors without mutations in *SF3B1* (90). In addition, *SF3B1* mutations were initially thought to occur in a mutually exclusive fashion to *BAP1* mutations, suggesting that there were two distinct pathways of tumor progression (91). However, mutations in both *BAP1* and *SF3B1* have been recently observed in a small number of UM, which contradicts this paradigm (57). More recently, an unusual subset of M3-UM with mutations in *SF3B1* that lack *BAP1* mutations has been identified; however, due to the small number of cases the impact on survival is unknown (57).

**2.2.3 *EIF1AX***

Eukaryotic translation initiation factor 1AX-linked *(EIF1AX*) located on Xp22.12 encodes an essential eukaryotic translation protein required for ribosome dissociation and stabilizing the binding of Met-tRNA to 40S ribosomal subunits. *EIF1AX* mutations in UM occur across hotspots on exons 1 and 2 at a frequency of 14-34% (39, 45, 87-89, 92), generally occurring only in D3-UM cases. Mutations in *EIF1AX* are associated with a more favorable prognosis and play a protective role to prevent metastasis even when taking into consideration clinical and histological risk factors (88, 92). Although they were initially thought to be mutually exclusive of *SF3B1* mutations (93), a targeted sequencing study identified two rare cases of D3-UM that harbored both *SF3B1* and *EIF1AX* mutations, as well as two cases of *EIF1AX* mutant M3-UM (57). In the two UM cases showing both *SF3B1* and *EIF1AX* mutations, metastatic death occurred within 5 years of the diagnosis of the primary UM (57).

**2.2.4 *SRSF2***

The TCGA-UM study was the first to identify change-of-function mutations in ‘Serine and Arginine Rich Splicing Factor 2’ *SRSF2*, with a proclivity for in-frame deletions in 4% of UM (39). Tumors with *SRSF2* mutations lacked either *SF3B1* or *EIF1AX* mutations, with each subset having unique methylation profiles (39). When analyzed alongside RNA-seq data for the corresponding sample, the in-frame deletions of SRSF2 altered translation initiation, skipping exons in *EIF4A2* and *FYN* (39).In a separate study investigating the prevalence of *SRSF2* mutations in *SF3B1* wild-type UM, heterozygous in-frame *SRSF2* deletions affecting amino acids 92–100 were detected in two UM (94), and again were mutually exclusive to *BAP1*, *SF3B1*, and *EIF1AX* mutations (94).These mutations are thought to play a crucial role in tumorigenesis by mis-splicing that affects elongation initiation factors and signaling gene transcripts (95). Additionally, mutations in the splicing factor *SRSF2* resulting in a change-of-function have also been identified in two separate studies in the same region reported to disrupt splicing in myelodysplastic syndrome (96, 97).

1. **RNA Gene Expression Profiling in UM**

Gene expression profiling (GEP) is a technique that simultaneously measures mRNA expression of multiple genes. GEP was employed by Tschentscher et al. 2003, who established an association between different levels of gene expression and chromosome 3 status in UM (98). The relationship between gene expression and survival was later also explored by Onken et al. 2004, leading to a classification of UM as low-grade (Class 1) or high-grade (Class 2) (99). These data identified 12 signature genes that could distinguish between low and high metastatic risk UM, and led to the development of a commercial test in which the 12 signature genes and 3 housekeeping genes formed the ‘Decision DxUM’ panel marketed by Castle Biosciences in the USA for prognostication of UM patients (100). The GEP classification system for UM was later revised to consider low-risk/class 1 patients, who developed metastases. This led to the creation of Class 1a and 1b UM, with the latter indicating an intermediate risk of metastasis (101). Despite commercial success in USA, recent data suggest that GEP cannot distinguish between primary UM and secondary metastases from other primary tumors (102, 103). Therefore, GEP should only be performed where there is a histological diagnosis of melanoma.

1. **Epigenetics of UM**

Epigenetics is the study of heritable modifications that act to regulate gene expression rather than alter the genetic code itself. Epigenetic mechanisms include non-coding RNA regulation, histone modification and, the most commonly studied, DNA methylation.

* 1. ***Methylation Profile of UM***

DNA methylation involves the covalent binding of a methyl group to the C5 position of the DNA base cytosine to form 5-methylcytosine; most commonly at cytosine-phosphate-guanine (CpG) sites where the methylated cytosine precedes a guanine nucleotide. DNA methylation acts to silence gene expression, and aberrations in DNA methylation are associated with tumorigenesis (104); with transcriptional repression resulting in gain of function of oncogenes or in loss of function of tumor suppressor genes (105).

In UM the role of epigenetics, in particular DNA methylation, on tumor initiation and progression have been less well defined. In general, cancer studies often focus on altered methylation patterns in gene promoter regions due to the high incidence of clustered CpG sites, known as CpG islands, in this region (106). Indeed, initial methylation analyses in UM focused on identifying tumor suppressor genes regulated by promoter methylation contributing to UM tumorigenesis. A number of genes were studied including *SNRPN (small nuclear ribonucleoprotein polypeptide N)* (107), *RARB (retinoic acid receptor-β2*) (107, 108), *FHIT* (108), *APC (adenomatous polyposis coli)* (108), *RASSF1 (Ras association domain family member 1)* (108) and *RASEF (Ras and EF-hand domain containing)* (109), with reported rare to moderate methylation events. Methylation of *hTERT (human telomerase reverse transcriptase)* (108) was shown to be a regular event in UM; and *TIMP3* *(tissue inhibitor of metalloproteinase 3)* methylation whilst shown to be a rare event in one study (108), was highlighted as a regulator of TIMP3 protein expression in UM cell lines in another (110).

More widely studied genes include *CDKN2A (cyclin dependent kinase inhibitor 2A)* (107, 108, 111, 112) with promoter methylation being reported in UM primary tissue and cell lines; however methylation varied from 4 to 33% (44). A more recent study reported higher levels of methylation, which correlated with reduced p16 mRNA (113). Since *CDKN2A* mutations are rare events in UM, epigenetic modification of this gene may be an alternative mechanism of repression (113). Another gene widely studied, *RASSF1A,* an alternative transcript of the *RASSF1* gene, was shown by Maat et al. 2007, to be hypermethylated in cell lines, primary UM tissue and within the metastatic lesion (114), and to correlate with metastasis (114-117). Both CDKN2A and RASSF1A methylation, have been shown to be reversible following treatment with 5-aza-2’-deoxycytidine; and therefore may be prospective targets for future epigenetic treatments (111, 117).

Neumann et al. 2011, studying EFS (embryonal Fyn-associated substrate) methylation, observed full or partial methylation of this gene in 11/16 tissues from M3-UM and D3-UM tumors (106). Full methylation was predominantly seen in M3-UM (87.5%), and was correlated with metastatic death in a larger confirmatory sample set. Complete *EFS* methylation was shown to be tissue specific and biallelic, which was suggested to unlikely be a random event, and instead be a site directed *de novo* methylation mechanism rather than a two-step mutation selection process (106).

Of note, one of the only studies to show hypomethylation in UM was by Field et al. 2016, who observed that 12 CpG sites in the gene *PRAME* *(preferentially expressed antigen in melanoma)* were hypomethylated in PRAME+ compared with PRAME- tumors; and was associated with increased metastatic risk in both Class 1 and Class 2 tumors (118). *PRAME* hypomethylation resulted in activated PRAME expression, which could potentially be targeted by PRAME-directed immunotherapy harnessing the reactivity of PRAME-specific T-cells (119).

Finally, the TCGA-UM study identified unique DNA methylation profiles associated with molecular subtypes of UM. They showed M3-UM with BAP1 loss correlated with a global DNA methylation profile that was distinct from D3-UM (39). Furthermore, they showed separation of D3-UM into low and intermediate risk subgroups, *EIF1AX*- and *SRSF2/SF3B1*- mutant tumors, respectively, by their distinct methylation profiles. Further studies also report distinct UM methylation profiles (120), and even phylogenetic methylation clusters that strongly correlate with GEP (96).

Gene regulation by DNA methylation as a target for diagnostic and prognostic biomarkers, and future treatment targets has great potential. However, the methylation of genes is rarely a singular event, and is accompanied by a complex network of functionally connected molecular events, that are further complicated by tumor heterogeneity (121). Additional work is required in this area to better understand and release the full potential of DNA methylation in UM therapy.

* 1. ***MicroRNA in UM***

MicroRNA’s (miRNA) are small non-coding RNAs that are believed to function as either oncogenes or tumor‐suppressor genes in various cancer types (122). In UM, aberrant miRNAs have been associated with metastatic disease determined from primary tumor samples, UM cell lines, plasma and serum; using a range of techniques including microarrays, PCR and next generation sequencing (NGS) (123-130). So far, there is no clear consensus as to which miRNAs are most important in UM, with many studies identifying multiple miRNAs that may play a role in disease progression and prognosis. However, with the ability of miRNAs to target multiple effector genes (approx. 500 genes per miRNA family), and with genes often carrying more than one conserved miRNA interaction sequence, it is highly likely that networks of miRNAs cooperate to regulate gene expression (131).

Early studies in UM focused on miRNA expression patterns between the molecular subtypes of UM, and showed that tumors clustered easily into two groups based on miR expression with let-7b and miR-199a being major discriminators (130). Radhakrishnan et al. 2009, also described miRNA expression differences between metastasizing (11 miRNAs) and non-metastasizing tumors (19 miRNAs) (125).

Other studies have focused on profiling metastatic exosome and immune regulatory miRNAs. Eldh et al. 2014, showed UM patients with metastases had a greater release of liver-derived exosomes carrying miRNA clusters that were unique, compared with other cellular exosome sources (132). Triozzi et al. 2016, observed dynamic miRNA expression changes in the plasma of six patients monitored at the time of diagnosis until the onset of metastasis, with several immune miRNAs decreasing in line with changes in immune cell profiles (128).

Biologically, the miR-34 family has been implicated in UM disease progression. miR-34a was predicted to target c-Met protein expression, a receptor known to be upregulated in metastatic UM (133, 134). Overexpression of miR-34a was shown to inhibit cell proliferation and migration by downregulating c-Met protein expression (134). This was similarly seen with miR-34b/c with c-Met, p-Akt and cell cycle proteins targeted (135). In fact, miR-34a response elements (MREs), along with miR-137 and miR-182 MREs, have been used to direct a TRAIL-expressing adenoviral vector to target UM, both in cell lines and xenografts giving scope for potential future therapies (136).

More recently, with the publishing of the UM dataset from TCGA (39), larger bioinformatic studies have been undertaken, utilizing the sample size power of the TCGA-UM to inform miRNA predictions. For example, a panel of 13 differentially expressed miRNAs have been identified, including miR-17-5p, miR-21-5p, miR-151a-3p, from primary UM by microarray analysis that distinguish between patients at high and low risk of developing metastasis. Integration with TCGA-UM dataset, suggest these miRNAs interact with many genes involved in cell cycle regulation, including EIF2 and EGF signaling (126). In addition, machine learning and bioinformatics approaches have been used to classify tumors using ratiometric miRNA analysis with high sensitivity and specificity, and identify the most highly deregulated miRNAs in high risk UM; these included miR-506-514 cluster, miR-592 and miR-199a-5p targeting pathways such as PI3K-Akt and MAPK1 (137, 138). Finally, a multicenter cross-sectional study recently revealed a panel of six circulating miRNAs that not only successfully distinguish metastatic UM from localized UM, but also distinguish uveal nevi from localized disease (139).

With multiple approaches and recent studies revealing a plethora of miRNA targets, it is crucial that our understanding of the functionality and biological consequences of these miRNAs in UM is advanced.

1. **Prognostication modelling using genetic markers in UM**

In UM, prognostication plays an essential role in patient care: it aids in discussions about advanced care planning, and predicts *whether* and *when* metastatic disease will occur. Moreover, it provides patients with reassurance if the outlook is good and allows patients at a high-risk of metastasis to receive the care they require. Initially, prognostication utilized tumor measurements only to calculate risk. However, the addition of genetic and histological factors greatly improves prognostication.

**5.1 LUMPO**

The Liverpool Uveal Melanoma Prognosticator Online (LUMPO; [www.lumpo.net](http://www.lumpo.net)) was the first multi-parameter prognostic tool developed to estimate survival probability in UM patients. LUMPO combines anatomical, histological and genetic predictors, specifically SCNA of chromosomes 3 and 8q, and produces an age- and gender-matched all-cause mortality survival curve. The tool was validated in 2012 using data from 3653 UM patients, with a follow-up of more than 20 years at the Liverpool Ocular Oncology Centre (LOOC) (140). LUMPO underwent external validation in Poland and the USA in 2015 and 2016, respectively, both demonstrating that LUMPO accurately estimated all-cause mortality for UM patients, despite differences in the treatments and availability of cytogenetic data between centers (141, 142). More recently an anonymized dataset of 1836 UM patients from seven international ocular oncology centers were analyzed with LUMPO3 to predict the 10-year survival for each patient in each external dataset (143). Despite slight differences between cohorts, LUMPO3 was considered an accurate and valuable tool predicting all-cause mortality in patients with UM.

**5.2 PRiMeUM**

Prediction of Risk of Metastasis in Uveal Melanoma (PRiMeUM) is an interactive web-based tool developed by Vaquero-Garcia et al. in 2017 (144). PRiMEUM provides a personalized risk estimate of developing metastases within 48 months of primary UM treatment. A Cox proportional hazard model was used to evaluate factors that can predict metastasis including clinical and chromosomal characteristics using a cohort of 1227 UM cases (144). From this they developed a multivariate model with a reported accuracy of 80%, 83% and 85% using cytogenetic, clinical and a combination of cytogenetics and clinical information, respectively. This model is in a relatively early stage of development, and would benefit from external validation similar to that undertaken for LUMPO, in order to examine the discrimination performance of the model on a separate cohort of samples.

**CONCLUSION**

UM is a rare but aggressive intraocular tumor with a propensity for liver metastases, presently incurable. In stark contrast to cutaneous melanoma, UM lacks the UV radiation mutational signature and has a low mutational burden. Consequently, UM lacks the sensitivity to immune-checkpoint blockers that are highly effective in cutaneous melanoma. The vast majority of UM have initiating mutations in *GNAQ*, *GNA11*, *CYSLTR2* and *PLCB4*, which despite not having any prognostic significance, represent potential targets for therapy. Patient prognosis can be accurately determined by analysis of gene expression/aberrations in chromosomes 1, 3, 6 and 8, and further subdivided by the presence or absence of mutations in *BAP1*, *SF3B1* and *EIF1AX*. By exploring the biological changes and altered cell signaling caused by initiating and driver mutations, effective systemic treatments may be identified for patients with metastatic disease. Additionally, epigenetics and the consequences of miRNA dysregulation are other avenues to explore in terms of developing targeted therapeutics, although research into this is in its infancy and to date has been undertaken on primary UM. Only by combining our efforts, working with metastatic UM and collating all of this information to better understand this enigmatic and elusive cancer, can we come closer to an effective treatment for patients with metastatic disease.

**EXPERT OPINION**

After the successes of the TCGA and 100,000 Genome studies in the USA and UK, respectively, deep sequencing of clinical samples is transforming the way cancer patients are being managed, enabling them to receive personalized treatments. The genomics industry is growing exponentially worldwide, and the combination of sequencing data with a patient’s medical records provides an invaluable resource for clinicians and researchers alike. In cancer alone, it is estimated that around half of all malignancies have the potential for a therapy or a clinical trial. Whilst the genetic etiology of UM enables clinicians to tailor surveillance programs to a patient’s individual metastatic risk, at present no targeted treatments exist, and little is known about how UM progresses from primary to metastatic disease.

Focused research on the genomic profiling of UM cell-free tumor DNA, circulating tumor cells and, ultimately, the metastatic disease will provide a more detailed snapshot of how disseminated UM cells differ from the primary tumor, and what strategies they apply to evade the immune system. Identifying the ‘how and why’ this happens will inform new treatment modalities and more effective ways of monitoring residual disease. Immunotherapy is an emerging treatment option for some cancers. T-cells and dendritic cells have already been identified in UM, and thereby metastatic UM is a possible target for immunotherapy. Dendritic-cell vaccination (DCV) treatment with dendritic cells loaded with tumor-associated antigens, such as gp100 and tyrosinase, has received encouraging results in phase I and phase II trials in an adjuvant setting after resection of the primary tumor (145, 146). A phase III trial is presently underway to determine whether DCV can prevent or delay progression among high-risk UM patients (NCT01983748) (147). Checkpoint inhibitors – e.g. anti-cytotoxic T-lymphocyte-associated Protein 4 (CTLA-4), anti-PD-1 (PD-1) and anti-programmed cell death protein 1 (PD-L1), which have been successful in the treatment of metastatic cutaneous melanoma-, have proven disappointing to date in improving disease control for UM patients (148-150). It is likely that in-depth profiling of the immune microenvironment of metastatic UM will provide some answers as to why these tumor cells can effectively ‘hide’ from the immune system. Further, by identifying targetable cellular pathways within metastatic UM, there is the potential for adjuvant therapies, to be administered to high-risk patients after the treatment of the primary tumor.

There are currently no approved therapeutic regimens for metastatic UM. Meta-analysis of 29 phase II trials carried out between 2000 and 2016, using a range of treatment regimens, were found to be inefficacious in the disseminated disease (151). Chemotherapy regimens are ineffective at controlling disease progression with dismal response rates ranging from 0–15% (152-154). Given the prevalence of driver mutations in *GNAQ* and *GNA11* in UM, these GTPases represent ideal targets for therapy. However, inhibiting signaling pathways activated downstream of these mutations has been explored with little success. The use of MEK inhibitors is not effective in UM, irrespective of the inhibiting agent or combination partner (155). Similarly, PKC inhibition as single treatment appears to have only limited clinical benefit (156). Given the inefficacy of monotherapies in controlling metastatic disease, it is probable that combination therapies are key to effective systemic strategies in metastatic UM. Advances in our understanding of the epigenetics, consequences of miRNA dysregulation and ways in which to effectively target the initiating signaling and driver spliceosome pathways will enable the development of precision therapies in the future.

There are currently vast differences in the approach to prognostication in UM. It is clear from the literature that there are ‘grey’ areas that do not fit either the Class 1/Class 2 or M3/D3 risk classifications. Over the next five years, providing prognostication for UM patients is likely to evolve from the binary classifications of high- and low-risk using a single testing methodology, to a multiparameter approach. Only by applying such a multi-faceted algorithm that examines a multitude of clinical, histological and genetic factors (e.g. SCNA, mutations, the transcriptome and immune markers) can we more accurately and better stratify UM patients into clear risk groups, and simultaneously enhance our knowledge and the treatment of this disease.

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