Genetic prognostication in uveal melanoma

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Chapter 2

Genetic Prognostication in Uveal Melanoma

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ABSTRACT

Uveal melanoma (UM) is a rare tumor with a high propensity to metastasize. Although no effective treatment for metastases yet exists, prognostication in UM is relevant for patient counselling, planning of follow-up, and stratification in clinical trials. Besides conventional clinicopathologic characteristics, genetic tumor features with prognostic significance have been identified. Non-random chromosome aberrations such as monosomy 3 and gain of chromosome 8q are strongly correlated with metastatic risk, while gain of chromosome 6p indicates a low risk. Recently, mutations in genes such as *BAP1*, *SF3B1*, and *EIF1AX* have been shown to be related to patient outcome. Genetics of UM is a rapidly developing field, which not only contributes to the understanding of the pathogenesis of this cancer, but also results in further refinement of prognostication.

Concomitantly, advances have been made in the use of genetic tests. New methods for genetic typing of UM have been developed. Despite the considerable progress made recently, many questions remain, such as those relating to the reliability of prognostic genetic tests, and the use of biopsied or previously-irradiated tumor tissue for prognostication by genetic testing. In this article, we review genetic prognostic indicators in UM, also comparing available genetic tests, addressing the clinical application of genetic prognostication, and discussing future perspectives for improving genetic prognostication in UM.

1. INTRODUCTION

Uveal melanoma (UM) arises from melanocytes residing in the uveal tract, which comprises the iris, ciliary body, and choroid. UM accounts for most (85%) ocular melanomas and is the most common primary intraocular malignancy in adults.[1] The annual age-adjusted incidence in the United States is 5.1 per million, with a male-to-female ratio of 1.1:1.[2] The mean age at diagnosis is 61 years and most patients develop UM after the age of 50.[3] Approximately 95% of uveal melanomas occur in Caucasians,[3] especially in those with a light iris color, fair skin, propensity to sunburn, and a tendency to develop common/atypical cutaneous nevi and cutaneous freckles.[4-7] Congenital oculodermal melanocytosis (nevus of Ota), which affects 0.04% of the white population,[8] is associated with a 1 in 400 risk of uveal melanoma. [9] Choroidal nevi are estimated to have a 1-in-4300 to 1-in-8845 per year risk of malignant transformation.[10-12] Evidence correlating ultraviolet light exposure with UM is inconclusive. Arc welding has been reported to be a risk factor;[13] however, welding arcs are also a source of blue light, which has recently been proposed as a risk fator for the development of UM.[14]

In the last decades, many advances have been made in the treatment of the primary tumor, which include various forms of radiotherapy, phototherapy and local resection. These eye-sparing methods have largely replaced enucleation, which is now reserved for large UMs, tumors involving the optic nerve and eyes with a poor visual prognosis. Early detection of UM may enhance opportunities for eye-conserving therapy.[15, 16] Damato et al. have provided tentative evidence that early treatment of tumors may prevent metastatic disease and improve survival in patients with small tumors.[17] However, survival of patients with metastasized UM has not improved because effective treatment is lacking. The overall 10-year metastasis rate is 40% with almost 50% of patients eventually dying from metastases, which usually involve the liver. The median survival time after the diagnosis of metastases ranges from 4 to 15 months.[18]

Despite the lack of efficient treatment for metastasized UM, prognostication in UM is valuable since it enables clinicians to reassure patients who have a low risk of metastasis and to target special measures at high-risk patients, who are likely to have clinically undetectable micrometastases at the time of diagnosis of the primary UM.[19] These patients may be stratified in clinical trials to determine the efficacy of adjuvant treatments for UM metastases. Moreover, reliable prognostication allows risk-based planning of screening for metastases, preventing unnecessary investigations in those with a good prognosis.

Various patient and tumor characteristics have been identified as survival predictors in UM. For example, the prognosis is better in children than in adults, independently of other risk factors.[20, 21] Clinical features indicating increased metastatic risk include large tumor size, ciliary body involvement, and extraocular extension.[22-25] These form the basis of the Tumor, Node, Metastasis (TNM) staging system of the American Joint Committee on Cancer (AJCC).[26] This prognostication system has recently been validated in large multinational studies.[27,] AJCC Ophthalmic Oncology Task Force [28] Histopathologic predictors of metastasis include epithelioid melanoma cytomorphology,[29] high mitotic count,[30] lymphocytic infiltration,[31] extravascular matrix loops [32] and vascular invasion.[33] Early studies of the genetics of UM indicate that non-random alterations of chromosomes 3, 6, and 8 are common and have prognostic significance. Recently, molecular classification of UMs based on gene-expression profiling has been shown to correlate with survival. Several studies have demonstrated that genetic markers have better prognostic accuracy than clinical and histopathologic biomarkers. Although considerable progress has been made in the genetic characterisation of UM, questions remain with regard to the accuracy of markers, the reliability of genetic tests, and the use of biopsy specimens or previously-irradiated tumor tissue for prognostication by genetic testing.
In this article, we overview genetic prognostic indicators in UM, compare current genetic tests, discuss genetic testing in biopsied and irradiated tumors, and propose methods for improving genetic prognostication in UM.

2. GENETIC PROGNOSTIC MARKERS

Genomic instability is one of the hallmarks of cancer.[34, 35] However, in comparison to cutaneous melanoma and other cancers, UM has relatively few mutations.[36-39] In UM, one can identify recurring non-random chromosome aberrations, which are not the result of chromosomal instability but which are specific alterations that are linked to tumor development and progression.[39]

2.1 Chromosome alterations

In 1996, a strong association was observed between loss of one copy of chromosome 3 and the development of metastatic disease.[40] Several studies had previously reported chromosome 3 aberrations in UM and had described the recurrent and nonrandom nature of these alterations[41-51] Prescher et al. detected monosomy 3 in 56% of a series of 54 UMs and reported a 50% metastasis rate at

3-years follow-up in patients whose tumor harbored this aberration, while none of the patients with a disomy 3 melanoma had developed metastatic disease.[40] Subsequent studies in larger cohorts showed monosomy 3 in 25% to 65% of UMs and confirmed the strong association with metastatic disease (Table 1A).[52-62] Monosomy 3 is known to be associated with clinicopathologic features indicative of a poor prognosis, such as large tumor diameter, ciliary body involvement, epithelioid melanoma cytomorphology,[53, 54, 63] and inflammation.[64, 65] Nevertheless, monosomy 3 is also predictive of metastatic death independent of clinicopathologic factors [54] and has been shown to be superior to clinicopathologic factors as a prognostic indicator.[40, 66-68] In UM metastases, the presence of monosomy 3 has been associated with decreased survival from the time of diagnosis of disseminated disease.[69]

Some studies proposed that complete monosomy is more strongly correlated to metastasic risk than partial monosomy 3.[56, 70] However, when considering cases with borderline results as 'normal' and only defining tumors with definite loss of chromosome 3 as such, Damato and associates reported similar rates of metastatic death for cases with partial or total loss of chromosome 3,[55, 71] which was corroborated in a study by Ewens et al.[59]

Another type of loss of heterozygosity (LOH) of chromosome 3, isodisomy 3,[51] which occurs in 5% to 10% of cases, conveys a metastatic risk that is similar to monosomy 3.[72] Isodisomy is the presence of two identical copies of a chromosome, both from the same parent. This implies that the pathologic effect of monosomy 3 is not due to haploinsufficiency, but due to complete loss of various tumor suppressor proteins, presumably by mutations on certain loci on the remaining copy of chromosome 3 (see below). It is supposedly this abnormal copy that is duplicated in tumors with isodisomy 3.[72] The way monosomy 3 affects tumor development and progression has not yet been elucidated. Since monosomy 3 UMs exhibit a higher level of aneuploidy than disomy 3 tumors, it has been suggested that monosomy 3 leads to increased genomic instability.[39]

Another chromosome that is frequently altered in UM is chromosome 8.[42-44, 47] Gain of the long arm of chromosome 8 (8q), which often results from isochromosome formation, is associated with poor prognosis and occurs in 37% to 63% of primary UM.[52, 54, 55, 58, 59, 61, 62, 68] Isochromosome 8q leads to gain of

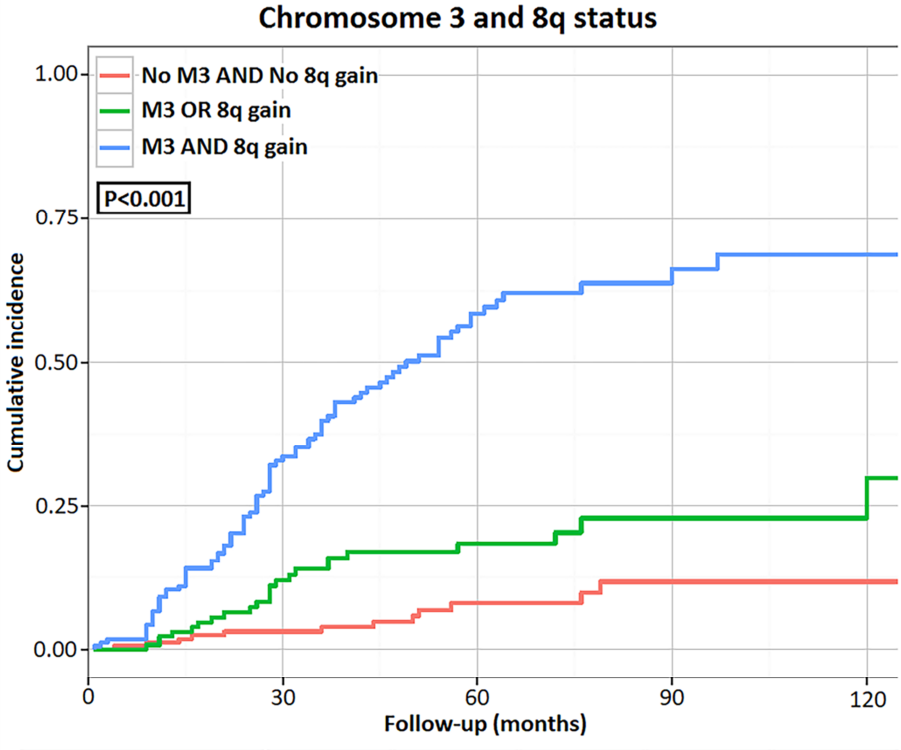
Table 1. Frequency of common chromosome aberrations with evident prognostic significance and gene mutations in primary uveal melanoma. Studies are listed in chronologic order. A: chromosome aberrations. B: gene mutations.
A:

Study	Chromosome aberration (%)			
	Loss of 1p	Monosomy 3	Gain of 6p	Gain of 8q
Prescher et al. 1996		56		
Sisley et al. 1997		50		54
Scholes et al. 2003		51		
Kilic et al. 2006	24		18	53
Damato et al. 2007		47		37
Damato et al. 2010	34	61	54	63
Shields et al. 2011		25		
Thomas et al. 2012		56		
Van den Bosch et al. 2012	30	61	42	61
Ewens et al. 2013	19	45	32	51
Ewens et al. 2014		65		
Koopmans et al. 2014	31	62	51	58
Dogrusöz et al. 2017		53		47
Range	19-34	25-65	18-54	37-63

B:

Study	Gene mutation (%)				
	GNAQ	GNA11	BAP1	SF3B1	EIF1AX
Van Raamsdonk et al. 2009	46				
Harbour et al. 2010			47		
Van Raamsdonk et al. 2010	48	34			
Daniels et al. 2012	47	44			
Furney et al. 2013	25	58	58	15	8
Harbour et al. 2013	42	52	38	19	
Koopmans et al. 2013	50	43			
Martin et al. 2013	45	40		21	18
Dono et al. 2014	42	33	32	10	19
Ewens et al. 2014	46	35	50	10	16
Koopmans et al. 2014			47		
Decatur et al. 2016	44	44	45	24	17
Moore et al. 2016	43	49	35	18	13
Van de Nes et al. 2016			51		
Yavuzyigitoglu et al. 2016	49	45	46	24	21
Range	25-50	33-58	32-58	10-24	8-21

material because it results in 3 copies of 8q while there is only 1 copy of 8p. An increasing dosage of 8q has been shown to convey an even greater risk of metastatic death.[52, 73] Gain of 8q commonly accompanies monosomy 3 and the concomitant occurrence of these aberrations is associated with a higher risk of metastasis than either of the aberrations alone.[52, 54, 55, 74] We corroborated this in a recently published study in collaboration with the Copenhagen University Hospital Rigshospitalet, in which we reported on combining AJCC staging and chromosome 3 and 8q status to improve prognostication.[61] In the cohort of 470 tumors with known chromosome 3 and 8q status, tumors harboring monosomy 3 as well as chromosome 8q gain showed an increased risk of metastatic death (Figure 1).



Time points (months)	0	30	60	90	120
	Numbers at risk				
No M3 AND No 8q gain	169	126	66	26	15
M3 OR 8q gain	135	85	49	15	8
M3 AND 8q gain	166	75	26	8	3

Figure 1. Cumulative incidence curves showing death due to uveal melanoma metastases in relation to chromosome 3 and 8q status. Adopted from reference# 61.

Although less frequently occurring than loss of chromosome 3 and gain of chromosome 8q, loss of the short arm of chromosome 1 (1p) is quite common in UM (19-34%),[55, 58, 59, 62, 68] especially in metastasizing tumors (33%).[75] In keeping with that finding, loss of 1p is associated with monosomy 3 [55, 76, 77] and the concurrent loss of 1p and chromosome 3 has been correlated with a decreased disease-free survival.[78]

Chromosome 6 was the first chromosome in which alterations were reported in UM.[79] The loss of the long arm of chromosome 6 (6q) is more common in metastasizing than in non-metastasizing primary UM,[75] while, in contrast to all aforementioned chromosomal alterations, gain of the short arm of chromosome 6 (6p) has a protective effect.[55, 71, 80] However, tumors with a normal chromosome 3 status and normal chromosome 6p status show a better prognosis than those with 6p gain.[39] Between 18% and 54% of UM exhibit gain of chromosome 6p,[55, 58, 59, 62, 68] which is almost exclusive to monosomy 3, suggesting distinct evolutionary pathways of tumor development.[76, 81, 82] Although chromosome 8q gain is related to monosomy 3, it is also found in tumors with gain of 6p.[39] While it has been hypothesized that monosomy 3 is the first step in the malignant transformation of UM,[83] and that 8q gain occurs after monosomy 3 or 6p gain,[39, 81] a recently published study reported monosomy 3 heterogeneity in tumors that are homogeneous for 8q gain; the authors therefore concluded that monosomy 3 is preceded by gain of 8q.[84] A study by Singh et al. indicated that gain of the telomeric part of 8q has a central role in UM tumorigenesis and reported this aberration in 92% of their studied tumors. Their analysis showed that this aberration is followed by either gain of the centromeric 8q and loss of chromosome 3, or by gain of chromosome 6p, as well as 7q, 11p, and 22q.[85]

2.2 Gene expression profiling

Since UM is characterized by non-random chromosome aberrations with distinct prognostic implications, it was anticipated that UM could be separated into prognostic groups based on gene expression profiling (GEP). In 2003, Tschentscher et al. performed unsupervised hierarchical cluster analysis of gene expression data on 20 primary tumors using a microarray gene chip of 12,500 probes and defined two distinct molecular classes, correlating with chromosome 3 status.[86] Zuidervaart et al., in an independent study, performed an mRNA expression array on 12 UM cell lines, and identified four genes that were subsequently used on 19 primary UM samples to separate them into two groups, based on the expression

of these genes.[87] A subsequent study of gene expression by Onken et al. in 40 primary UM used a microarray chip containing approximately 45,000 probes and confirmed the clustering of UM into two molecular groups. This study showed that the two observed specific genetic expression profiles (GEP) predicted survival.[88] Class 1 tumors were found to correlate with a low risk of metastatic death with a 92-month survival rate of 95% as compared to 31% in class 2 UM (the high-risk tumors). In a subgroup analysis of 10 tumors, chromosome 6p gain was found in four of five class 1 tumors and in none of the class 2 tumors, while loss of chromosome 3 occurred in four of five class 2 cases and in none of the class 1 UMs. All class 2 tumors with loss of chromosome 3 also showed gain of chromosome 8q, which was found in only two class 1 tumors.[88]

Recently, class 1 tumors have been subdivided into class 1A (2% 5-year metastatic risk) and class 1B (21% 5-year metastatic risk),[89] based on the differential expression of the *CDH1* and *RAB31* genes.[90] Class 2 tumors occur more commonly in older patients [88] and are related to monosomy 3,[91] greater thickness, epithelioid cell type,[92] extravascular matrix loops,[93] and a higher proliferation rate (Ki-67 score).[94] Class 2 tumors have been subclustered into class 2A and class 2B tumors. Class 2B cases harbor a deletion of chromosome 8p that makes the tumors even more aggressive and results in an earlier onset of metastasis compared to class 2A tumors.[95] Recently, expression of *PRAME* has been associated with increased metastatic risk in class 1 as well as class 2 tumors.[90]

The association between GEP class and survival has been validated independently in several studies.[92, 96, 97] For clinical purposes, a practical 15-gene assay based on the 12 most highly discriminating genes and 3 control genes, which can be performed on small biopsied tumor samples, has been developed,[98] and validated in a large multicenter study.[99] It has been claimed that analysis of mRNA is more accurate in prognostication than clinicopathologic parameters or chromosome 3 testing.[91, 99] However, similar to the original reports of Tschentscher et al.,[86] Onken et al.,[88] and van Gils et al.,[96] the mRNA expression pattern corresponds very strongly with chromosome 3 status.[100] In accordance with earlier reports by Damato's group on combining clinical, histologic, and genetic predictors to improve prognostication in UM,[101, 102] a recent study from Harbour's group [103] and another independent study[104] indicated that largest basal diameter provides prognostic information that is independent of GEP (see section 5).

2.3 Gene mutations

Unlike cutaneous melanoma, UM does not harbor mutations in *BRAF* or *NRAS* genes,[105-109] but instead is characterized by mutations in the *GNAQ* gene (chromosome 9q) and its paralogue *GNA11* (chromosome 19p); these genes encode alpha subunits of the heterotrimeric G proteins associated with the transmembrane G protein-coupled receptors.[110-114] Mutations in these genes are thought to result in the constitutive activation of the mitogen-activated protein kinase (MAPK) pathway and protein kinase C (PKC) pathway, which are involved in cell growth, cell proliferation, differentiation and apoptosis.[110, 111, 113, 115, 116] The MAPK pathway is activated in up to 90% of primary UM [108] and mutations of *GNAQ* and *GNA11* have been reported in 83% to 91% of primary UM, occurring in a mutually-exclusive manner.[113, 117, 118] Mutations in *GNAQ* are reported to occur in 25-50% of tumors, while *GNA11*-mutant cases account for 33-58% (Table 1B).[60, 111, 113, 117-125]

GNAQ and *GNA11* mutations are thought to be initiating events in UM pathogenesis since they are present in the majority of UM, regardless of chromosome aberrations or GEP class, and are also found in benign melanocytic lesions such as blue nevi.[110, 111, 113] Van Raamsdonk et al. found a mutation in either *GNAQ* or *GNA11* in 61% of the 139 blue nevi they have tested,[113] and reported an 83% mutation frequency for *GNAQ* in 29 tested blue nevi in an earlier study.[111] Although most studies could not find a correlation between *GNAQ* or *GNA11* and survival,[113, 118, 126] a recent study by Griewank et al. reported a predominance of *GNA11* mutations in UM metastases, and a poorer disease-specific survival of *GNA11*-mutant tumors in a cohort of 30 UM patients with metastases.[127] In 101 UMs treated by primary enucleation in the LUMC, we found monosomy 3 in 70% of *GNA11*-mutant UMs (n=53) versus 48% in *GNAQ*-mutant UMs (n=48) (Pearson's chi-squared test, p=0.03) (unpublished data). Although we noticed a trend towards worse survival for *GNA11*-mutant tumors compared to *GNAQ*-mutant cases, this difference was not signicant (log-rank test, p=0.27) (unpublished data).

As mentioned above, the strong correlation between loss of heterozygosity of chromosome 3 and an adverse prognosis raised the suspicion that loss of function of tumor suppressor genes on chromosome 3 may result in a malignant phenotype. Early efforts to identify the critical region of chromosome 3 yielded varying results.[128-130] Blasi et al. found a translocation involving chromosome region 3p13 as the only clinical aberration in a primary UM cell culture and

suggested that this region could harbor a pathogenically-relevant tumor suppressor gene.[128] Tschentscher et al. investigated partial deletions of chromosome 3 and found two regions (3q24-26 and 3p25) that were frequently lost.[129] A study by Parrella et al. identified the same region (3p25.1-25.2), and overlapping results were reported by Cross et al. and van Gils et al., who also speculated on a segment (3p12-3p14) similar to the one addressed earlier by Blasi et al. [96, 128, 130, 131]

In 2010, inactivating hemizygous somatic mutations of the *BAP1* (BRCA1-associated protein 1) gene on chromosome 3p21.1 were identified in 47% (27/57) of cases. *BAP1* mutation was found in most metastasizing UMs, occurring in 84% (26/31) of class 2 tumors and in only 4% (1/26) of class 1 cases.[132]. Subsequent studies showed that inactivating mutations of *BAP1* occur in 32-58% of primary UM.[60, 62, 119-122, 124, 125, 133] Mutation of *BAP1* is also strongly correlated with chromosome 3 status, ocurring in 89% of monosomy 3 tumors and in no disomy 3 tumors, in a cohort of 66 Ums.[133]

Loss of *BAP1* gene expression has been shown to correlate well with the lack of BAP1 protein expression, which has been proposed as a clinically valuable prognostic tool.[62, 100, 133-135] Metastases arise when there is a combination of loss of one chromosome 3 and a mutation in the *BAP1* gene on the other chromosome, leading to loss of expression of BAP1.[62, 100]

The BAP1 protein is a ubiquitin carboxyterminal enzyme that affects the activity of other proteins through deubiquitination. For example, it regulates gene expression epigenetically by removing ubiquitin molecules from histone H2A. It has been demonstrated that loss of BAP1 function leads to the loss of the melanocytic cell phenotype and loss of differentiation in UM.[136]

Germline mutations in *BAP1* [137, 138] have been identified in 2-3% of UM patients.[139-141] These patients tend to have a family history of UM. A recent study reported *BAP1* germline mutations in approximately 20% of familial cases of UM.[142] Patients with *BAP1* germline mutations have larger tumors, with more common ciliary body involvement, both of which are related to a higher risk of metastasis.[140] These mutations may be present in UM occurring at a younger age.[143] In addition, patients with germline *BAP1* mutations are at higher risk of other cancers such as lung adenocarcinoma, renal cell carcinoma, meningioma, and malignant mesothelioma,[137, 144-146] prompting the need for treating physicians to recognize familial cases of UM and to identifying patients with germline *BAP1* mutations.

In contrast to *BAP1*, mutations in the *SF3B1* (splicing factor 3 subunit B1) gene on chromosome 2q are associated with favorable prognostic parameters such as younger age at diagnosis and fewer epithelioid cells, whilst being inversely associated with adverse prognostic features such as monosomy 3 and the class 2 gene expression profile.[123] Patients with mutations in this gene account for 10% to 24% of UM cases.[60, 119-125] In the study by Furney et al., patients with *SF3B1* mutations showed a better prognosis than patients with *SF3B1*-wildtype tumors,[121] while in another study significance could not be reached [123] and in a study with a relatively short follow-up (48 months) no association with metastatic disease was reported.[60] In a long-term study by Yavuzyigitoglu et al. using whole-exome sequencing, an association of mutated *SF3B1* and favorable prognosis was observed in the overall group (n=133, 32 *SF3B1*-mutant) during the first few years of follow-up; however, this difference was less evident at longer follow-up since patients with *SF3B1* were noticed to develop metastases at a later stage. Within the disomy 3 cohort, patients with *SF3B1* mutations had an increased metastasic risk when compared to patients without this mutation and developed metastases at a median follow-up of 8.2 years. *SF3B1* mutation was therefore correlated with late-onset metastasis and was the only parameter independently associated with worse survival in disomy 3 tumors in the multivariate analysis. Most (11/14) disomy 3 patients who developed metastases had an *SF3B1* mutation, while *BAP1* mutations were found in two other disomy 3 patients who developed metastases.[125] These mutations were missense mutations and the tumors stained positively for BAP1 using immunohistochemistry. Although it may be assumed that a nonfunctional protein is produced, this should be validated by functional assays.

Mutations in the *EIF1AX* (eukaryotic translation initiation factor 1A, X-linked) gene on chromosome Xp are found in 8% to 21% of primary UMs and are associated with a decreased risk of metastasis.[60, 119-121, 123-125] Ewens et al. reported a 10-fold lower metastasic risk for disomy 3/*BAP1*-wild type/*EIF1AX*-mutant tumors, when compared to disomy 3/*BAP1*-wild type/*EIF1AX*-wild type cases.[60] The association of *EIF1AX* mutations and a favorable clinical outcome was confirmed in two recently published studies.[119, 125] Together, these reports show that mutations in *BAP1*, *SF3B1* and *EIF1AX* occur in a mutually exclusive manner, which has been underlined by a study that reported on the results of whole-genome sequencing in 33 samples.[147] Moreover, mutations in these three genes are associated with differing risks of developing metastasis. Tumors with *BAP1* mutations show a high

and early metastatic risk whereas tumors with mutated *SF3B1* are associated with late-onset metastasis and *EIF1AX*-mutant tumors have a very low metastatic risk.[125]

3. GENETIC TESTS

Diverse genetic techniques, such as karyotyping, fluorescence in situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA), array-based comparative genomic hybridization (aCGH), single-nucleotide polymorphism (SNP) assay, and GEP are commonly utilized to determine genomic tumor characteristics with prognostic value in UM. Relevant aspects to take into consideration with regard to the application of a certain test are the type of tumor specimen (fresh tumor tissue/frozen/formalin-fixed paraffin embedded), the available genetic material from the tumor specimen (DNA/RNA), the prognostic accuracy, and the costs of the test. In this section, we discuss briefly the most important tests that can be utilized for genetic prognostication in UM and mention their respective advantages and disadvantages (Table 2).

Initial studies reporting on the prognostic value of aberrations in chromosomes 3 and 8 [40-43] used karyotyping of short-term cultured UM cells, which was also utilized in later studies to further characterize UM cytogenetically.[68, 148] The advantage of karyotyping is that it provides information on all chromosomes in a single assay and allows the identification of structural and balanced chromosome abnormalities, in addition to numerical changes. However, tumor specimens must be fresh since viable dividing cells are required. Furthermore, this method is labor-intensive test and has to be performed by an experienced cytogeneticist. Another disadvantage of karyotyping is that it can only reliably detect gross aberrations due to its overall low resolution of approximately 5 to 10 Mega base pairs (Mbp).[149-151] Kilic et al. have reported a 100% 10-year mortality in patients with loss of chromosome 3p detected by karyotyping, and a 30% mortality rate in patients without this aberration.[68] The relatively high percentage of mortality in patients without detected loss of chromosome 3 may be explained by the low sensitivity of karyotyping in detecting LOH in cases of isodisomy 3 (copy-neutral LOH).[51]

Another approach to chromosomal testing is FISH, which can be performed on aged, frozen and paraffin-embedded specimens as well as fresh samples. FISH uses a technique where a specific colored probe is used that binds

Table 2. Main advantages and limitations of genetics tests commonly utilized in UM prognostication.

Genetic test	Main advantages	Main limitations
Karyotyping	- information on all chromosomes in single assay - identification structural and balanced chromosome abnormalities	- fresh tumor specimens with viable dividing cells needed - labor-intensive - experienced cytogeneticist required - low resolution (only reliable for gross aberrations) - prone to sampling error (tumor heterogeneity) - can not detect isodisomy 3
FISH	- can be performed on fixed samples - relatively easy technique	- only identification targeted (regions of) chromosomes - can not detect isodisomy 3 - prone to sampling error (tumor heterogeneity)
aCGH	- can be performed on fixed samples - provides copy numbers of all chromosomes - high resolution (can detect smaller aberrations than karyotyping and FISH)	- can not detect isodisomy 3 - prone to sampling error (tumor heterogeneity)
SNP	- can be performed on fixed samples - high resolution - detects isodisomy 3 - relatively inexpensive	- prone to sampling error (tumor heterogeneity)
MSA	- can be performed on fixed samples - detects isodisomy 3 - inexpensive	- prone to sampling error (tumor heterogeneity) - low resolution
MLPA	- can be performed on fixed samples - suitable for small samples (biopsies) - detects aberrations of chromosomes 1p,3,6, and 8 in single reaction - relatively inexpensive	- prone to sampling error (tumor heterogeneity) - can not detect isodisomy 3
GEP	- can be performed on fixed samples - gene expression information on all chromosomes - analyzes the tumor microenvironment - claimed to be the most accurate test	- costly - probably prone (claimed to be insensitive) for sampling error (tumor heterogeneity)

to a specific chromosome site. FISH can be performed on tissue secions as well as isolated nuclei.[152] Two advantages of FISH over karyotyping are, first, that it does not require the presence of viable dividing cells to yield a successful result and, second, it does not have to be performed by an experienced cytogeneticist.[151] FISH has been shown to be a reliable technique for detecting chromosome 3 and 8 aberrations [153] and has been used, alone or in combination with conventional karyotyping, for the assessment of the chromosomal status of UM.[52, 54, 66, 148, 152, 154, 155]

Limitations of FISH are that it only allows evaluation of alterations in the targeted (region of) a chromosome [156] and its inability to detect isodisomy of chromosome 3 and structural abnormalities such as partial deletions (in particular if only centromeric probes are used).[54, 157] An example of this is provided by Lake et al. in metastasizing UM, where MLPA identified two cases with multiple deletions in 3p and 3q, which were previously missed by FISH.[158] Although it has been reported that FISH and MLPA have similar predictive powers,[159] Damato et al. have shown that MLPA is more sensitive than FISH in detecting partial deletions of chromosome 3,[71, 160, 161] also validating the use of this technique for prognostication in UM in a large cohort of 452 choroidal melanomas.[55] MLPA can be performed on smaller samples than FISH, which makes it suitable for use in biopsy material.[157] Although MLPA can be used on formalin-fixed tumor specimens, the use of fresh or snap-frozen material is preferred.[162] However, similar to karyotyping and FISH,[152, 155, 163, 164] MLPA is prone to sampling errors caused by tumor heterogeneity.[71, 165] The effect of sampling errors on predictive value of these tests was not specifically determined.

Another technique used for genetic testing in UM is aCGH.[74, 166, 167] In aCGH, tumor DNA and reference DNA are labelled differently and hybridized with cloned DNA fragments (±100-200 kb) of which the exact chromosomal location is known.[168, 169] aCGH provides genome-wide information on copy-number variations and can detect smaller aberrations than karyotyping and FISH.[167, 170] However, as with karyotyping, FISH, and MLPA, aCGH is unable to detect copy-neutral LOH, and therefore can not identify isodisomy 3.

A more modern technique is the use of SNP arrays, in which variations of single nucleotides are evaluated. A major advantage of SNP is its ability to detect isodisomy 3, since it can distinguish the two copies of chromosomes inherited from each parent. In a study by Onken et al. SNP was more accurate than FISH and aCGH in detecting LOH of chromosome 3 due to its ability to identify isodisomy

3.[72] Another technique that can be utilized to detect isodisomy 3 is microsatellite analysis (MSA), which evaluates the presence of informative microsatellite marker regions of repetitive DNA on chromosomes. However, this technique is also susceptible to sampling error resulting from tumor heterogeneity.[171]

Recently, whole-exome sequencing (WES) and whole-genome sequencing (WGS) have been applied for research purposes to evaluate the genetic landscape of UM.[125, 147] WES sequences all exons of the genome, thereby identifying genetic variants that alter protein sequences, while WGS also sequences the non-coding regions. Although WGS provides more information than WES, it has higher costs and is more time-consuming. However, costs keep decreasing, probably soon allowing WGS for a fair cost.

In contrast to the above-mentioned genetic tests, which analyze DNA, GEP evaluates mRNA expression to stratify tumors into two main prognostic classes, class 1 and class 2.[88] In the initial paper correlating GEP classes to survival, Onken et al. stated that this molecular classification may potentially be superior to chromosomal analysis in predicting high-risk cases.[88] However, the studies showing a higher accuracy of GEP were performed with relatively few patients and FISH and aCGH were used instead of more reliable chromosome tests to detect chromosome copy number variations. As mentioned earlier, these techniques are unable to detect isodisomy of chromosome 3, which is related to the development of metastatic disease.[72] Multiple chromosome changes are very strongly associated with a specific GEP and it has been shown that combining prognostic information provided by different chromosomes increases the predictive accuracy of chromosomal testing.[52, 54, 55, 74, 166]

Although GEP is more costly than tests based on chromosomal analysis, one of the advantages of GEP over chromosomal testing has been proposed to be its insensitivity for sampling errors due to tumor heterogeneity. This is supposedly due to the fact that it evaluates the tumor environment, which is less variable across the tumor than the cytogenetic markers.[98] Nevertheless, a recently published study by Augsburger et al. evaluating the GEP classification of biopsy samples from different sites within a tumor, reported a discordance rate of 11%.[172] Although GEP is able to provide prognostic information even in very small samples,[173] Augsburger et al. found that discordant GEP results occur most frequently (24%) in small tumors (thickness<3.5mm).[172] To decrease the risk of misclassification, the Collaborative Ocular Oncology Group has recommended taking several samples if the tumor consists of morphologically different areas.[99]

In concordance with this recommendation, a recently published case report showed discordant GEP results of samples with different histopathologic features.[174]

4. CLINICAL ASPECTS OF GENETIC PROGNOSTICATION

Genetic testing for prognostication in UM is now applied in many ophthalmic oncology centers. The clinical application of genetic testing has raised certain issues, which we will highlight in this section. Below we discuss implications of genetic prognostication for follow-up and therapy of patients. We also address clinical issues regarding the use of biopsy material and the application of genetic testing in irradiated tumors, highlight statistical issues considering the interpretation of the results of genetic prognostication, and discuss the psychological aspects of genetic prognostication for patients.

4.1 Implications for follow-up and therapy

Genetic prognostication will play an important role in the stratification of patients into clinical trials in order to evaluate the efficacy of novel adjuvant treatments. It allows the identification of high-risk patients who may benefit the most from adjuvant therapies and thereby guides enrollment of patients into clinical trials to test therapies targeting micrometastases. Prognostic stratification allows clinicians to taylor follow-up according to metastatic risk: those with a low-risk can be spared from follow-up examinations, saving costs, while more intensive surveillance can be offered for high-risk patients. However, there is a lack of consensus regarding the type and frequency of systemic screening,[175] and the possible survival benefit of earlier detection of clinical metastases for the individual patient has been questioned since no effective treatment for metastatic UM yet exists.

In a study by Kim et al., the median survival after diagnosis of primary UM of 90 patients with metastatic UM detected by surveillance was only 4.5 months longer than the survival of 259 patients who were diagnosed with metastatic UM at the time that they developed symptoms. The percentage of patients receiving treatment for their metastases and the treatment type was comparable between the groups. The authors concluded that the difference in survival was due to lead-time bias.[176] An earlier study similarly reported a longer survival after detection of metastases (8.9 vs 4.3 months) in patients who had undergone surveillance examinations.[177] However, the survival time after diagnosis of the primary tumor

was comparable. More recent studies evaluating the effects of novel treatment options have reported prolonged survival in patients whose metastases were detected earlier.[178, 179] Nevertheless, no clear survival benefit from screening for metastases was reported in a literature review by Augsburger et al.,[180] while a recently published review concluded that adjuvant therapy has not been shown to improve survival in UM.[181]

Studies on the analysis of genetic differences between high-risk and low-risk tumors have led to an evolved understanding of the pathophysiology of UM. The discovery of *GNAQ/GNA11* and *BAP1* mutations has contributed to the unraveling of the molecular landscape of UM and provided opportunities for targeted therapy of metastatic disease.[116, 182, 183] Progress in the molecular characterization of UM may not only enhance prognostication but may also contribute to the development of targeted therapy and may in the near future even allow for individualized treatment based on mutational analysis of the tumor.

4.2 Genetic testing in biopsies

UMs treated by enucleation provide adequate tumor specimens for genetic testing. Since the entire tumor is available, samples from different parts of the tumor can be sent in for genetic analysis. However, most UMs are currently treated by globe-preserving techniques such as plaque radiotherapy and proton beam irradiation while enucleation is reserved for larger tumors.[184] In tumors treated by eye-conserving methods, biopsies may be taken to obtain tumor material for genetic prognostication. Although there is a risk of localised bleeding, vitreous hemorrhage, retinal detachment and tumor seeding after a tumor biopsy, these risks are small and fine-needle aspiration biopsy (FNAB) is considered a safe procedure.[56, 154, 185-187]

Tumor size may be a limiting factor since larger tumor volume makes it easier to acquire enough material for testing. Even in enucleated cases, greater tumor size was found to be correlated with a higher success rate of FISH in 213 primarily enucleated tumors.[148] McCannel et al. performed transscleral FNAB in 170 cases and reported that sufficient material for FISH was obtained in 91% of tumors with a thickness over 5 mm, while this percentage was only 53% for tumors less than 3 mm thick.[186] In a study of FNAB performed in a cohort of 150 UM, Singh et al. found that a sufficient yield of tumor material was similarly related to tumor size, with more successful tests in larger tumors (basal diameter >5.0 mm, height >2.5 mm), as well as to the biopsy approach (success rates, transcorneal: 100%,

transscleral: 96%, transvitreal: 86%).[188] In contrast, Shields et al. determined the chromosome 3 status in FNAB specimens by analyzing microsatellite markers and found that a transvitreal approach yielded sufficient material in almost all cases (97%, 31/32) while a transscleral approach showed a success rate of 67% (16/24).[189] However, a study in 38 patients showed comparable results for transvitreal and transscleral FNAB approaches, with sufficient material for cytopathological analysis in 71% and 66% of tumors, respectively.[190] Recent improvements in surgical techniques and laboratory methods increased the success rate of genetic testing in biopsied samples as shown by the group of Coupland.[191] They analyzed their samples biopsied between 2011 and 2013 and noticed an increase in success rate from 79% to 93%.

The type of needle may also influence tissue yield, which is affected by the diameter of the needle bore so that a larger specimen is obtained by FNAB with 25-gauge needles than with 27-gauge or 30-gauge needles.[192] In a study of 18 cases of UM, transscleral FNAB using a 30-gauge needle yielded sufficient material for FISH for chromosome 3 testing in 50% of cases.[193] Midena et al. obtained sufficient material for chromosome 3 testing with FISH with a 25-gauge transscleral FNAB in 7 of 8 cases [185] and in 81% of tumors in a subsequent larger cohort (n=32).[154]

As already indicated, the biopsy technique may influence the size of the specimen and the success rate. Transretinal biopsies using a 25-gauge vitrector have been shown to harvest larger tissue samples when compared to FNABs, improving the chance of obtaining adequate tumor samples for histologic examination and cytogenetic analysis.[192, 194] Bagger et al. have shown that the theoretical tissue yield of a 25-gauge vitrector-based biopsy is higher than the tissue yield of FNABs using 25-gauge, 27-gauge, and 30-gauge needles.[192] They have reported a low risk of complications for this procedure.[195]

The question has arisen as to whether a single biopsy is truly representative of the entire tumor. As mentioned previously, genetic heterogeneity in UM has been reported [165] and may cause genetic misclassification of tumors when genetic testing is performed on biopsies. Naus et al. reported the application of FISH in FNABs to be a reliable method for assessing chromosome 3 and 8q status in 40 UM samples;[196] however, other studies reported heterogeneity for chromosome 3 as determined by FISH in 14% [152] to 32% of cases.[197] Regarding MLPA for chromosome 3 status, interpretation of results was complicated by genetic heterogeneity in 13% of cases.[165] In contrast, in a recent study by Coupland et al.

who performed MLPA (n=14) and MSA (n=14) on 28 biopsies and matching tumor sections, concordant results for chromosome 3 status were reported for all cases.[198] As mentioned above, it has been suggested that because GEP evaluates the tumor microenvironment, which is expected to be less variable across the tumor, one might expect that GEP is less prone to sampling errors caused by genetic heterogeneity. Nevertheless, a discordance rate of 11% was reported recently by Augsburger et al., who compared two random samples from the same tumor. To increase the chance of obtaining sufficient tumor material and to minimize the risk of genetic misclassification of the tumor, various authors have proposed performing vitrector-based biopsies or taking multiple FNAB samples.[172, 192]

4.3 Genetic testing in irradiated tumors

Most patients with UM are treated by irradiation, with a biopsy being performed prior to the radiotherapy. However, in some centers, biopsies are not taken routinely and genetic testing is performed only on secondarily-enucleated tumors in which the radiotherapy failed. In addition, some patients undergo endoresection of the tumor after radiation therapy of large melanomas, which yields tumor material for genetic analysis.[199] However, as the radiobiological effects of irradiation on tumor cells causes necrosis and fibrosis,[200-205] the probability of successfully performing a genetic test on these tumors is questioned. We recently published a study evaluating success rates of karyotyping and FISH, and found that both tests are more likely to be successful in primarily-enucleated tumors (n=291) than in enucleated tumors following radiotherapy (n=36, 28 Ruthenium-106 brachytherapy).[148] Karyotyping was successful in 79% of primarily-enucleated cases, while this was the case in only 25% of the previously-irradiated tumors. FISH was done when karyotyping had not shown monosomy 3 or had failed and was more often than karyotyping successful in irradiated cases (17/35, 49%) (Table 3). Horsman et al. achieved successful karyotyping in 20 of 23 (87%) primarily-enucleated tumors and 7 of 12 (58%) previously-irradiated tumors (gold plaque brachytherapy).[47] The greater success rate when compared to our cohort may be due to a difference in the time interval between radiotherapy and time of enucleation, which may play a determinative role in whether genetic testing is successful, as more necrosis and fibrosis has been reported when the time interval between irradiation and enucleation was larger.[202, 203]

Table 3. Success rates of different genetic tests in irradiated uveal melanomas.

Study	Genetic test	Type of specimen	Type of irradiation	Time between irradiation and enucleation/ endoresection/ biopsy or genetic testing	Success rate in irradiated tumors
Horsman et al. (1993)	Karyotyping (n=12)	Enucleation	Gold plaque brachytherapy	*Not specified*	58%
Wackernagel et al. (2013)	CGH (n=15)	14 Endoresection 1 Enucleation	10 Gamma-Knife radiotherapy 5 Ruthenium-106 brachytherapy	~ 5 months (*median)*	100%
Gold et al. (2014)	GEP (n=3)	Biopsy	2 Iodine-125 brachytherapy 1 Proton beam irradiation	~ 27 months *(median)*	100%
Coupland et al. (2015)	MLPA (n=8) MSA (n=1)	6 Enucleation 2 Endoresection Enucleation	5 Ruthenium-106 brachytherapy 3 Proton beam irradiation Proton beam irradiation	~ 11 months *(median)* ~ 34 months	100% 100%
Dogrusöz et al. (2015)	Karyotyping (n=36) *with additional* FISH (n=35)	Enucleation	28 Ruthenium-106 brachytherapy 5 Proton beam irradiation 3 Stereotactic radiotherapy	26 months *(median)*	25% 49%

Symbol: ~: indicates a rounded value or estimation since in the respective studies the time periods were mentioned as days or weeks

Secondly, not only the time interval but also the cause for the secondary enucleation may influence the success of genetic testing. In our study, we found that, although not significantly different, karyotyping as well as FISH tended to be more often successful in irradiated tumors that were enucleated because of tumor recurrence, compared to enucleations due to tumor non-responsiveness or radiation-related complications.[148] The fact that the recurrent tumor is unaffected by radiobiological damage inflicted by irradiation may explain this difference. Thirdly, the success of genetic typing in irradiated tumors may also depend on the type of test. Wackernagel et al. performed CGH in 15 irradiated UMs (5 Ruthenium-106 brachytherapy, 10 Gamma-Knife radiotherapy) and obtained successful results in all cases,[206] while Gold et al. successfully performed GEP after radiotherapy in 3 cases (2 Iodine-125 brachytherapy, 1 proton beam irradiation).[207] Coupland et al. successfully determined the genetic status of 8 previously irradiated (5 Ruthenium-106 brachytherapy, 3 proton beam irradiation) solid (enucleation/endoresection) specimens using MLPA and in one enucleated specimen that was previously treated with proton beam irradiation using MSA.[198] However, these three studies were done in small cohorts of only a few cases. Another important issue regarding genetic testing after irradiation is whether the results are representative of the primary genetic status of the tumor, since radiotherapy may cause genetic alterations. Hussein et al. performed survival analysis in 102 patients and found a metastatic death rate of 0% in 63 patients with disomy 3, while 35% of 39 monosomy 3 patients died of UM metastases.[208] This suggests that genetic testing by MLPA/MSA after proton beam irradiation is reliable and accurately predicts disease outcome. The fact that biopsies were taken less than a month after proton beam irradiation may have contributed to their accurate testing results, since the time between irradiation and sampling may affect the success of genetic testing. Coupland et al. reported concordant results for chromosome 3 status when comparing the biopsy specimens with the enucleated specimens in 4 tumors that were treated by Ruthenium-106 brachytherapy and secondarily enucleated due to tumor recurrence.[198] Wackernagel et al. found concordant results for chromosome 3 and 8 status determined by CGH pre-radiotherapy and post-radiotherapy in 5 cases with a median time interval between radiotherapy and genetic analysis of 76 days.[206] Further studies evaluating genetic testing pre-radiotherapy and post-radiotherapy in larger cohorts with longer follow-up are necessary to determine the reliability of the different genetic tests after radiotherapy. Because of the lack of validation

in post-radiotherapy tumors, the use of DecisionDx-UM GEP in irradiated samples has been considered ineligible by the manufacturer of this commonly used GEP kit.[209]

4.4 Statistical considerations

Prognostic tools must take account of lead-time bias, competing risks, bias caused by missing data, loss of precision arising from categorization of continuous data, and other factors. For example, with respect to lead-time bias, larger tumors are associated with a shorter life expectancy partly because they have been growing and metastasizing for a longer time.[102] As for competing risks, the censoring (i.e., exclusion from analysis) of patients dying of unrelated disease may exaggerate the apparent metastatic mortality.[210] For this reason, it is necessary to take account of the life-expectancy of the general population, matched by age and sex.[102] Bias may also arise if non-random missing data is simply excluded, which is why some statistical models estimate the likely values of missing data according to other prognostic factors.[101]

Loss of precision occurs when continuous data are categorized into groups so that, for example, an 0.1 mm difference in tumor diameter results in a great adjustment of survival probability.

Not all factors associated with mortality are useful for prognostication so that a multivariable analysis is needed to determine which factors to include in the statistical model.[101, 211] The sample size and the number of events (i.e., deaths) should be large enough for the model to have adequate statistical power.

To ensure that the prognostic tool is relevant to patients who were not included in its development, the statistical model should be evaluated on a test dataset that is separate from the training dataset, unless methods such as bootstrapping are used. Every prognostic tool should ideally be validated externally by different centres, which should ensure that tumor diameter and thickness are measured in a standardized manner and that structures such as ciliary body are defined consistently.[212]

Genetic tumor type only indicates whether or not the tumor has metastatic potential. If it does, then factors such as tumor size and mitotic count may indicate the likely survival time.[211] If genetic studies suggest that the tumor has no metastatic potential, then anatomic and histological predictors should in theory not influence the prognosis; however, these biomarkers may sometimes cast doubt on a genetic test result (e.g., ostensible disomy 3 in a large tumor with

ciliary body involvement, extraocular spread, epithelioid cells, closed loops and a high mitotic count).

4.5 Psychological aspects of genetic prognostication

Most ocular oncology centers offer UM patients genetic testing for prognostication. Since an effective treatment for UM metastasis is lacking, the main benefits of genetic prognostication are reassurance or life-planning. On the other hand, genetic testing for prognostication may have negative psychological consequences. One can expect patients with a poor prognosis will experience psychological distress and regret their decision to know their prognosis.

In a study in 298 UM patients, 97% accepted genetic prognostication and none of the patients later regretted their decision to have testing.[213] Patients reported that they gained a sense of control, which was linked to the hope that screening and early treatment would improve their survival. The authors gained the impression that patients with a good prognosis benefitted the most. In another study, 36 of the 38 patients who received a prognostic test stated that they wanted to know the results, which to the authors indicated no obvious regret of the decision to undergo testing.[214] The majority of patients (58%, n=14 of 24) who received a conclusive result (monosomy 3/disomy 3) perceived prognostic testing as useful. However, significantly more patients who received a disomy 3 result (69%, n=9 of 13) perceived testing as useful as compared to those with a monosomy 3 tumor (46%, n=5 of 11). Disomy 3 and monosomy 3 patients perceived genetic testing as useful for different reasons: patients with a disomy 3 tumor indicated that the result provided relief/hope, while patients with a monosomy 3 tumor generally indicated that the genetic testing result inspired them emotionally and/or gave a reason to prepare for a shortened life. When assessing depressive symptoms and quality of life (mental/physical), the authors did not find significant differences between patients who received prognostic testing versus those who did not undergo testing, nor between monosomy 3 and disomy 3 patients. Similarly, Hope-Stone et al. did not find differences in experiences of uncertainty between patients who received a poor prognosis and those receiving a good prognosis. The authors concluded that a good prognostic result does not necessarily relieve feelings of uncertainty.[215]

In a recently published longitudinal study of 96 patients, depression, anxiety, and decision regret prior to prognostication and at 3 and 12 months afterwards was assessed.[216] In contrast to the aforementioned studies, the authors observed

decision regret in 10% and depression symptoms in 9% of their patients at 12 months follow-up. Decision regret was not correlated with an unfavorable prognosis but was associated with depression, which may make patients question their decisions.

5. CONCLUSIONS AND FUTURE PERSPECTIVES: IMPROVING GENETIC PROGNOSTICATION

Since the identification of monosomy 3 as a prognostic marker, using karyotyping,[40] considerable progress has been made in the genetic prognostication of UM. New genetic markers have been identified, which, besides being used as a prognostic indicators, have also enhanced our understanding of the pathophysiology of UM. The genes that initiate tumor formation and metastasis have been identified, and this has resulted in the discovery of new targets for therapy. Assessment of the applicability of genetic tests in specific types of tumor specimens such as biopsy samples and irradiated tumors have expanded the group of tumors in which genetic testing for prognostication is possible. However, more research is necessary to determine which biopsy type and approach yields the best success rate with respect to genetic testing and prognostication. Also more studies need to be conducted to validate genetic testing in irradiated tumors.

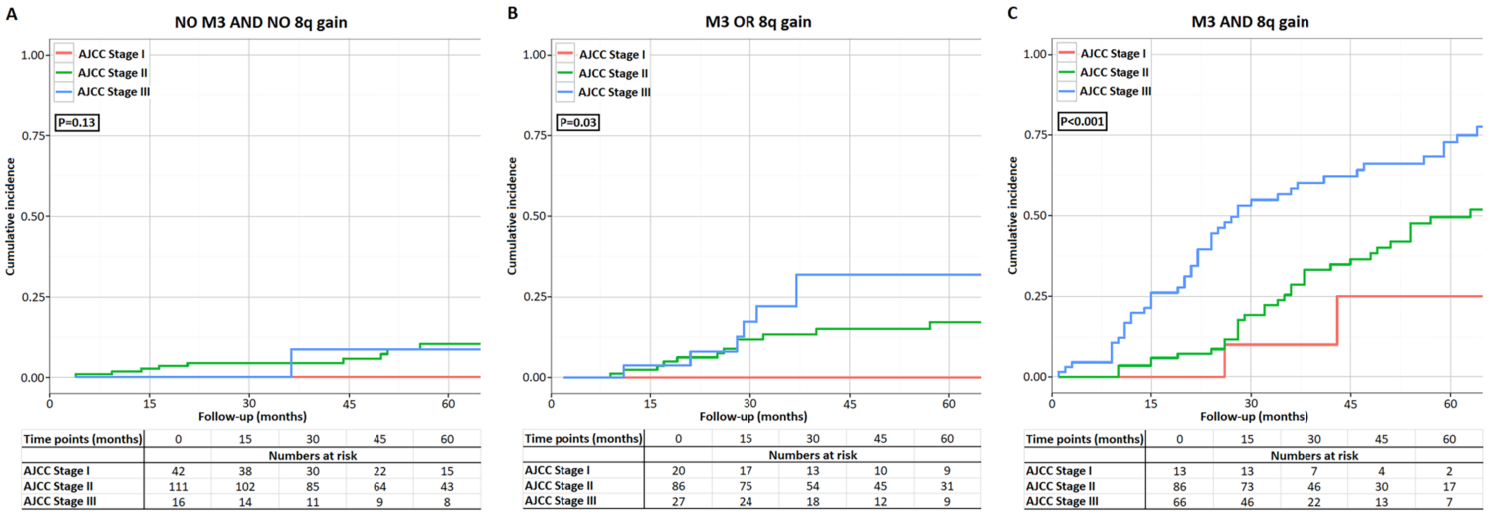
The field of prognostication in UM is a rapidly advancing one. Although chromosome status and GEP are shown to be accurate methods for prognostication, both carry a risk of tumor misclassification. Recent discoveries of specific gene mutations have made further risk stratification possible and helped to explain exceptional cases. Combining different genetic prognostic measures with other tumor features and patient demographics clearly enhances prognostic accuracy. MLPA for chromosome 3 has been shown to have increased accuracy when combined with information on the status of chromosome 8q as well as tumor diameter and histologic parameters characteristic of high-grade malignancy.[71] Damato and associates have created the Liverpool Uveal Melanoma Prognosticator Online (LUMPO) tool, which takes into account age, sex, chromosome 3 status, tumor size, tumor location, extraocular extension, cell type, presence of extracellular closed-loop matrices and mitotic count to estimate the survival probability for an individual patient.[101, 102, 217] This tool has been used effectively to detect patients with a predicted 5-year mortality of at least 50% for

inclusion in a prospective study evaluating metastasis screening by MRI in 188 high-risk cases.[218]

Similar to the work of Damato and colleagues, combining genetic alterations with the AJCC stage, which is based on tumor size, extraocular extension and ciliary body involvement, has been proposed as a means of enhancing prognostic accuracy.[219] We have expanded a prior study [220] by combining data from our center with their data (the Copenhagen University Rigshospitalet) and we have demonstrated that the prognostic values of chromosome 3 and 8q status as well as the AJCC stage are enhanced when these prognostic parameters are applied together.[61] We have shown that adding information on the AJCC staging improves the prognostic value of chromosome 3 and 8q status (Figure 2). Since chromosome 3 status corresponds closely to the GEP classes, it is expected that combining AJCC staging with GEP would enhance prognostication. In line with these findings, two recently published studies have reported that the tumor diameter has prognostic significance that is independent of GEP class and show that adding information on the tumor diameter to GEP improves the prognostic value of GEP.[103, 104] Further stratification of risk estimates provided by genetic parameters may also be possible by combining information of several genetic determinants.

Combining chromosome status with specific mutations such as *BAP1/ SF3B1/EIF1AX* mutations also improves prognostic acccuracy [60, 119, 125] and recently *PRAME* has helped to stratify metastatic risk in GEP class 1 as well as class 2 tumors.[90]

These recent findings indicate that genetic prognostication is an advancing field in which continued research is expected to further enhance prognostic accuracy and improve patient counselling, planning of follow-up, trial enrollment, and the identification of new therapeutic targets.



Time points (months)	0	15	30	45	60
	Numbers at risk				
AJCC Stage I	42	38	30	22	15
AJCC Stage II	111	102	85	64	43
AJCC Stage III	16	14	11	9	8

Time points (months)	0	15	30	45	60
	Numbers at risk				
AJCC Stage I	20	17	13	10	9
AJCC Stage II	86	75	54	45	31
AJCC Stage III	27	24	18	12	9

Time points (months)	0	15	30	45	60
	Numbers at risk				
AJCC Stage I	13	13	7	4	2
AJCC Stage II	86	73	46	30	17
AJCC Stage III	66	46	22	13	7

Figure 2. Cumulative incidence curves showing the effect of adding the AJCC staging on the prognostic value of chromosome 3 and 8q status. A: NO M3 and NO 8q gain tumors, B: M3 OR 8q gain tumors, C: M3 AND 8q gain tumors. Adopted from reference# 61.

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