Metastatic Disease in Polyploid Uveal Melanoma Patients Is Associated With *BAP1* Mutations

Serdar Yavuzyigitoglu,[1,2] Hanneke W. Mensink,[3] Kyra N. Smit,[1,2] Jolanda Vaarwater,[1,2] Robert M. Verdijk,[4] Berna Beverloo,[2] Hennie T. Brüggenwirth,[2] Ronald van Marion,[4] Hendrikus J. Dubbink,[4] Dion Paridaens,[4] Nicole C. Naus,[1] Annelies de Klein,[2] and Emine Kiliç[1]; for the Rotterdam Ocular Melanoma Study Group (ROMS)

[1]Department of Ophthalmology, Erasmus University Medical Center, Rotterdam, The Netherlands
[2]Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands
[3]The Rotterdam Eye Hospital, Rotterdam, The Netherlands
[4]Department of Pathology, Erasmus University Medical Center, Rotterdam, The Netherlands

Correspondence: Emine Kiliç, Erasmus University Medical Center, Department of Ophthalmology, Room Number Ee-1610, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands;
e.kilic@erasmusmc.nl.

SY and HWM contributed equally to the work presented here and should therefore be regarded as equivalent authors.

Study Group: See the appendix for the members of the Rotterdam Ocular Melanoma Study Group (ROMS).

Submitted: November 9, 2015
Accepted: February 8, 2016

Citation: Yavuzyigitoglu S, Mensink HW, Smit KN, et al.; for the Rotterdam Ocular Melanoma Study Group. Metastatic disease in polyploid uveal melanoma patients is associated with *BAP1* mutations. *Invest Ophthalmol Vis Sci*. 2016;57:2232–2239. DOI:10.1167/iovs.15-18608

Purpose. Most of the uvea melanoma (UM) display a near-diploid (normal, ~2N) karyotype with only a few chromosomal changes. In contrast to these simple aberrations 18% of the UM samples show a polyploid character (>2N) and this was associated with an unfavorable prognosis. This study attempts to gain insight in the prognostic value of polyploidy in UM.

Methods. In 202 patients the ploidy status of the UM was determined using cytogenetic analysis, fluorescence-in-situ-hybridization (FISH), multiplex ligation dependent probe amplification (MLPA), and/or single nucleotide polymorphism (SNP) array analysis. Immunohistochemistry was used to determine the BAP1 expression and mutation analyses of *BAP1* (coding regions) and the mutation hotspots for the *SF3B1*, *EIF1AX*, *GNAQ*, and *GNA11* genes was carried out using Sanger sequencing or whole-exome sequencing.

Results. Twenty-three patients had a polyploid UM karyotype (11.4%). Patients with a polyploid tumor had larger tumors (15.61 vs. 13.13 mm, $P = 0.004$), and more often loss of heterozygosity of chromosome 3 ($P = 0.003$). No difference in occurrence of mutations between polyploid and diploid tumors was observed for *BAP1*, *SF3B1*, *EIF1AX*, *GNAQ*, and *GNA11*. Polyploidy did not affect survival ($P = 0.143$). BAP1 deficiency was the only significant independent prognostic predictor for patients with polyploid tumors, with a 16-fold increased hazard ratio (HR 15.90, $P = 0.009$).

Conclusions. The prevalence of mutations in the UM related genes is not different in polyploid UM compared with diploid UM. Moreover, similar to patients with diploid UM, *BAP1* mutation is the most significant prognostic predictor of metastasis in patients with polyploid UM.

Keywords: uveal melanoma, BAP1, polyploidy, chromosomal abnormalities, oncology

Uveal melanoma (UM) is the most common primary intraocular malignancy in adults with an annual incidence of approximately 7 to 10 per million.[1] In approximately one-half of the patients UM metastasizes via the blood with a preference for the liver.[1] The prognostic factors linked to metastatic disease include clinical variables (increased age, large tumor size), histopathologic findings (epithelioid cell type, closed vascular patterns), genetic, and chromosomal abnormalities (loss of chromosome 3, gain of chromosome 8q).[2–5]

For UM, the karyotype is usually near-normal (diploid) with only few nonrandom chromosomal changes, such as loss of chromosome 3 (monosomy 3) and gain of chromosome 8q.[6] Besides these near-diploid (~2N) tumors, UM with polyploidy (>2N) have also been described. Based on DNA content, a prevalence of 13% to 18% of polyploid UM has been observed.[7–9] In addition to the prevalence, the prognostic value of the ploidy was also described, in which polyploidy was associated with an unfavorable prognosis.[7,9] However, despite the impact on survival, polyploidy in UM is not mentioned in recent literature or investigated with the current knowledge of UM.

Nowadays in UM research, the focus is more on genetic variations. Monosomy 3 in combination with the loss of function of the tumor suppressor BAP1 (BRCA-associated protein 1) is strongly associated with metastases.[10–13] In contrast, mutations in the *SF3B1* (splicing factor 3 subunit B1) gene and the *EIF1AX* (eukaryotic translation factor 1A) gene are reported mainly in disomy 3 (no loss of chromosome 3) tumors.[14–16] Therefore, mutations in *SF3B1* or *EIF1AX* have been suggested as favorable prognostic factors in UM, with low risk of metastasis.[10,14–17] Mutations in the oncogenes *GNAQ* (Guanine nucleotide-binding protein, q polypeptide) and *GNA11* (Guanine nucleotide-binding protein, subunit alpha-11) are present in the majority of UM and are not associated with patient prognosis.[18–20]

This study attempts to describe the differences between polyploid and diploid UM regarding clinical variables, histopathologic findings, chromosomal abnormalities, and genetic

Table 1. Patient and Tumor Characteristics of the Study Cohort, and Associations Between Ploidy of the Tumor With Other Clinical, Histopathologic, and Genetic Variables

Variables, $n = 202$	Ploidy Status Tumor: Polyploid, Mean or *n*	Ploidy Status Tumor: Diploid, Mean or *n*	*P* Value
Age at diagnoses	62.52 ± 2.67	61.17 ± 1.08	0.689†
Sex			
Male	9	90	0.314‡
Female	14	89	
Localization			
Choroid	15	136	0.263‡
Ciliary body	8	43	
Largest tumor diameter	15.61 ± 0.76	13.13 ± 0.25	**0.004**†
Tumor thickness	8.87 ± 0.75	7.68 ± 0.26	0.131†
TNM classification			
Category 1	1	20	–
Category 2	5	66	
Category 3	10	80	
Category 4	7	13	
Cell type			
Spindle	4	59	0.129‡
Epithelioid/mixed	19	120	
Extracellular matrix patterns			
Absent	12	90	0.925‡
Present	11	86	
Chromosome 3			
Relative loss (<4N*)	23	98	**<0.001**‡
Normal (4N*)	0	81	
Chromosome 3			
LOH	20	98	**0.003**‡
No LOH	3	81	
Chromosome 8q			
Normal (2N)	0	69	**<0.001**‡
Absolute gain (>2N)	23	110	
Chromosome 8q			
Normal (4N*)	4	69	0.047‡
Gain (>4N*)	19	110	
BAP1			
Normal	8	69	0.223‡
Deficient	14	68	
SF3B1			
Wild-type	19	83	0.546‡
Mutated	4	25	
EIF1AX			
Wild-type	22	88	0.125§
Mutated	1	22	
GNAQ			
Wild-type	11	52	0.946‡
Mutated	12	55	
GNA11			
Wild-type	13	60	0.969‡
Mutated	10	47	

P values ≤ 0.005 was considered significant after correction for multiple testing. TNM classification was not compared since tumor diameter and thickness are analyzed separately. Bold numbers indicate significant *P* values at $P \leq 0.005$.

* Copy numbers for the polyploid UM.

† Mann-Whitney *U* test was used for associations with continuous data.

‡ χ^2 test or § Fisher's exact test was used for associations with categorical data.

mutations (*BAP1*, *SF3B1*, *EIF1AX*, *GNAQ*, and *GNA11*). We also aimed to investigate the prognostic value of polyploidy and prognostic factors within polyploid UM.

Methods

Study Population

Tissue specimens were obtained from 324 patients with UM who were enucleated or had a biopsy between 1993 and 2014. Informed consent was given prior to enucleation and the study was performed according to the guidelines of the Declaration of Helsinki. This study was approved by the local ethics committee. The clinical data from time of diagnosis until December 2014 were updated from the patients' chart. All tumors were histopathologically confirmed. Tumor node metastasis (TNM) classification of the UM was adapted from the AJCC Cancer Staging Manual.[21] Iris melanomas were excluded from this study.

DNA Extraction and Copy Number Analysis

DNA was extracted directly from fresh tumor tissue or frozen tumor using the QIAmp DNA-mini kit (Qiagen, Hilden, Germany) according to the manufacturers' instructions. Chromosome abnormalities were described following the recommendations for cytogenetic nomenclature.[22] The tumors were processed for fluorescence in situ hybridization (FISH), multiplex ligation dependent probe amplification (MLPA), and/or single nucleotide polymorphism (SNP) array analysis (Illumina HumanCytoSNP-12 v2.1 BeadChip and Illumina 610Q BeadChip; Illumina, San Diego, CA, USA), as described previously.[23] Cut-off limits for deletion detected by FISH (>15% of the nuclei with one signal) or amplification (>10% of the nuclei with 3 or more signals) were adapted from the available literature.[23]

Tumor Ploidy

For this study, 202 of 324 patients were included for whom the combination of several techniques was available to determine the ploidy status of the tumor. A threshold of greater than 20% polyploid cells was maintained for cytogenetic analyses. Based on cytogenetic or SNP array analyses, selected chromosomes, which were suspected to be 3N or 4N, were analyzed with FISH to determine the ploidy fraction. A threshold of greater than 10% of the nuclei with three or more signals of these control chromosomes was used to classify a tumor as polyploid.[23] Based on literature, we assumed baseline ploidy of polyploid UM as tetraploid (4N).[24] Chromosomal copy number changes were calculated based on the ploidy baseline. For polyploid samples, chromosomes were treated as loss only in combination with loss of heterozygosity (LOH). For chromosome gain, both quantitative gain (>2N) as well as relative gain (>4N) were included in the analyses of polyploid UM.

Mutational Analyses

Mutation analyses using Sanger sequencing or obtained from whole-exome sequencing (WES) was available for 126 samples.[25] Variants found in the WES data were validated by Sanger sequencing. *BAP1, SF3B1*, *EIF1AX, GNAQ*, and *GNA11* mutation analyses and BAP1 immunohistochemistry (IHC) were carried out as reported previously.[13,18,25,26] For five polyploid samples no fresh or frozen tissue was available, therefore DNA was isolated from formalin-fixed, paraffin-embedded (FFPE) tissue.

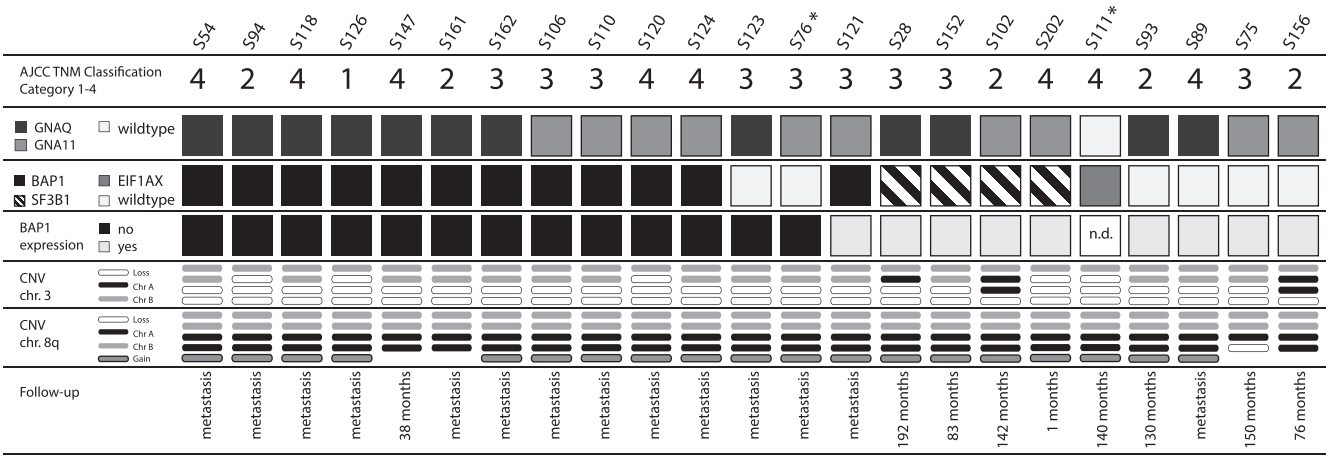

Figure 1. Overview of mutations and copy number variation in polyploid UM. The TNM classification is represented on the first line. *First row* of blocks represent the *GNAQ* and *GNA11* mutation status; *dark gray*, *GNAQ* mutation; *light gray*, *GNA11* mutation; n.d. = not determined; All mutations were exclusive. *Second row* of blocks represent the *BAP1*, *SF3B1*, and *EIF1AX* mutation status; *black, BAP1* mutation; *striped*, *SF3B1* mutation; *gray*, *EIF1AX* mutation; and *white*, wild-type for the three genes. All mutations were exclusive. Third row of blocks represent the BAP1 expression; *white*, BAP1 expressed; *black*, BAP1 not expressed. *Fifth row* represent the alleles of chromosome 3; CNV = copy number variation (baseline is four copies); *black*, allele A; *light gray*, allele B; *white*, loss of chromosome. *Sixth row* represent the alleles of chromosome 8q; *black*, allele A; *light gray*, allele B; *white*, loss of chromosome; *dark gray*, gain chromosome(s). * In these samples the *BAP1* mutation status could not be determined.

These DNAs were screened for mutations in the UM genes using the ION Torrent Personal Genome Machine (PGM) with the supplier's materials and protocols (Life Technologies, Carlsbad, CA, USA). A custom primer panel was designed using the ION AmpliSeq Designer 3.2 (Thermo Fisher Scientific, Waltham, MA, USA). Reads were visualized with Integrative Genomics Viewer (v2.3; Broad Institute, Cambridge, MA, USA). Variants were validated with Sanger sequencing using primers (Supplementary Table S1) and protocols for FFPE DNA. Protocols and designs regarding FFPE DNA sequencing are available upon request. *De novo* missense mutations were investigated with SIFT (in the public domain, http://sift.jcvi.org/) and PolyPhen-2 (in the public domain, http://genetics.bwh.harvard.edu/pph2/index.shtml) for predictions on possible functional impact and pathogenicity of the amino acid change.

Statistical Analyses

Disease-free survival (DFS) was calculated as date of first initial treatment to date of clinically proven metastasis from UM. The Log-rank test was used for categorical variables, and Cox proportional hazard analysis for continuous variables. Statistical significant variables conducted from univariate analysis were analyzed using Cox proportional hazard multivariate analysis. *P* values of 0.05 or lower were considered significant for survival analyses. χ^2 or Fisher's exact test was used for associations with categorical data; Mann-Whitney *U* test was used for associations with continuous data. *P* values of 0.005 or lower were considered significant for correlation analyses. All statistical analyses were performed with SPSS 21.0 Software (IBM, Armonk, NY, USA).

Results

Patient and Tumor Characteristics

Polyploid UM was detected in 23 of 202 patients (11.4%). Nine patients were male and 14 were female with a mean age at diagnosis of 62.5 years. Mean tumor diameter was 15.6 mm with a mean tumor thickness of 8.9 mm. Histopathologically, 19 tumors contained epithelioid cells and 11 formed extracellular matrix patterns. All tumors ($n = 23$) showed a relative loss of chromosome 3 (<4N), resulting in LOH for chromosome 3 in 20 tumors (Fig. 2), whereas three tumors (S28, S102, and S156) still contained two different alleles despite the relative loss (Figs. 1, 2). For chromosome 8q all polyploid UM had more than two copies, 19 tumors had a relative gain (>4N), all copies were present in three tumors (4N; S147, S156, and S161) and one tumor (S75) had three copies of chromosome 8q (Fig. 1). An overview of the clinical, histopathologic, and chromosomal variables are shown in Table 1.

Genetic Analyses UM Genes

Within the patients with polyploid tumors 12 patients harbored a *BAP1* mutation, which were hemi- or homozygous in all cases. In 13 of 22 patients, the tumors did not express BAP1 (examples provided in Fig. 3). In one case (S111) the lack of tumor material restricted us to investigate BAP1 both for mutations and expression. One patient (S76) could not be investigated for *BAP1* mutations, but did reveal loss of BAP1 expression. In one patient (S123), the tumor did not harbor a mutation in the coding exons, but had a loss of expression of BAP1 in the tumor. One patient (S121) harbored a missense mutation in the tumor, p.E30G, whereas the IHC did show expression of BAP1. This mutation was predicted as 'Deleterious' by SIFT software (J. Craig Venter Institute, Rockville, MD, USA) and 'Probably damaging' by PolyPhen-2 software (Harvard Medical School, Boston, MA, USA). All three patients (S76, S121, and S123) were treated as BAP1-deficient tumors in further analysis. *SF3B1* was mutated in four samples targeting the hotspot p.R625 in three cases and p.V576del in one case. *EIF1AX* harbored a missense mutation, p.G15D, in one case, which was predicted as 'Deleterious' by SIFT software and 'Probably damaging' by PolyPhen-2 software. Twelve tumors harbored a *GNAQ* p.Q209 hotspot mutation, 10 harbored a *GNA11* p.Q209 hotspot mutation, and one tumor was wild-type for exon 4 and 5 of both genes. An overview of the mutations with the corresponding polyploid tumor is shown in Figure 1.

Mutations in *BAP1*, *SF3B1*, *EIF1AX*, *GNAQ*, and *GNA11* in the patients with diploid UM were described previously.[13,18,25]

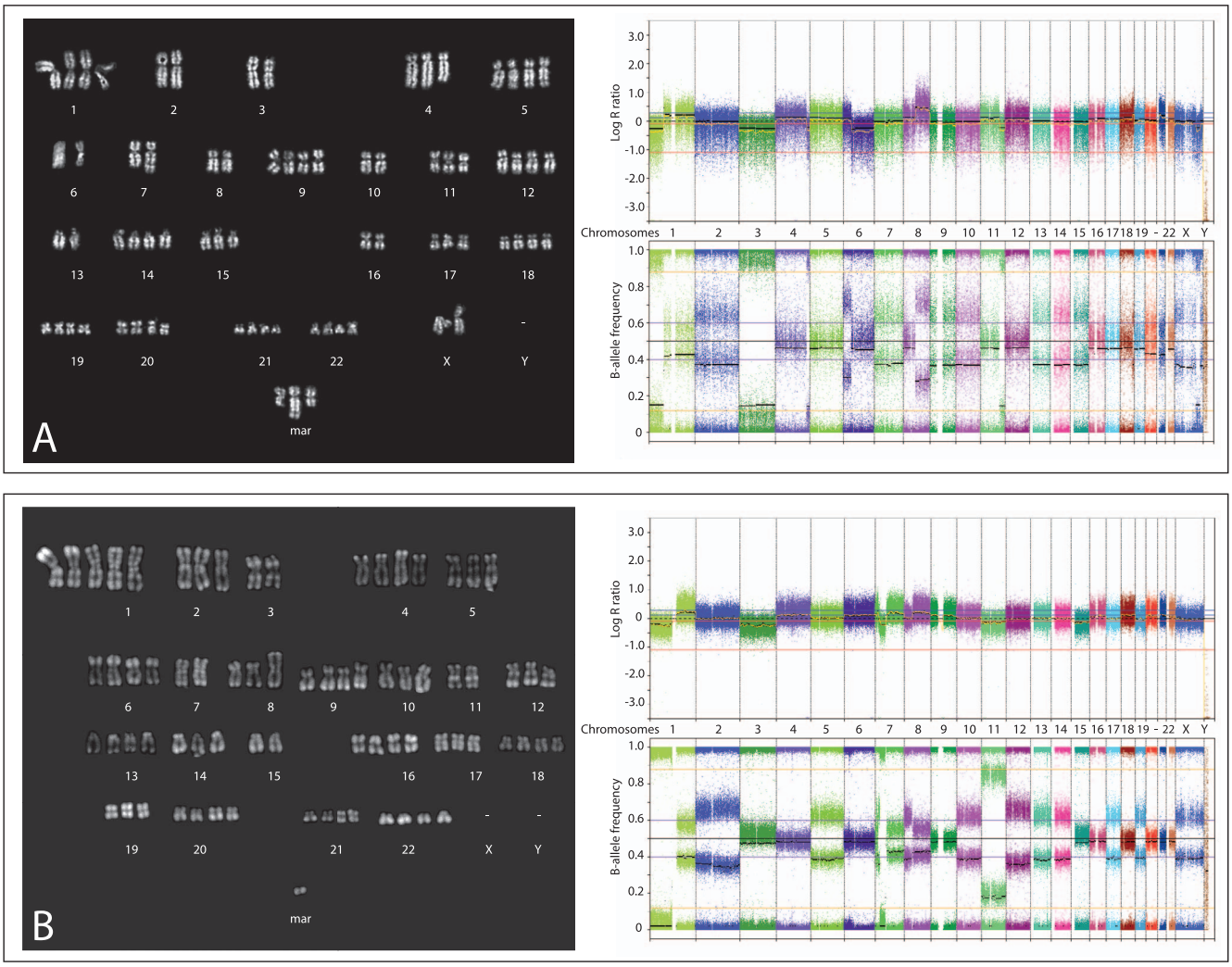

Figure 2. **(A)** Karyogram of S152 demonstrates a polyploid cell with three or four copies of most chromosomes and two copies of chromosome 3; the corresponding whole-genome SNP array (all chromosomes on the X-axis) demonstrates a relative loss of chromosome 3 as observed in the Log R ratio (negative values indicate a relative loss and positive values a relative gain). Loss of heterozygosity of chromosome 3 can be deduced from the B-allele frequency (the B-allele frequency is either 1.0 or 0.0). **(B)** Karyogram of S28 demonstrates a polyploid cell with three or four copies of most chromosomes and two copies of chromosome 3; the corresponding whole-genome SNP array demonstrates a relative loss as observed in the Log R ratio; however, without the loss of heterozygosity as demonstrated by the SNP's (B-allele frequency of ~0.5).

Statistical Analyses

Based on tumor ploidy, patients did not differ in age at diagnoses, sex, tumor localization, tumor thickness, cell type, and presence of extracellular matrix patterns. Patients with a polyploid tumor had significantly larger tumors than patients with a diploid tumor (15.6 vs. 13.1 mm, $P = 0.004$; Table 1). For chromosomal abnormalities we classified the copy number changes for polyploid UM in two ways. For chromosome loss, we determined the relative loss from baseline and also loss with LOH. For chromosome gain, we determined absolute gain from disomy state and relative gain from baseline. Patients with polyploid UM showed more loss of chromosome 3 ($P < 0.001$), which was still significant after correcting for LOH ($P = 0.003$). For chromosome 8q, the polyploid UM contained more often absolute gain (>2N; $P < 0.001$). Relative gain of chromosome 8q was observed more often in polyploid UM ($P = 0.047$), and after correcting the P value for multiple testing to P less than or equal to 0.005 this was not considered significant. Mutational frequencies did not differ between polyploid and diploid UM for *BAP1*, *SF3B1*, *EIF1AX*, *GNAQ*, and *GNA11*.

Survival Analyses

To test whether polyploidy in UM was associated with worse disease-free survival we performed survival analyses for the total group ($n = 202$). Ploidy was not associated with prognosis, because patients with polyploid tumors, as a group, did not differ from patients with diploid tumors based on the survival (Fig. 4A). Univariate analyses results are shown in Table 2. Also in the multivariate Cox-regression analyses, polyploidy was not associated with disease-free survival. Larger basal tumor diameter (HR 1.110; $P = 0.015$) and BAP1 deficiency (HR 5.132; $P < 0.001$) were the only independent significant predictors for disease-free survival in the total cohort (Table 2).

Survival analysis was also performed for patients with polyploid UM to investigate prognostic predictors within this subset ($n = 23$). Loss of heterozygosity of chromosome 3 ($P = 0.050$), BAP1 deficiency ($P = 0.001$), and *SF3B1* wild-type mutation status ($P = 0.035$) were significantly associated with decreased disease-free survival. Other variables were not significantly associated with disease-free survival (Table 2).

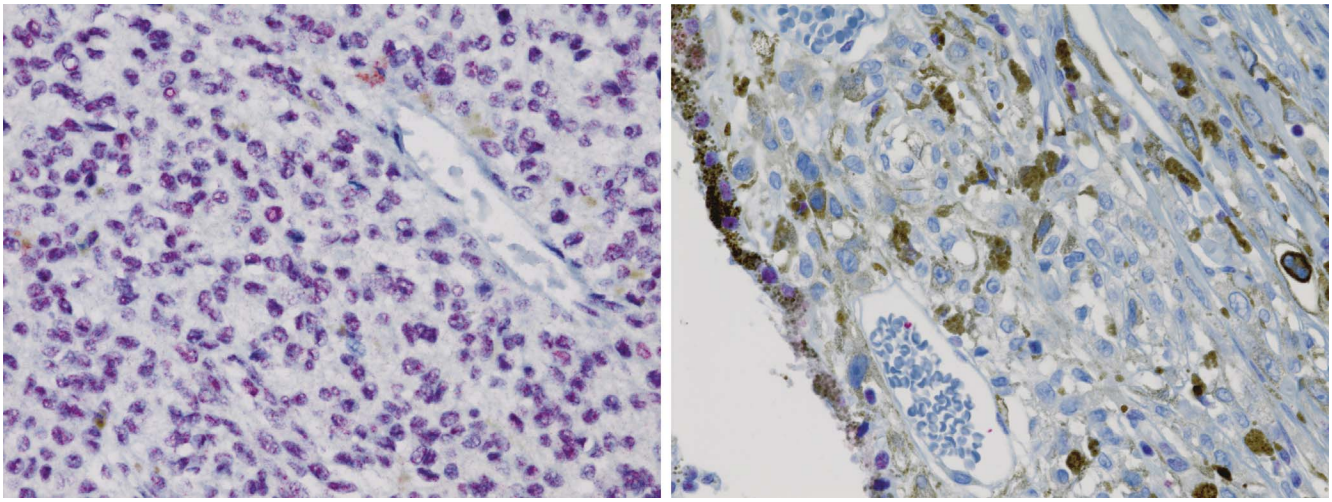

FIGURE 3. Examples of BAP1 immunohistochemistry of two polyploid uveal melanoma cases. *Left picture*: BAP1 expression in the tumor cells of S28 (×400). *Right picture*: lack of BAP1 expression in the tumor cells of the case S162 (×400). Note the positive staining in the retinal pigment epithelium cells and macrophages.

Chromosome 3, BAP1, and *SF3B1* status, together with tumor diameter (because this was associated with polyploid UM) were included into the multivariate Cox analyses. This showed BAP1 deficiency as the only significant independent prognostic predictor for patients with polyploid tumors, with a 16-fold increased HR (HR 15.90, $P = 0.009$; Table 2).

DISCUSSION

In our cohort polyploidy occurred in 11.4% of the patients with UM. Previously, we as well as other groups have reported ranges between 13% and 18%,[8,9] and this difference in prevalence can be explained by the different methods which were used to determine the ploidy status and the DNA index (DI) thresholds which were adapted to classify a tumor as polyploid. Meecham et al.[7] report polyploidy in 13% of the UM with flow cytometry measurements and a threshold of DI greater than 1.4 for polyploidy. Toti et al.[9] report polyploidy in 18% of the UM cases, while maintaining a threshold of DI greater than 1.3 for polyploidy, which would explain the higher prevalence of polyploidy. Mooy et al.[8] reports tetraploidy (4N) in 17% of the cohort; however, this subset also contains preirradiated tumors, which they also correlate to a higher prevalence of aneuploidy.

When compared with patients with diploid UM, we found larger tumor diameter in the polyploid patient group. Polyploid UM also contained more LOH of chromosome 3. We could not confirm previous findings, which stated that polyploidy as a group is associated with worse patient survival.[7,9] However, we did find that BAP1 deficiency was the most significant factor associated with survival in patients with a polyploid UM, similar to diploid UM.

BAP1 expression has been shown as an independent prognostic marker in UM.[10,12,13] The gene is located on chromosome 3, and is mutated mainly in tumors with loss of chromosome 3,[15] resulting in the loss of BAP1 expression.[13] One sample harbored a missense mutation (p.E30G), whereas the staining did reveal BAP1 expression. This mutation was predicted 'Deleterious' and 'Probably damaging' by the prediction software's. Moreover, this mutation is located at the first β-sheet of the protein and also next to three amino acids (p.E31–p.Y33), which form a binding site for ubiquitin,[27] making it likely that the replacement of the negatively charged

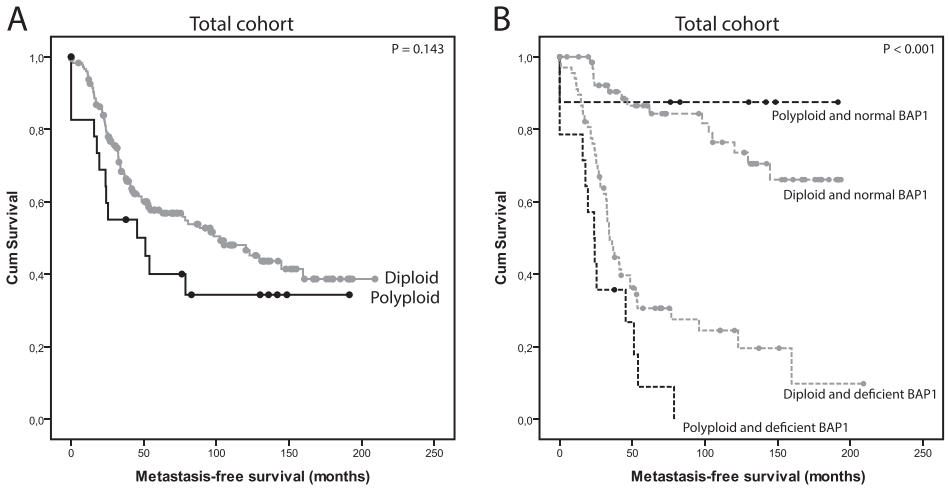

FIGURE 4. Kaplan-Meier survival analyses for; (**A**) polyploid UM compared to diploid UM ($P = 0.143$) and (**B**) survival analyses between polyploid and diploid UM stratified for *BAP1* status ($P < 0.001$).

Table 2. Univariate and Multivariate Analyses of the Uveal Melanoma Parameters in Relation to Patient Survival for the Total Cohort and Patients With a Polyploid Tumor Karyotype

	Univariate		Multivariate		
Covariate	**95% CI**	***P* Value**	**HR**	**95% CI**	***P* Value**
Total cohort, $n = 202$					
Age at diagnoses	1.006–1.036	**0.004**			0.683
Largest tumor diameter	1.030–1.158	**0.003**	1.110	1.021–1.206	**0.015**
Tumor height	0.997–1.113	0.065	–	–	–
Cell type					
Spindle	121.7–160.9	**<0.001**	–	–	0.912
Mixed/epithelioid	77.5–108.6				
Closed vascular loops					
Present	59.4–94.3	**<0.001**	–	–	0.072
Absent	117.1–149.6				
Ploidy					
Diploid	99.6–128.0	0.143	–	–	0.568
Polyploid	49.2–117.9				
Chromosome 3					
Loss, with LOH	50.6–80.1	**<0.001**	–	–	0.549
Normal	147.1–175.7				
Chromosome 8q					
Normal	155.1–190.7	**<0.001**	2.171	0.958–4.920	0.063
Gain, relative	60.7–87.9				
BAP1					
Normal	141.2–172.5	**<0.001**	5.132	2.623–10.04	**<0.001**
Deficient	44.4–75.5				
SF3B1					
Wild-type	84.3–121.3	**0.028**	–	–	0.166
Mutated	114.9–165.5				
EIF1AX					
Wild-type	75.9–106.8	**<0.001**	0.145	0.19–1.116	0.064
Mutated	168.3–214.6				
Polyploid, $n = 23$					
Age at diagnoses	0.987–1.073	0.175	–	–	–
Largest tumor diameter	0.937–1.261	0.270	–	–	0.156
Tumor height	0.909–1.229	0.471	–	–	–
Cell type					
Spindle	22.0–149.2	0.624	–	–	–
Mixed/epithelioid					
Closed vascular loops					
Present	17.1–96.2	0.129	–	–	–
Absent	52.5–128.6				
Ploidy					
Diploid					
Polyploid					
Chromosome 3					
Loss, with LOH	*	**0.050**	–	–	0.357
Normal					
Chromosome 8q					
Normal	66.5–167.4	0.136	–	–	–
Gain, relative	35.0–107.0				
BAP1					
Normal	123.7–211.5	**0.001**	15.90	1.97–128.3	**0.009**
Deficient	16.4–42.5				
SF3B1					
Wild-type	*	**0.035**	–	–	0.294
Mutated					

Table 2. Continued

	Univariate		Multivariate		
Covariate	**95% CI**	***P* Value**	**HR**	**95% CI**	***P* Value**
EIF1AX					
Wild-type	*	0.228	–	–	–
Mutated					

CI, confidence interval of survival (months/HR); HR, hazard ratio (expB). *P* value of $P \leq 0.05$ was considered significant. Log-rank test and Cox regression analyses were used to obtain univariate analyses for categorical and continuous data respectively. Multivariate analyses was conducted with Cox regression analyses with variables significantly associated with survival in the univariate analyses. Bold numbers indicate significant *P* values at $P \leq 0.05$.

* No statistics could be computed because all cases were censored.

glutamic acid with the neutral glycine causes a structural malformation of the protein resulting in a deficiency of BAP1. The BAP1 expression can be explained, because the mutation is a missense mutation and does not lead to protein degradation. Also, the affected amino acid is located in the N-terminal UCH domain, whereas the antibody used for the staining target the C-terminal end of the BAP1 protein.[13] In this study, tumors with *BAP1* mutations and/or loss of BAP1 expression were categorized as deficient BAP1. In this way we observed that deficient BAP1 was the only independent prognostic marker in patients with polyploid UM.

For the other UM relevant genes we found *GNAQ* or *GNA11* mutations in all but one polyploid UM as one would expect based on the occurrence described in diploid UM.[19,20] *SF3B1* mutations were observed in four of our polyploid tumors. Patients with *SF3B1* mutation in the UM have been associated with the low-risk prognostic features; disomy 3, spindle-cell type, and low age at diagnoses.[15,16] None of the patients in our polyploid group with *SF3B1* mutations had developed metastatic disease and were alive at the end of the study (follow-up range, 1–192 months). Nevertheless, both to our own experience as well as by other groups patients can be identified with disomy 3 tumors harboring an *SF3B1* mutation that developed metastasis.[15,16,25,28] In our polyploid cohort the numbers are too low and the follow-up for some tumors is too short in order to draw conclusions regarding the influence of *SF3B1* mutations in the tumor on disease-free survival. *EIF1AX* mutations are mainly reported in disomy 3 tumors and are correlated with a good prognosis for these patients, and present in the tumor of one patient in our series of polyploid UM, who is metastasis-free and alive at a follow-up of 136 months.[10,16,17,25] The missense mutation found in the tumor of this patient affects amino acid 15 (p.G15D), a missense mutation described in other UM samples as well (the COSMIC database; id = COSM3973544; in the public domain, http://cancer.sanger.ac.uk/cosmic/). The first 18 amino acids at the N-terminus of the eiF1A protein are essential in the interaction with the 40S subunit,[29] thus making it very likely that this mutation results in an altered function of the protein. In this current study, we have shown that the prevalence of mutations in the UM genes do not differ between tumors with diploid and polyploid karyotypes, indicating a similar behavior and progression toward metastatic disease, suggesting polyploid UM are not a subclass in UM.

Caution should be taken in the interpretation of chromosomal abnormalities and using one technique only this could possible lead to misclassifications. Uveal melanoma are characterized by nonrandom recurring chromosomal losses and gains.[6] Loss of chromosome 3 has been correlated to metastasis,[3–5] but in polyploid UM with loss of one or multiple copies of chromosome 3 this does not automatically result in LOH. This is shown in our polyploid tumors, which all contain a relative loss of chromosome 3, while three tumors do not display a LOH (Figs. 1, 2). These three patients without LOH are still alive with a median DFS of 11 years (range, 76–192 months), which is comparable to the survival of patients with disomy 3 tumors.[3] Onken et al.[30] described that LOH of chromosome 3 is superior to quantitative loss of monosomy 3, and that is also the case in polyploid UM in our study. We emphasize the importance of SNP-array to investigate the zygosity of UM, to reduce false-negative (disomy 3 with LOH) and false-positive (relative monosomy 3 without LOH) prognostification. However, we cannot use the same reasoning for chromosome gain. Increase in copies of chromosome 8q is shown to be associated with shorter DFS.[31] In polyploid UM, all tumors contain more than two copies of 8q, while four tumors do not have a relative gain based on the baseline of four copies. One of these four patients developed liver metastasis at 54 months and is still alive after a partial hepatectomy 20 months later, two died due to another cause at 38 and 149 months respectively, and one is alive and metastasis-free at 76 months. Because the survival of these patients is not homogenous we cannot draw conclusions regarding the pathogenicity of absolute gain without relative gain (tri- and tetrasomy) of chromosome 8q.

In conclusion, here we show that polyploid UM do not differ from diploid UM based on prevalence of mutations in the UM genes, and that similar to patients with diploid UM, BAP1 is the most significant prognostic predictor of metastasis in patients with polyploid UM (HR 15.90). Yet, the increased chromosome count and frequent losses in polyploid tumors can cause wrongful interpretations of chromosomal data and should therefore be analyzed for ploidy status.

Acknowledgments

Supported by the SWOO-Flieringa Foundation (Rotterdam, The Netherlands) and the Professor Henkes Foundation, (Rotterdam, The Netherlands).

Disclosure: **S. Yavuzyigitoglu**, None; **H.W. Mensink**, None; **K.N. Smit**, None; **J. Vaarwater**, None; **R.M. Verdijk**, None; **B. Beverloo**, None; **H.T. Brüggenwirth**, None; **R. van Marion**, None; **H.J. Dubbink**, None; **D. Paridaens**, None; **N.C. Naus**, None; **A. de Klein**, None; **E. Kiliç**, None

References

1. Bakalian S, Marshall JC, Logan P, et al. Molecular pathways mediating liver metastasis in patients with uveal melanoma. *Clin Cancer Res*. 2008;14:951–956.
2. Kujala E, Makitie T, Kivela T. Very long-term prognosis of patients with malignant uveal melanoma. *Invest Ophthalmol Vis Sci*. 2003;44:4651–4659.
3. Prescher G, Bornfeld N, Hirche H, Horsthemke B, Jockel KH, Becher R. Prognostic implications of monosomy 3 in uveal melanoma. *Lancet*. 1996;347:1222–1225.

4. Tschentscher F, Husing J, Holter T, et al. Tumor classification based on gene expression profiling shows that uveal melanomas with and without monosomy 3 represent two distinct entities. *Cancer Res*. 2003;63:2578–2584.
5. White VA, Chambers JD, Courtright PD, Chang WY, Horsman DE. Correlation of cytogenetic abnormalities with the outcome of patients with uveal melanoma. *Cancer*. 1998;83:354–359.
6. Prescher G, Bornfeld N, Friedrichs W, Seeber S, Becher R. Cytogenetics of 12 cases of uveal melanoma and patterns of nonrandom anomalies and isochromosome formation. *Cancer Genet Cytogen*. 1995;80:40–46.
7. Meecham WJ, Char DH. DNA content abnormalities and prognosis in uveal melanoma. *Arch Ophthalmol-Chic*. 1986;104:1626–1629.
8. Mooy C, Vissers K, Luyten G, et al. DNA flow-cytometry in uveal melanoma - the effect of preenucleation irradiation. *Br J Ophthalmol*. 1995;79:174–177.
9. Toti P, Greco G, Mangiavacchi P, Bruni A, Palmeri MLD, Luzi P. DNA ploidy pattern in choroidal melanoma: correlation with survival. A flow cytometry study on archival material. *Br J Ophthalmol*. 1998;82:1433–1437.
10. Ewens KG, Kanetsky PA, Richards-Yutz J, et al. Chromosome 3 status combined with *BAP1* and *EIF1AX* mutation profiles are associated with metastasis in uveal melanoma. *Invest Ophthalmol Vis Sci*. 2014;55:5160–5167.
11. Harbour JW, Onken MD, Roberson EDO, et al. Frequent mutation of *BAP1* in metastasizing uveal melanomas. *Science*. 2010;330:1410–1413.
12. Kalirai H, Dodson A, Faqir S, Damato BE, Coupland SE. Lack of BAP1 protein expression in uveal melanoma is associated with increased metastatic risk and has utility in routine prognostic testing. *Br J Cancer*. 2014;111:1373–1380.
13. Koopmans AE, Verdijk RM, Brouwer RWW, et al. Clinical significance of immunohistochemistry for detection of *BAP1* mutations in uveal melanoma. *Modern Pathol*. 2014;27:1321–1330.
14. Furney SJ, Pedersen M, Gentien D, et al. *SF3B1* mutations are associated with alternative splicing in uveal melanoma. *Cancer Discov*. 2013;3:1122–1129.
15. Harbour JW, Roberson EDO, Anbunathan H, Onken MD, Worley LA, Bowcock AM. Recurrent mutations at codon 625 of the splicing factor *SF3B1* in uveal melanoma. *Nat Genet*. 2013;45:133–135.
16. Martin M, Masshofer L, Temming P, et al. Exome sequencing identifies recurrent somatic mutations in *EIF1AX* and *SF3B1* in uveal melanoma with disomy 3. *Nat Genet*. 2013;45:933–936.
17. Dono M, Angelini G, Cecconi M, et al. Mutation frequencies of *GNAQ*, *GNA11*, *BAP1*, *SF3B1*, *EIF1AX* and *TERT* in uveal melanoma: detection of an activating mutation in the *TERT* gene promoter in a single case of uveal melanoma. *Br J Cancer*. 2014;110:1058–1065.
18. Koopmans AE, Vaarwater J, Paridaens D, et al. Patient survival in uveal melanoma is not affected by oncogenic mutations in *GNAQ* and *GNA11*. *Br J Cancer*. 2013;109:493–496.
19. Van Raamsdonk CD, Bezrookove V, Green G, et al. Frequent somatic mutations of *GNAQ* in uveal melanoma and blue naevi. *Nature*. 2009;457:599–U108.
20. Van Raamsdonk CD, Griewank KG, Crosby MB, et al. Mutations in *GNA11* in uveal melanoma. *N Engl J Med*. 2010;363:2191–2199.
21. Edge SE, Buyrd DR, Compton CC, Frits AG, Greene FL, Trotti A. *AJCC Cancer Staging Manual*. New York: Springer; 2010.
22. *ISCN (2013): An International System for Human Cytogenetic Nomenclature*. Basel: S. Karger; 2013.
23. Vaarwater J, van den Bosch T, Mensink HW, et al. Multiplex ligation-dependent probe amplification equals fluorescence in-situ hybridization for the identification of patients at risk for metastatic disease in uveal melanoma. *Melanoma Res*. 2012;22:30–37.
24. Davoli T, de Lange T. The causes and consequences of polyploidy in normal development and cancer. *Annu Rev Cell Dev Bi*. 2011;27:585–610.
25. Yavuzyigitoglu S, Koopmans AE, Verdijk RM, et al. Uveal melanomas with *SF3B1* mutations: a distinct subclass associated with late-onset metastases [published online ahead of print February 25, 2016]. *Ophthalmology*. doi:10.1016/j.ophtha.2016.01.023.
26. Van Beek JG, Koopmans AE, Vaarwater J, et al. Metastatic disease in uveal melanoma: importance of a genetic profile? *Melanoma Res*. 2015;25:447–449.
27. Misaghi S, Galardy PJ, Meester WJ, Ovaa H, Ploegh HL, Gaudet R. Structure of the ubiquitin hydrolase UCH-L3 complexed with a suicide substrate. *J Biol Chem*. 2005;280:1512–1520.
28. Luscan A, Just PA, Briand A, et al. Uveal melanoma hepatic metastases mutation spectrum analysis using targeted next-generation sequencing of 400 cancer genes. *Br J Ophthalmol*. 2015;99:437–439.
29. Weisser M, Voigts-Hoffmann F, Rabl J, Leibundgut M, Ban N. The crystal structure of the eukaryotic 40S ribosomal subunit in complex with eIF1 and eIF1A. *Nat Struct Mol Biol*. 2013;20:1015–1017.
30. Onken MD, Worley LA, Person E, Char DH, Bowcock AM, Harbour JW. Loss of heterozygosity of chromosome 3 detected with single nucleotide polymorphisms is superior to monosomy 3 for predicting metastasis in uveal melanoma. *Clin Cancer Res*. 2007;13:2923–2927.
31. van den Bosch T, van Beek JG, Vaarwater J, et al. Higher percentage of FISH-determined monosomy 3 and 8q amplification in uveal melanoma cells relate to poor patient prognosis. *Invest Ophthalmol Vis Sci*. 2012;53:2668–2674.

Appendix

ROMS: Rotterdam Ocular Melanoma Study Group

Serdar Yavuzyigitoglu, MD, PhD Student, Department of Ophthalmology, Department of Clinical Genetics, Erasmus Medical Center, The Netherlands.

Kyra N. Smit, MSc, PhD Student, Department of Ophthalmology, Department of Clinical Genetics, Erasmus Medical Center, The Netherlands.

Natasha van Poppelen, MD, PhD Student, Department of Ophthalmology, Department of Clinical Genetics, Erasmus Medical Center, The Netherlands.

Jolanda Vaarwater, Laboratory Technician, Department of Ophthalmology, Department of Clinical Genetics, Erasmus Medical Center, The Netherlands.

Dion A. Paridaens, Ophthalmologist, MD, PhD, Rotterdam Eye Hospital, Rotterdam, The Netherlands.

Hanneke W. Mensink, Ophthalmologist, MD, PhD, Rotterdam Eye Hospital, Rotterdam, The Netherlands.

Nicole C. Naus, Ophthalmologist, MD, PhD, Department of Ophthalmology, Erasmus Medical Center, The Netherlands.

Jackelien G. van Beek, Ophthalmologist, MD, Department of Ophthalmology, Erasmus Medical Center, The Netherlands.

Robert M. Verdijk, Ophthalmic Pathologist, MD, PhD, Department of Pathology, Erasmus Medical Center, The Netherlands

Annelies de Klein, Clinical Cytogeneticist, PhD, Department of Clinical Genetics, Erasmus Medical Center, The Netherlands.

Emine Kiliç, Ophthalmologist, MD, PhD, Department of Ophthalmology, Erasmus Medical Center, The Netherlands.