8q Gain Has No Additional Predictive Value in *SF3B1*MUT Uveal Melanoma but Is Predictive for a Worse Prognosis in Patients with *BAP1*MUT Uveal Melanoma

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Purpose: Gain of chromosome 8q has been associated with poor prognosis in uveal melanoma (UM), and an increase in the absolute number of 8q-copies correlated with an even shorter survival. Splicing factor 3b subunit 1 (*SF3B1*)-mutated (*SF3B1*MUT) tumors display structural chromosomal anomalies and frequently show a partial gain of chromosome 8qter. A recent subset of *SF3B1*MUT UM with early-onset metastases has been identified, prompting the investigation of the relationship between survival, 8q gain, and *SF3B1*MUT UM.

Design: Retrospective cohort study.

Subjects: Patients diagnosed with UM who underwent enucleation or received a biopsy at the Erasmus MC Cancer Institute or the Rotterdam Eye Hospital, The Netherlands were included.

Methods: Fifty-nine patients with *SF3B1*MUT tumors and 211 patients with BRCA1 associated protein 1 (*BAP1*)-mutated (*BAP1*MUT) tumors were included in this study. Copy number status and gene expression were assessed using either a single nucleotide polymorphism array, fluorescence in situ hybridization, and karyotyping, or a combination of these techniques. Disease-free survival was determined and a cut-off of 60 months was used to define early-onset metastatic disease.

Main Outcome Measures: Disease-free survival.

Results: Forty-eight patients with *SF3B1*MUT UM (81%) had chromosome 8q gain (3 copies, 78%; 4 copies, 22%). Kaplan−Meier analysis of *SF3B1*MUT UM did not indicate a difference in survival in patients with or without gain of 8q ($P = 0.99$). Furthermore, the number of 8q copies was not associated with survival when comparing early ($P = 0.97$) versus late ($P = 0.23$) metastases group. In contrast, the presence of 8q gain (86%) was correlated with a decreased survival in *BAP1*MUT UM ($P = 0.013$).

Conclusions: We did not find a correlation between 8q gain and early-onset metastasis in *SF3B1*MUT tumors. Gain of 8q has no additional predictive value in *SF3B1*MUT tumors. In contrast, 8q gain is predictive of a worse prognosis in patients with *BAP1*MUT tumors. Thus, gain of chromosome 8q has additional predictive value for *BAP1*MUT tumors, but not for *SF3B1*MUT tumors.

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Uveal melanoma (UM) is the most common primary intra-ocular malignancy in adults.[1] Recurrent mutations in secondary driver genes BRCA1 associated protein 1 (*BAP1*), splicing factor 3b subunit 1 (*SF3B1*), and eukaryotic translation initiation factor 1A X-linked (*EIF1AX*),[2] as well as characteristic copy number variations (CNV)[3] and gene expression profiles[4] are used in prognostication. Previously, studies using fluorescence in situ hybridization (FISH) probes and array comparative genomic hybridization revealed that gain of chromosome 8q (> 2 copies) in UM has a strong correlation to a short survival compared with normal or low copies (< 2 copies) of chromosome 8q.[5,6] An increased number of 8q copies was also associated with a worse prognosis (the more copies of 8q, the worse the prognosis).[5] Furthermore, chromosome 8q status in UM patients with different iris colors was assessed in a large cohort study.[7] Authors describe the correlation of chromosome 8q gain to worse survival in patients with light iris color, but not in those with dark iris color.[7] Previously, we reported that CNV

profiles are mutation-specific and *BAP1*-mutated (*BAP1*^MUT) UM are characterized by gains or losses of entire chromosomes, chromosome arms, and recurrent isochromosomes.[2,3] Splicing factor 3b subunit 1-mutated (*SF3B1*^MUT) UM are characterized by multiple structural chromosomal aberrations at the distal ends of chromosomes (gain of chromosomes 6p and 8q as well as loss of chromosomes 6q and 11q). The *EIF1AX*-mutated UM are characterized by gain of chromosome 6p. In *BAP1*^MUT UM, gain of 8q is the result of the gain of an entire chromosome or isochromosome formation. However, in *SF3B1*^MUT UM, 8q gain is the outcome of structural chromosome changes resulting in a gain of only the distal end of 8q.[3] Both *BAP1*^MUT and some of the *SF3B1*^MUT UM patients develop early-onset metastatic disease,[8] prompting us to investigate the relationship between gain of 8q, survival, and *BAP1*^MUT or *SF3B1*^MUT UM.

Methods

This is a retrospective cohort study of patients diagnosed with UM between 1994 and 2022 and included in the Rotterdam Ocular Melanoma Study Group (ROMS) database. All patients underwent enucleation or received a biopsy at the Erasmus MC Cancer Institute or the Rotterdam Eye Hospital, The Netherlands. Copy number variations were assessed with ≥ 1 of the following methods: single nucleotide polymorphism (SNP) array, karyotyping, or FISH. Uveal melanoma with concomitant mutations in *BAP1*, *SF3B1*, or *EIF1AX* were excluded. In total, 59 patients with a confirmed *SF3B1*^MUT tumor were included. In 211 samples, *BAP1* status was confirmed with either a *BAP1* mutation and negative BAP1 immunohistochemistry or both. Because tumors with an *EIF1AX* mutation typically do not metastasize,[9] we excluded these tumors in this study. The disease-free survival (DFS) (determined as the interval from treatment until metastasis or metastasis and subsequent death due to UM or until the last follow-up) was determined in all patients and a cut-off of 60 months was used to identify early-onset metastatic disease in UM patients.[8] Informed consent was obtained from all patients. The study adhered to the guidelines of the Declaration of Helsinki and was approved by the Medical Ethics Committee of the Erasmus Medical Centre (OZR nr 2009-17, MEC-2009-375, 12 November 2009).

Copy Number Analysis

For the SNP array, 200 ng of tumor DNA was used for whole-genome copy number analyses by SNP arrays. For the entire set, 6 types of Illumina Human SNP array platforms were used according to the manufacturers protocol (CytoSNP-12 v2.0 BeadChip [n = 12], CytoSNP-12 v2.1 [n = 35], OmniExpress-12 v1 [n = 29], CytoSNP-850K BeadChips [n = 67] and GSAMD_V1 [n = 34] and GSAMD_V2 [n = 23] [Bead Studio Illumina]). The resolution to detect CNV of all chip types is ≥ 30 kb. Nexus Copy Number 10.0 (BioDiscovery, Inc) was used to calculate the total percentage aneuploidy of the entire genome, the total copy number events, and to visualize the whole-genome SNP array data. A summary plot showing the SNP-array-derived chromosomal patterns of primary UM can be found in the supplementary material Figure S1 (available at www.ophthalmologyscience.org). We used cytogenetic and FISH data from our ROMS cohort, which have been previously described by Van den Bosch et al[5] and Yavuzyigitoglu et al.[3] Because techniques have changed over the years when including patients in the ROMS cohort, the different techniques were mostly used alternatively in patients, e.g., the earliest patients included only have cytogenetic/FISH data (from 1992 until 2010), and the latest patients included all only have SNP array data (from 2010 onward), though almost all tumors from the earliest patients were later analyzed with the SNP array. Gain of the distal ends of chromosome 8q was defined at location 8q23qter, which was used as the cut-off point by Yavuzyigitoglu et al.[3]

Survival Analysis

The DFS was determined from the date of diagnosis until the date of metastasis or last follow-up. Patients were censored when they
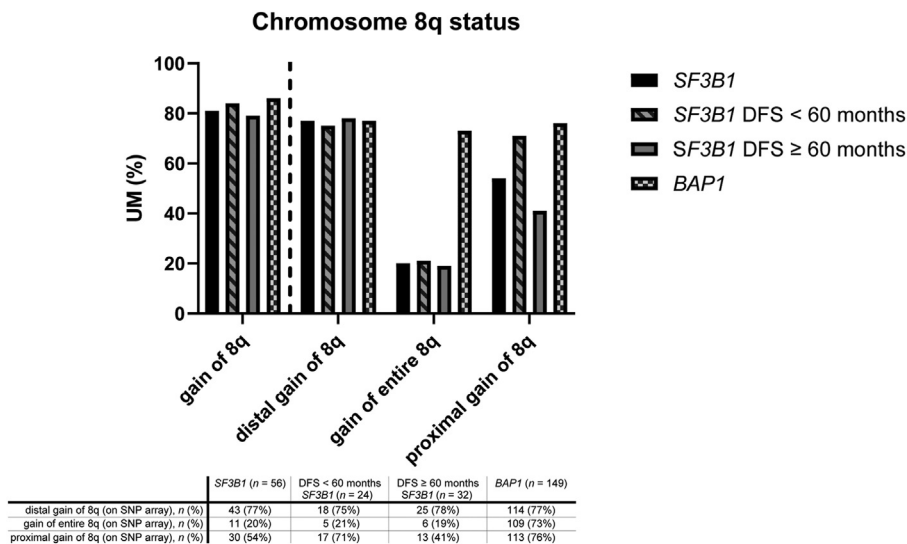

Figure 2. Bar chart of 8q status in splicing factor 3b subunit 1-mutated (*SF3B1*^MUT) and BRCA1 associated protein 1-mutated (*BAP1*^MUT) tumors, as well as in *SF3B1*^MUT tumors with disease-free survival (DFS) < 60 months and DFS ≥ 60 months. SNP = single nucleotide polymorphism; UM = uveal melanoma.

	SF3B1 (n = 56)	DFS < 60 months SF3B1 (n = 24)	DFS ≥ 60 months SF3B1 (n = 32)	BAP1 (n = 149)
distal gain of 8q (on SNP array), n (%)	43 (77%)	18 (75%)	25 (78%)	114 (77%)
gain of entire 8q (on SNP array), n (%)	11 (20%)	5 (21%)	6 (19%)	109 (73%)
proximal gain of 8q (on SNP array), n (%)	30 (54%)	17 (71%)	13 (41%)	113 (76%)

Table 1. Characteristics of All SF3B1^{MUT} and BAP1^{MUT} Tumors in the Rotterdam Ocular Melanoma Study Group Cohort

Characteristics	SF3B1 (n = 59)	SF3B1 8q Gain (n = 48)	SF3B1 No 8q Gain (n = 11)	3 copies 8q (n = 37)	≥ 4 copies 8q (n = 11)	BAP1 (n = 211)	BAP1 8q Gain (n = 181)	BAP1 No 8q Gain (n = 30)	3 Copies 8q (n = 97)	≥ 4 Copies 8q (n = 84)
Male, n (%)	32 (54)	26 (54)	6 (55)	20 (54)	6 (55)	105 (50)	94 (52)	11 (37)	53 (55)	41 (49)
Age, yrs (mean ± SD)	55.3 ± 15.3	55.9 ± 15.3	52.7 ± 15.4	54.5 ± 14.2	60.9 ± 18.6	65.2 ± 12.8	65.3 ± 12.7	65.0 ± 14.0	65.0 ± 13.1	65.7 ± 12.2
DFS, mos (mean ± SD)	80.3 ± 57.5	78.2 ± 57.6	89.6 ± 58.9	71.8 ± 50.1	99.7 ± 76.8	41.7 ± 45.0	39.9 ± 44.4	53.0 ± 47.7	43.3 ± 47.4	35.9 ± 40.5
8q gain, n (%)	48 (81)	-	-	-	-	181 (86)	-	-	-	-

BAP1 = BRCA1 associated protein 1; BAP1^{MUT} = BAP1 mutated; DFS = disease-free survival; SD = standard deviation; SF3B1 = splicing factor 3b subunit 1; SF3B1^{MUT} = SF3B1 mutated.

were lost to follow-up, when death from a cause other than UM occurred, or when death without any cause was reported. Kaplan−Meier survival analyses were performed to estimate survival probabilities with either metastasis or death due to metastases. Cox proportional hazard regression analysis was performed to identify the value of the prognostic factors, adjusted for age and sex. Differences between groups were assessed using the *t* test. The analyses were performed using the statistical software GraphPad Prism 9.4.0 (GraphPad Software, Inc). A *P* value of < 0.05 was considered statistically significant.

Results

Characteristics

Fifty-nine tumors were included in the *SF3B1* group. Gain of chromosome 8q (> 2 copies of 8q) was present in 48 tumors (81%) (Fig 2, Table 1). One *SF3B1*^{MUT} UM had proximal 8q gain without a gain in the distal ends. Cox proportional hazard analysis could not confirm the reported independent[5] prognostic value of gain of chromosome 8q in general (> 2 copies) in *SF3B1*^{MUT} UM (hazard ratio [HR]: 0.9366 [95% confidence interval {CI}: 0.2809−4.231]), nor for the number of additional 8q copies (3 copies of 8q, HR: 1.145 [95% CI: 0.3174−5.412]; ≥ 4 copies of 8q, HR: 0.5687 [95% CI: 0.07347−3.494]) in *SF3B1*^{MUT} tumors. Kaplan−Meier survival analysis also indicated no difference in survival in patients with or without 8q gain (*P* = 0.99). Furthermore, no difference was found in survival between 2, 3, or ≥ 4 copies (*P* = 0.69) (Fig 3).

Chromosome 8q in *BAP1*^{MUT} Tumors

Although 8q gain is seen often in *SF3B1*^{MUT} UM, it also frequently occurs in *BAP1*^{MUT} UM. We assessed the survival of 211 UM patients with either immunohistochemically BAP1-negative tumors and tumors with pathogenic *BAP1* mutations or both. One hundred thirty patients with *BAP1*^{MUT} UM developed metastases. Gain of chromosome 8q was present in 181 tumors (86%) (Fig 2, Table 1), including 6 *BAP1*^{MUT} UM with only gain in the proximal 8q without distal gain, and this correlated with worse survival in *BAP1*^{MUT} UM (*P* = 0.013) (Fig 4). However, when the analysis was repeated with a cut-off DFS of 2 and 3 years, no significant differences were found Figure S5 (available at www.ophthalmologyscience.org). Based on the Cox regression model, tumors with 3 copies of 8q were not associated with a decreased survival when compared with 2 copies of 8q (HR: 1.645 [95% CI: 0.9486−3.034]), though ≥ 4 copies of 8q showed a significant decrease in survival when compared with normal copies of 8q (HR: 2.172 [95% CI: 1.246−4.023]).

Chromosome 8q in *SF3B1*^{MUT} Tumors with DFS < 60 and DFS ≥ 60 Months

Next, *SF3B1*^{MUT} tumors were classified based on DFS (DFS < 60 months [n = 25] and DFS ≥ 60 months [n = 34]) (Table 2). The distal gains of chromosome 8q (defined as 8q23qter[3]) were similar in patients with a DFS < 60
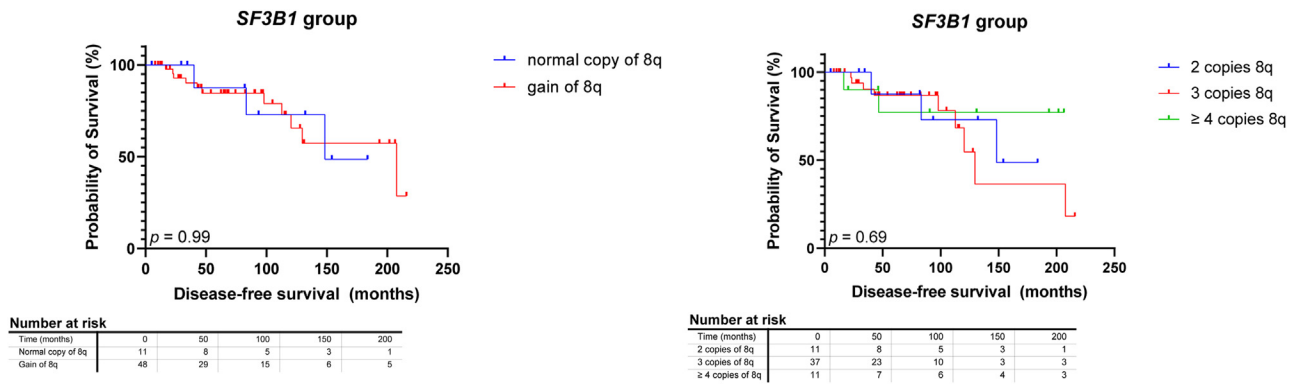
Figure 3. Kaplan−Meier survival curves in splicing factor 3b subunit 1-mutated ($SF3B1^{MUT}$) tumors stratified to chromosome 8q status and chromosome 8q copy numbers. No differences were found between chromosome 8q status ($P = 0.99$) and chromosome 8q copy numbers ($P = 0.69$).

months (74%) and DFS $\geq$ 60 months (78%) based on SNP array data (Fig 2). Gain of the entire chromosome 8q was similar in patients with a DFS < 60 months (21%) and DFS $\geq$ 60 months (19%) (Fig 2). Proximal gain of chromosome 8q (defined as gain at least between 8q11-8q23) was more prominent in patients with a DFS < 60 months (71%) compared with patients with a DFS $\geq$ 60 months (41%), though most of these also had distal gains (therefore, these can also be seen as an incomplete gain of the entire chromosome). Fifteen patients with $SF3B1$ mutation have metastatic disease, including 8 patients with a DFS < 60 months (88% with 8q gain) and 7 patients with a DFS $\geq$ 60 months (71.4% with 8q gain).[8] Six patients in the DFS < 60 months group died of other causes.

Discussion

Many studies identified gain of chromosome 8q as one of the prognostic factors in UM.[10−13] In a previous study, we have already assessed 220 UM with the FISH technique and concluded that chromosome 8q gain is an important independent prognostic factor in UM.[5] Furthermore, a higher percentage of gain of 8q in tumor cells was significantly correlated to a worse prognosis. This was later confirmed by Cassoux et al[6] with multiplex ligation-dependent probe amplification assay in 338 UM, and by Versluis et al[14] with digital polymerase chain reaction in 66 enucleated UM, where they found that an increased 8q copy number was correlated with metastatic risk. Wierenga et al recently showed that gain of chromosome 8q has a significant role in survival in UM patients with light iris color compared with those with dark iris color.[7]

One of the most important commercial methods to prognosticate UM is the gene expression profiling test,[4] which uses the expression of 15 genes to classify UM into 2 groups, a low metastatic risk group (reported as class 1) and a high metastatic risk group (reported as class 2). Class 2 tumors frequently had either $BAP1$ mutations and monosomy 3 (M3) or both, whereas most of class 1 tumors had 2 copies of chromosome 3.[15] Additionally, Robertson et al[2] divided UM into 4 molecular and clinical clusters. Uveal melanoma with M3 was associated with a poor prognosis, whereas UM with disomy 3 (D3) showed a more favorable prognosis. Disomy 3 tumors have the least chromosomal aberrations, where gain of 8q can be
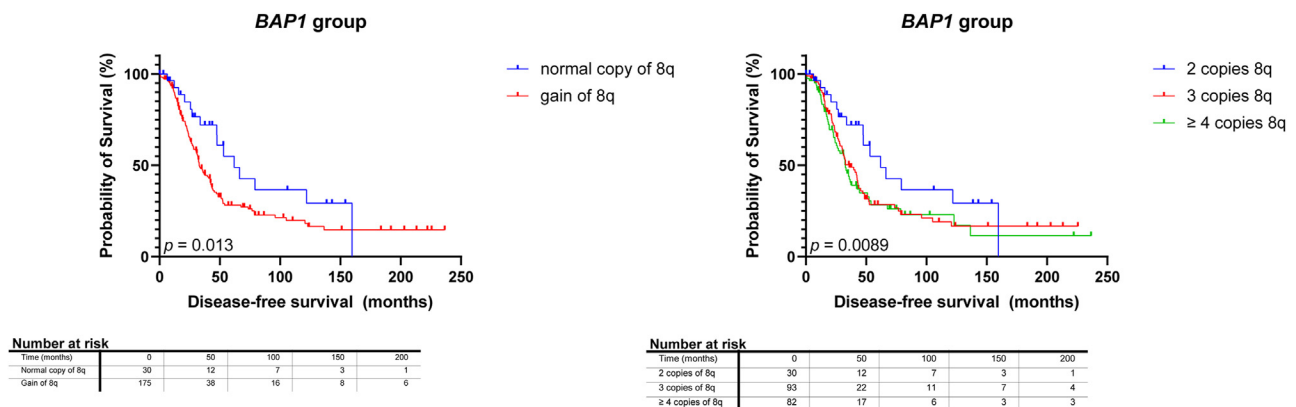

Figure 4. Kaplan−Meier survival curves in BRCA1 associated protein 1-mutated ($BAP1^{MUT}$) tumors stratified to chromosome 8q status and chromosome 8q copy numbers. Gain of chromosome 8q was correlated with a worse prognosis ($P = 0.013$) with increased copy numbers of 8q correlating with shorter disease-free survival ($P = 0.0089$).

Table 2. Characteristics of 8q Status of All *SF3B1*[MUT] Grouped Based on DFS in the Rotterdam Ocular Melanoma Study Group Cohort

SF3B1[MUT] Tumors	DFS < 60 mos (n = 25)	DFS ≥ 60 mos (n = 34)
2 copies of 8q, n (%)	4 (16)	7 (21)
3 copies of 8q, n (%)	16 (64)	21 (61)
≥ 4 copies of 8q, n (%)	5 (20)	6 (18)
8q gain, n (%)	21 (84)	27 (79)

DFS = disease-free survival; SF3B1[MUT] = splicing factor 3b subunit 1-mutated.

present, but most commonly partial or total gain of chromosome 6p.[2] Alternatively, M3 tumors showed gain of 6p and partial gain of 8q, often a result of isochromosome formation.[3] Therefore, chromosome 8q gain is associated with M3 and correlated with poor prognosis. Furthermore, although not always done in the past, UM can also be prognosticated by mutation status. As 8q gain often coexists with M3, gain of 8q is often seen in *BAP1*[MUT] tumors. Even though both *SF3B1*[MUT] and *BAP1*[MUT] tumors display 8q gain,[2,3] the correlation between the amount of 8q copies and mutation status in UM has yet to be assessed.

In this study, no association was found between 8q gain and *SF3B1*[MUT] tumors in relation to survival. Furthermore, we show that 8q gain is not discriminating for the survival of early versus late metastasizing *SF3B1*[MUT] tumors. Uveal melanoma with gain of 8q in combination with D3 is correlated to a lower metastatic risk compared with those with gain of 8q and M3.[6] A possible explanation could be because these tumors with 8q gain in combination with D3 could be classified as *SF3B1*[MUT] tumors (as *SF3B1*[MUT] tumors have D3 in general). However, although rare, *BAP1*[MUT] tumors (with positive BAP1 immunohistochemistry) can also have D3, making it more difficult to correlate 8q to prognosis based only on gene expression profiling classification.[2,16] Furthermore, as *BAP1*[MUT] tumors have a peak in metastatic disease (large peak at approximately 3 years) within the first 5 years after diagnosis, we repeated the analysis at 2 and 3 years, though no significant differences were found (Fig S5). Additionally, chromosome 8q status in this study was assessed with various methods over the years. Where karyotyping and FISH were used in the past, the SNP array has become the first line of choice to detect CNV,

as it offers relatively fast and objective results as well as a minimal required amount of tissue. Single nucleotide polymorphism array can detect submicroscopic alterations which can be missed with either karyotyping and FISH or both. Therefore, the lack of 8q gain in some of the patients in this study could be explained due to the difference in techniques used over the years, though most tumors have had SNP array analysis. Furthermore, in the ROMS cohort, only 1 *SF3B1*[MUT] tumor with 8q gain had loss of chromosome 8p whereas 40% of *BAP1*[MUT] tumors with 8q gain had loss of chromosome 8p, strengthening the findings of small chromosomal aberrations in the *SF3B1* group. The current study is limited by its small sample size, and consequently the small number of events. Conducting further studies with a larger cohort would enhance the validity and robustness of these findings.

Therefore, in the current study, we have shown that the prognostic relevance of chromosome 8q status is only observed in *BAP1*[MUT] tumors, and not in *SF3B1*[MUT] tumors. In addition, *BAP1*[MUT] tumors showed a trend toward decreased survival with increased numbers of 8q copies compared with normal copies of 8q. Furthermore, *BAP1*[MUT] tumors contribute to > 50% of all UM and are characterized by gains and losses of entire chromosomes or chromosome arms. In contrast, *SF3B1*[MUT] UM karyotypes are more complex and CNV often have recurrent distal breakpoints on chromosomes 6 and 8. This difference in CNV patterns could indicate a separate tumorigenesis mechanism in both groups. In this study, the correlation between 8q gain and survival was observed only in *BAP1*[MUT] tumors, not *SF3B1*[MUT] tumors. Therefore, we conclude that the reduced survival reported in UM with chromosome 8q gain is primarily influenced by *BAP1*[MUT] tumors, which have not been specifically identified in this context.

To conclude, we have evaluated gain of chromosome 8q and its role on DFS in *SF3B1*[MUT] and *BAP1*[MUT] UM in the ROMS cohort. There is no correlation between 8q gain and early-onset metastasis in *SF3B1*[MUT] tumors. We do not see evidence of additional predictive value of gain of 8q in *SF3B1*[MUT] tumors. In contrast, 8q gain is only predictive of a worse prognosis in *BAP1*[MUT] tumors. To establish and clarify the role of 8q gain in UM in general, a uniform approach toward analyzing CNV, mutation status and transcriptomic data in relation to a DFS over a longer follow-up period in a larger cohort study should be considered. Validation in clinically well-typed patient cohorts with CNV profiles, transcriptome, and mutation data would strengthen these findings.

Footnotes and Disclosures

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