IMPORTANCE Studies on uveal melanomas (UMs) have demonstrated the prognostic value of 8q gain and monosomy 3, but the prognosis of UMsWith partial deletion of chromosome 3 remains to be defined.

OBJECTIVE To examine the association of partial chromosome 3 deletion in UMswith metastasis-free survival.

DESIGN, SETTING, AND PARTICIPANTS This retrospective cohort study of 1088 consecutive comparative genomic hybridization arrays performed from May 1, 2006, to July 31, 2015, assessed patients presenting with UMswith and without partial loss of chromosome 3 at a referral center. Data analysis was performed from September 1, 2017, to November 30, 2017.

EXPOSURE Uveal melanoma with or without partial loss of chromosome 3.

MAIN OUTCOMES AND MEASURES Metastasis-free survival and overall survival at 60 months.

RESULTS Of the 1088 consecutive comparative genomic hybridization arrays that were performed, 43 UM(s (4.0%) in 43 patients (median age, 58 years [range, 12-79 years]); 22 [51%] female) carried partial deletions of chromosome 3. Median follow-up was 66 months (range, 1.2-126.2 months). Metastasis-free survival at 60 months was 33.6% (95% CI, 15.8%-71.4%) for UMsthat carried a deletion of the BAPI (BRCA1 associated protein 1) locus (BAP1del; 24 tumors) and 80.5% (95% CI, 64.8%-100%) for UMswithout the loss of the BAPI locus (BAP1 normal [BAP1nl]; 19 tumors) (log-rank \( P = .001 \)). Overall survival at 60 months was 64.5% (95% CI, 43.5%-95.8%) in the BAP1del group vs 84.1% (95% CI, 69.0%-100%) in the BAP1nl group (log-rank \( P < .001 \)). In these 43 cases, metastasis-free survival at 60 months was 100% for UMswithout the loss of the BAPI locus or 8q gain, 70.0% (95% CI, 50.5%-96.9%) for UMsthat carried 1 of these alterations, and 12.5% (95% CI, 2.1%-37.7%) for those that carried both (log-rank \( P < .001 \)). Similarly, overall survival at 60 months was 100% for UMswithout loss of the BAPI locus or 8q gain, 80.8% (95% CI, 63.3%-100%) for UMsthat carried 1 of these alterations, and 46.7% (95% CI, 23.3%-93.6%) for those that carried both (log-rank \( P < .001 \)).

CONCLUSIONS AND RELEVANCE These findings suggest that partial deletion of chromosome 3 encompassing the BAPI locus is associated with poor prognosis. A cytogenetic classification of UMscould be proposed based on the status of the BAPI locus instead of the chromosome 3 locus, while also taking chromosome 8q into account.

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Uveal melanoma (UM) is the most common primary malignant ocular tumor in adults of European ancestry. Despite efficient treatment, up to 50% of the patients will eventually develop metastases. Reliable prognostic assessment allows a closer monitoring of high-risk patients. Pathologic prognostic factors include large tumor basal diameter, thickness, ciliary body involvement, extraocular extension, epithelioid cell histologic findings, high mitotic rate, and lymphocytic infiltration. The gene expression profile (GEP) DecisionDx-UM (Castle Biosciences), based on the expression level of 12 genes, is frequently used in North America to complete the prognostic assessment.

In the early 1990s, recurrent cytogenetic aberrations, including monosomy 3 (M3) and gain of 6p and 8q, were identified in UM samples. In 1996, M3 was empirically identified as a robust prognostic factor. Since then, genomic arrays have become routine tools to refine pathologic prognosis along with the GEP. We previously refined the prognostic value of M3 and gain of 8q by defining 3 groups: (1) high-risk patients whose tumors present an M3 and an 8q gain with a 2-year metastasis-free interval of 37%, (2) intermediate-risk patients with an M3 or an 8q gain (2-year metastasis-free interval of approximately 85%), and (3) low-risk patients without an M3 or an 8q gain (2-year metastasis-free interval of approximately 100%).

The most common hypothesis to explain the poor prognosis of M3 tumors is the presence of 1 or more tumor suppressor genes on chromosome 3. BAP1 (BRCA1 associated protein 1) (OMIM 603089), a tumor suppressor gene located on the 3p21.1 cytoband, is now established as a main actor of UM malignant transformation because it is frequently mutated in M3 tumors, and germline mutations are associated with UM predisposition. However, all or most BAP1-mutated UM s intriguingly present a M3 (or a loss of heterozygosity of the whole chromosome 3 owing to an isodisomy), suggesting that the role of chromosome 3 loss in UM tumorigenesis may not be restricted to BAP1 inactivation. Therefore, prognostication of UM samples with partial deletions of chromosome 3, as sometimes observed in our daily practice and by other authors, is problematic. The goals of the present study were to explore these UM s with partial deletions of chromosome 3, as assessed by comparative genomic hybridization (array CGH), to assess their prognosis and to determine the minimal region of deletion associated with poor prognosis.

Methods

Patients
This cohort study was approved by our institutional ethics committee Institut Curie, PSL Research University. Written informed consent for the use of tissue samples and data for research was signed by each patient. The study complied with the principles of the Declaration of Helsinki. All patients were referred to Institut Curie, PSL Research University, Paris, France, and followed up by physicians at this institution from May 1, 2006, to September 30, 2017. Clinical diagnosis of UM was based on the presence of typical clinical findings as previously described. Local treatment consisted of proton beam radiotherapy, iodine 125 brachytherapy, or enucleation depending on the size and location of the tumors. Tumor samples were obtained by enucleation, enucleation, or fine-needle aspiration at the time of clip or plaque positioning. Liver ultrasonography, liver magnetic resonance imaging, or body computed tomography was performed at diagnosis and every 6 months thereafter. Diagnosis of metastasis was systematically confirmed by a biopsy.

Genomic Analysis
Tumor DNA was extracted and processed from May 1, 2006, to July 31, 2015, as previously described. Array CGH was performed on 3 different platforms according to the period when the test was performed: bacterial artificial chromosome arrays as previously described, NimbleGen 4 × 72 K arrays (Roche NimbleGen Inc), and Agilent 180K CGH/LOH custom chip (Agilent Technologies Inc). Array CGH results were interpreted by 3 of the authors (M.R., K.A.R., G.P.). Partial deletion of chromosome 3 was defined as the loss of at least 1 region of chromosome 3 but not the totality, whatever its size and location. Genomic positions in this article are defined in hg18 human genome assembly. Data analysis was performed from September 1, 2017, to November 30, 2017.

Statistical Analysis
Clinical, pathologic, and genomic data at diagnosis and follow-up events (local and distant recurrences, second cancers, or death from UM or from any other cause) were collected. The French Death Registry was consulted for patients who had not been followed up in consultation during the past 12 months. Metastasis-free survival (MFS) at 60 months was defined as the proportion of patients alive and free of metastasis at 60 months of follow-up after local treatment of primary UM. Overall survival (OS) at 60 months was defined as the proportion of patients alive at 60 months of follow-up after local treatment of primary UM, whatever the cause of death. Survival distributions were estimated by the Kaplan-Meier method and compared using the log-rank test. All tests were bilateral and performed with a 2-sided significance level of P < .05. To identify variables associated with MFS, a Cox proportional hazards regression analysis of candidate prognostic factors was performed using a forward stepwise selection procedure. The added value of each variable to the Cox proportional hazards regression model was determined using a

Key Points

**Question** What is the association of partial chromosome 3 deletion in uveal melanomas with metastasis-free survival?

**Findings** In this cohort study, partial deletions of chromosome 3 encompassing the BAP1 locus were associated with lower metastasis-free survival at 60 months compared with uveal melanomas without such deletion.

**Meaning** These findings suggest that uveal melanomas that carry a partial deletion of chromosome 3 encompassing the BAP1 locus have a poor prognosis.
We prospectively reanalyzed the array CGH profiles in 1088 UMs and detected 43 UMs (4.0%) that harbored a partial deletion of chromosome 3 in 43 patients (median age, 58 years [range, 12-79 years]; 22 [51%] female) (eTable in the Supplement). Median follow-up in these 43 cases was 66 months (range, 1.2-126.2 months). Median tumor diameter was 16 mm (range 10-22 mm), and median thickness was 10 mm (range, 5.3-18.2 mm). Ciliary body was involved in 14 of 43 cases (32.6%) and optic nerve in 4 of 43 cases (9.3%). Cell morphologic type was epithelioid or mixed in 13 cases (30.2%). Primary tumors were treated by enucleation in 18 cases (41.9%). The MFS at 60 months was 84.1% (95% CI, 69.0%-100%) for the BAP1del group (Figure 2). The OS at 60 months was 84.1% (95% CI, 69.0%-100%) for the BAP1del genomic group and 64.5% (95% CI, 43.5%-95.8%) for the BAP1del group. The only variables associated with MFS in univariate analysis were loss of the BAP1 locus and gain of 8q. These 2 variables were independently associated with MFS in multivariate analysis (Table). We defined 4 groups based on the BAP1 locus (lost or not lost) and 8q (gained or not gained) statuses. Prognoses of the BAP1 locus lost with 8q normal and BAP1 locus not lost with 8q gained were similar; thus, we merged these 2 groups, as in a previous classification (eFigure 2 in the Supplement). By analogy with previous work, we defined 3 prognosis groups as follows: (1) a group at low risk of metastasis without loss of the BAP1 locus or 8q gain (9 cases), (2) an intermediate risk group with tumors that carried loss of the BAP1 locus (7 cases) or 8q gain (15 cases), and (3) a high-risk group with loss of the BAP1 locus and 8q gain (12 cases). The MFS at 60 months was 100% for the low-risk group, 70.0% (95% CI, 50.5%-96.9%) for the intermediate-risk group, and 12.5% (95% CI, 2.1%-73.7%) for the high-risk group (P < .001; Figure 4). The OS at 60 months was 100% for the low-risk group, 80.8% (95% CI, 63.3%-100%) for the intermediate-risk group, and 46.7% (95% CI, 23.3%-93.6%) for the high-risk group (P < .001).

Discussion
In this work, we explored a relatively large series of UMs with partial deletion of chromosome 3 and found that loss of the BAP1 locus may be associated with poor prognosis of M3 UM. This result was obtained by 2 different approaches investigating indirectly the prognostic value of the most frequently deleted regions of chromosome 3 and then directly assessing the prognostic value of the loss of the BAP1 locus in this series. The main consequence is to provide a potentially more accurate estimation of the prognosis of UMs that present with a partial deletion of chromosome 3. Our classification suggested efficiency in estimating metastatic outcome, identifying a group
with a good MFS with no recurrence and a group with a high risk (87.5%) of recurrence with a median follow-up of more than 5 years. Survival rates were close to those observed in a previous series of UMs that presented with an M3 or disomy 3 associated or not associated with 8q gain. This hypothesis has yet to be verified in subsequent studies because direct comparison could not be done here.

Other teams are using different genomic technologies to assess UM prognosis. Fluorescence in situ hybridization (FISH) is widely used, but it may miss the loss of the BAP1 locus if the probe is not centered on this gene, as observed in several articles. Furthermore, FISH is often performed without chromosome 8q assessment, leading to suboptimal prognosis estimation. Multiplex ligation-dependent probe amplification assay that covers the BAP1 locus is a good alternative to characterize recurrent genomic imbalances in UM, but multiple ligation-dependent probe amplification, as well as FISH and array CGH, only evaluate copy number and consequently do not identify isodisomic cases. Gene expression profile is a transcriptomic prognosis assay that is widely used in the United States. This assay distinguishes 2 subsets of UMs at low or high risk of metastasis by assessing the expression of 12 genes, including 4 that are located on the short arm of chromosome 3 (EIF1B [HGNC 30792], LMCD1 [OMIM 604859], ROBO1 [OMIM 602430], and SATB1 [OMIM 602075]) and 1 on the 3q (FXR1 [OMIM 600819]). Underexpression of these genes, possibly owing to M3, is associated with poor prognosis. A more accurate estimation by GEP is possible by adding the expression of PRAME (OMIM 606021), a gene located on an unstable region of chromosome 22 exposed to duplication, which was correlated with the 8q status in a pivotal study. To our knowledge, GEP has never been specifically tested in a large series of UMs with partial chromosome 3 deletions. Furthermore, to our knowledge, GEP has never been compared with the combined M3/8q signature in a large cohort, impeding any conclusion on the superiority of one modality or the other. BAPI immunohistochemistry is an alternative way to assess the prognosis of UMs. However, immunohistochemistry for BAPI does not correlate in all cases with the BAPI mutational status in UM and is therefore not a perfect surrogate.

In the present series, partial deletions of chromosome 3 were found in 4.0% of cases, which is comparable with some previous series but lower than others. Recruitment bias may explain part of this discrepancy, but it is probably explained by the variety of technologies and the different classifications that were used. Comparison of all these studies is therefore limited. Similarly, the prognosis of these tumors was not clear because a discrepancy was observed, with some series associating partial loss with good prognosis and others associating it with intermediate or poor prognosis. These differences may be explained by the absence of distinction depending on the loss of the BAPI locus compared with other losses.

One explanation for the low MFS associated with the loss of this locus may be that the loss of 1 BAPI allele contributes to the inactivation of this gene and subsequent aggressiveness of the tumor. However, the minimal region of deletion that was associated with the lowest MFS in our series (3p22.1-p44.2) includes 291 genes. Even though this region encompasses BAPI, other important genes may be present there and haploinsufficiency of these genes may affect tumorigenesis. The 2 alleles of a tumor suppressor gene are commonly inactivated in the 2-hit model by a combination of different mechanisms, including total or partial loss of a chromosome, deleterious point mutations, short insertions and deletions, large-scale insertions and deletions, and promoter methylations. It is highly intriguing that BAPI inactivation is frequently associated with monosomy 3 in UM, contrary to renal clear cell carcinomas and mesotheliomas, which carry losses of the short arm of chromosome 3 only or deleterious mutations of both alleles. Furthermore, haploinsufficiency of other genes on chromosome 3, possibly on its long arm, may play a role in UM tumorigenesis. This hypothesis may be of particular interest and should be put in perspective with the recent discovery of MBD4 (3q21.3) recurrent, inactivating mutations in UM.

To date, there is no standard treatment in the metastatic setting, but new drugs are being developed in UM.
efficient treatment becomes available, the next step will consist of testing this treatment in the adjuvant setting in high-risk patients.41 Accurate prognosis evaluation is essential for such trials, and assays able to assess the status of the BAP1 locus and 8q status may then be required. Next-generation sequencing appears to be the best option in the near future because it not only assesses copy number, heterozygosity, and mutational statuses of UMs at low cost and with a lower amount of DNA but also allows circulating tumor DNA to be examined.42-44 Moving toward the implementation of such technologies in our daily practice would allow ophthalmology to enter the modern age of precision medicine while reducing costs and refining UM prognosis.

Limitations
The conclusions of this work are limited by its retrospective nature, but prospective series are unrealistic given the rarity of such tumors. Instead, this work provides evidence to refine the current UM genomic classification, which may help ophthalmologists to better identify the metastatic evolution of their patients. Before generalization, other series from different centers are required. Furthermore, our series, composed of large tumors (median diameter of 16 mm and median thickness of 10 mm), is not reflective of the overall population of patients with UM, particularly because larger UMs are known to host a greater frequency of genomic alterations, including 8q gains.38,45 Other centers have reported genomic studies on biopsy samples of smaller UMs.46 However, this procedure is not consensual and must not be undertaken in inexperienced ophthalmology centers because of potential surgical complications. Multicenter collaborative studies of small UM genomics are required to address the question of partial chromosome 3 loss frequency at this stage of primary UM development. Another limitation of this study is that the array CGH technology is not adapted to detect chromosome 3 isodisomy, an infrequent alteration in UM, which may be associated with poor prognosis. A single-nucleotide polymorphism array can resolve this issue, but in the

| Characteristic                | No. of Cases | HR (95% CI)   | P Value |
|------------------------------|--------------|---------------|---------|
| **Univariate Analysis**      |              |               |         |
| Age, y                       |              |               |         |
| <60                          | 23           | 1 [Reference] | .17     |
| ≥60                          | 20           | 0.49 (0.17-1.4) | .14     |
| Sex                          |              |               |         |
| Male                         | 21           | 1 [Reference] | .31     |
| Female                       | 22           | 0.46 (0.16-1.33) | .43     |
| Diameter, mm                 |              |               |         |
| ≤15                          | 17           | 1 [Reference] | .31     |
| >15                          | 26           | 1.72 (0.6-4.95) | .43     |
| Thickness, mm                |              |               |         |
| ≤10                          | 22           | 1 [Reference] | .43     |
| >10                          | 21           | 1.49 (0.55-4) | .43     |
| Tumor location               |              |               |         |
| On the equator               | 28           | 1 [Reference] | .07     |
| Anterior to the equator      | 4            | 2.55 (0.69-9.36) | .99     |
| Posterior to the equator     | 10           | 0.36 (0.08-1.62) | .99     |
| Retinal detachment           |              |               |         |
| No                           | 4            | 1 [Reference] | .06     |
| Yes                          | 21           | 0.32 (0.09-1.11) | .06     |
| Histologic type              |              |               |         |
| Spindle cells                | 10           | 1 [Reference] | >.99    |
| Epithelioid or mixed         | 13           | 1 (0.28-3.55) | >.99    |
| BAP1 locus deletion          |              |               |         |
| No                           | 24           | 1 [Reference] | .001    |
| Yes                          | 19           | 5.91 (1.89-18.54) | .001    |
| 8q Gain                      |              |               |         |
| No                           | 16           | 1 [Reference] | .007    |
| Yes                          | 27           | 6.02 (1.36-26.61) | .007    |
| **Multivariate Analysis**    |              |               |         |
| BAP1 locus deletion          |              |               |         |
| No                           | 24           | 1 [Reference] | .001    |
| Yes                          | 19           | 6.65 (2.09-21.18) | .001    |
| 8q Gain                      |              |               |         |
| No                           | 16           | 1 [Reference] | .01     |
| Yes                          | 27           | 6.88 (1.53-30.86) | .01     |

Abbreviation: HR, hazard ratio.

Figure 4. Metastasis-Free Survival (MFS) and Overall Survival (OS) According to the 3 Different Prognosis Groups

Survival curves in uveal melanomas with a partial loss of chromosome 3 according to the 3 different prognosis groups.
future, next-generation sequencing will probably be the privileged technology to circumvent this issue. Although the BAPI locus hypothesis is a logical hypothesis, we cannot definitely affirm that BAPI is the target of such deletions. Chromosome 3 is dense in cancer genes, and the BAPI region, for instance, encompasses the tumor suppressor gene PBRM1 (OMIM 606083), which was recently found mutated in rare UMs. To confirm the BAPI locus hypothesis and the classification, validation series are required, ideally together with further work sequencing BAPI to confirm the presence of a second hit.

Conclusions

These findings suggest that partial deletion of chromosome 3 that encompasses the BAPI locus is associated with poor prognosis. Consequently, a new cytogenetic classification of UMs was proposed based on the status of the BAPI locus instead of chromosome 3. The frequent loss of the whole chromosome 3 in UMs raises the possibility of other genes associated with UM tumorigenesis on this chromosome.

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Importance of Partial Losses of Chromosome 3 in Uveal Melanoma in the BAPI Gene Region
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Close to 30 years ago, 3 research groups independently described chromosomal abnormalities in primary uveal melanoma, with all highlighting the presence of monosomy 3 in some of the examined cases. All groups proposed that monosomy 3 may play an important role in uveal melanoma progression. This theory was later confirmed by Prescher and colleagues, who examined the outcome of 54 patients with primary uveal melanoma, 30 (55%) of whom had monosomy 3 tumors, with 17 of these 30 patients (57%) dying of the disease within 3 years. In the meantime, numerous research groups have confirmed the significance of monosomy 3 loss in primary uveal melanoma. Damato and colleagues used cytogenetic testing of consenting patients with primary uveal melanoma to stratify these patients into risk groups with respect to metastasis development and to assess liver surveillance management. In the meantime, molecular genetic testing has been incorporated into algorithms that integrate other known strong prognostic factors to refine metastatic risk. During the past decade, understanding of the underlying mutations present in primary uveal melanoma and how these may be associated with the described chromosomal alterations has advanced significantly. Of particular importance is the gene BAPI (3p12.1-3p21.2) and its temporal and functional association with the loss of 1 copy of chromosome 3.

In this issue of JAMA Ophthalmology, Rodrigues and colleagues report on their retrospective cohort study of 1088 uveal melanomas, which had been examined using compar-