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1 **Association of Partial Chromosome 3 Deletion in Uveal Melanomas with**  
2 **Metastasis-free Survival**

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36

37

38 **Key Points**

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

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

44 **Meaning:** These findings suggest that uveal melanomas carrying a partial deletion of  
45 chromosome 3 encompassing the *BAP1* locus have a poor prognosis.

46

47 **Abstract**

48 **Importance**

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

52 **Objective**

53 To determine the association of partial chromosome 3 deletion in uveal melanomas with  
54 metastasis-free survival.

55 **Design**

56 Retrospective cohort of consecutive comparative genomic hybridization arrays from  
57 May 2006 to July 2015.

58 **Setting**

59 Monocentric study in a referral center.

60 **Participants**

61 Patients presenting with UMs with and without partial loss of chromosome 3.

62 **Main Outcomes and Measures**

63 Metastasis-free survival and overall survival at 60 months.

64 **Results**

65 Of the 1,088 consecutive comparative genomic hybridization arrays that were  
66 performed, 43 UMs (4%) carried partial deletions of chromosome 3. Median follow-up  
67 was 66 months. Metastasis-free survival at 60 months was 34% (95% confidence  
68 interval [CI], 15.8 to 71.4) for UMs carrying a deletion of the *BAP1* (*BRCA1*  
69 *associated protein-1*) locus (BAP1del; 24 tumors) and 81% (95% CI, 64.8 to 100) for  
70 UMs without the loss of the *BAP1* locus (BAP1 normal; BAP1nl; 19 tumors; log-rank  
71 p-value = .001). Overall survival at 60 months was 65% (95% CI, 43.5 to 95.8) *versus*

72 84% (95% CI, 69.0 to 100) in the BAP1del and the BAP1nl groups, respectively (log-  
73 rank p-value < .001). In these 43 cases, metastasis-free survival at 60 months was  
74 100% for UMs without loss of the *BAP1* locus or 8q gain, 70% (95% CI, 50.5 to 96.9)  
75 for UMs carrying one of these alterations and 13% for those carrying both (95% CI,  
76 2.1 to 73.7; log-rank p-value < .001). Similarly, overall survival at 60 months was  
77 100%, 81% (95% CI, 63.3 to 100) and 47% (95% CI, 23.3 to 93.6) in these three  
78 groups, respectively (log-rank p-value < .001).

### 79 **Conclusions and Relevance**

80 These findings suggest that partial deletion of chromosome 3 encompassing the  
81 *BAP1* locus is associated with poor prognosis. A cytogenetic classification of UMs  
82 could be proposed based on the status of the *BAP1* locus instead of chromosome 3,  
83 locus, while also taking chromosome 8q into account.

84

## 85 **Introduction**

86 Uveal melanoma (UM) is the most common primary malignant ocular tumor in adults  
87 of European ancestry<sup>1</sup>. Despite efficient treatment, up to 50% of the patients will  
88 eventually develop metastases<sup>2-4</sup>. Reliable prognostic assessment allows a closer  
89 monitoring of high-risk patients. Pathological prognostic factors include large tumor  
90 basal diameter, thickness, ciliary body involvement, extraocular extension, epithelioid  
91 cell histology, high mitotic rate and lymphocytic infiltration<sup>5</sup>. The gene expression  
92 profile DecisionDx-UM (GEP; Castle Biosciences, Friendswood, TX), based on the  
93 expression level of 12 genes, is frequently used in North America to complete the  
94 prognostic assessment<sup>6,7</sup>.

95 In the early 1990s, recurrent cytogenetic aberrations including monosomy 3 (M3),  
96 gain of 6p and 8q were identified in UM samples<sup>8</sup>. In 1996, M3 was empirically shown  
97 to be a robust prognostic factor<sup>9</sup>. Since then, genomic arrays have become routine  
98 tools to refine pathological prognosis along with the GEP. We previously refined the  
99 prognostic value of M3 and gain of 8q by defining three groups: (i) high-risk patients  
100 whose tumors present a M3 and an 8q gain with a 2-year metastasis-free interval  
101 (2y-MFI) of 37%; (ii) intermediate-risk with either a M3 or an 8q gain (2y-MFI: ~85%)  
102 and (iii) low-risk with neither M3 nor 8q gain (2y-MFI: ~100%)<sup>10</sup>.

103 The most common hypothesis to explain the poor prognosis of M3 tumors is the  
104 presence of one or more tumor suppressor genes (TSG) on chromosome 3. *BAP1*  
105 (*BRCA1 associated protein-1*), a TSG located on the 3p21.1 cytoband, is now  
106 established as a main actor of UM malignant transformation as it is frequently  
107 mutated in M3 tumors and germline mutations are associated with UM  
108 predisposition<sup>11-16</sup>. However, all or most *BAP1*-mutated UMs intriguingly present a  
109 M3 (or a loss of heterozygosity of the whole chromosome 3 due an isodisomy)

110 suggesting that the role of chromosome 3 loss in UM tumorigenesis may not be  
111 restricted to *BAP1* inactivation. Therefore, prognostication of UM samples with partial  
112 deletions of chromosome 3, as sometimes observed in our daily practice and by  
113 other authors, is problematic<sup>17</sup>. The goals of the present study were to explore these  
114 UMs with partial deletions of chromosome 3, as assessed by comparative genomic  
115 hybridization (array-CGH), in order to assess their prognosis and to determine the  
116 minimal region of deletion associated with poor prognosis.



## 117 **Materials and methods**

### 118 **Patients**

119 This study was approved by our institutional ethics committee. Written informed  
120 consent for the use of tissues and data for research was signed by each patient. The  
121 study complied with the principles of the Declaration of Helsinki. All patients were  
122 referred to our institution and followed up by our physicians. Clinical diagnosis of  
123 uveal melanoma was based on the presence of typical clinical findings as previously  
124 described<sup>10</sup>. Local treatment consisted of proton beam radiotherapy, iodine 125  
125 brachytherapy or enucleation, depending on the size and location of tumors. Tumor  
126 samples were obtained by enucleation, endoresection or fine-needle aspiration at the  
127 time of clip or plaque positioning. Liver ultrasound, liver magnetic resonance imaging  
128 or body computed tomography were performed at diagnosis and every 6 months  
129 afterwards. Diagnosis of metastasis was systematically confirmed by a biopsy.

### 130 **Genomic analysis**

131 Tumor DNA was extracted and processed as previously described<sup>10</sup>. Array-CGH was  
132 performed on three different platforms according to the period when the test was  
133 performed: bacterial artificial chromosome arrays as previously described<sup>18</sup>,  
134 NimbleGen 4x72 K arrays (Roche NimbleGen, Madison, Wisconsin, USA) and  
135 Agilent 180K CGH/LOH custom chip (Santa Clara, California, USA). Array-CGH were  
136 interpreted by three of the authors (MR, KAR, GP). Partial deletion of chromosome 3  
137 was defined as the loss of at least one region of chromosome 3, but not the totality,  
138 whatever its size and location. Genomic positions in this article are defined in hg18  
139 human genome assembly.

### 140 **Statistical analysis**

141 Clinical, pathological and genomic data at diagnosis and follow-up events (local and  
142 distant recurrences, second cancers, death from UM or from any other cause) were  
143 collected. The French Death Registry was consulted for patients lost to follow-up.  
144 The metastasis-free survival (MFS) at 60 months was defined as the proportion of  
145 patients alive and free of metastasis at 60 months of follow-up after local treatment of  
146 primary UM. The overall survival (OS) at 60 months was defined as the proportion of  
147 patients alive at 60 months of follow-up after local treatment of primary UM, whatever  
148 the cause of death. Survival distributions were estimated by the Kaplan–Meier  
149 method and compared using the log-rank test. All tests were bilateral and performed  
150 with a significant level of 5%. In order to identify variables associated with MFS, a  
151 Cox regression analysis of candidate prognostic factors was performed using a  
152 forward stepwise selection procedure. The added value of each variable to the Cox  
153 model was determined using a likelihood ratio test with a significant level of 5%.  
154 Statistical analysis was performed using R software V3.3 (<http://www.r-project.org/>).  
155

156 **Results**

157 We prospectively re-analyzed the array-CGH profiles in 1,088 UMs which had been  
158 processed between May 2006 and July 2015, and detected 43 cases (4.0%)  
159 harboring a partial deletion of chromosome 3 (eTable 1 in the supplement). Median  
160 follow-up in these 43 cases was 66 months (range: 1.2-126.2 months). Median age  
161 was 58 years-old (range 12-79), median tumor diameter was 16 millimeters (range:  
162 10-22) and median thickness was 10 millimeters (range: 5.3-18.2). Ciliary body and  
163 optic nerve were involved in 33% (14/43) and in 9% (4/43) of cases, respectively. Cell  
164 morphology was epithelioid or mixed in 30% of cases (13/43). Primary tumors were  
165 treated by enucleation in 42% (18/43) of cases. MFS and OS at 60 months were 61%  
166 (95% confidence interval [CI], 46 to 79.7) and 76% (95% CI, 62.8 to 92.3),  
167 respectively. A global overview of copy number profiles is provided in eFigure 1 in the  
168 supplement. Size of deletions ranged from 1.36 to 110.88 megabases.

169  
170 We first explored survival data in an unsupervised manner and observed three  
171 recurrently lost regions in at least eight metastatic samples: (i) from 3pter to p22.2, (ii)  
172 from 3p22.1 to p14.2 and (iii) from 3q13.2 to q24 (Figure 1). Of these, two regions  
173 were more frequently lost in metastatic cases than in non-metastatic ones: the 3pter-  
174 p22.2 region (8/13 *versus* 6/30 cases, respectively;  $p=.013$ ; odds ratio [OR]=6.1; 95%  
175 CI, 1.2 to 34.1) and the 3p22.1-p14.2 region, which encompasses *BAP1* (10/13  
176 *versus* 9/30 cases, respectively;  $p=.007$ ; OR=7.4; 95% CI, 1.5 to 51.8). These two  
177 regions were close and highly correlated between each other, as 8 out of 10  
178 metastatic cases presenting a 3p22.1-p14.2 loss also presented a 3pter-p22.2 loss.  
179 The 3p22.1-p14.2 region carries 290 other genes beside *BAP1*, but no recurrent  
180 mutations of these 290 genes were found in public and in-house databases<sup>12,19,20</sup>.

181

182 We then hypothesized that *BAP1* loss was the main driver of poor prognosis in M3.  
183 To explore this hypothesis, we compared tumors with a chromosome 3 partial  
184 deletion encompassing the *BAP1* locus (24 tumors; BAP1del) and tumors with a  
185 chromosome 3 partial deletion not encompassing the *BAP1* locus (19 tumors;  
186 BAP1nl). Tumors carrying a loss of the *BAP1* locus frequently showed large losses of  
187 the short arm of chromosome 3 (Figure 2). MFS at 60 months was 81% (95% CI,  
188 64.8 to 100) for the BAP1nl genomic group and 34% (95% CI, 15.8 to 71.4) for the  
189 BAP1del group (Figure 3;  $p=.001$ ). OS at 60 months was 84% (95% CI, 69.0 to 100)  
190 for the BAP1nl genomic group and 65% (95% CI, 43.5 to 95.8) for the BAP1del group  
191 ( $p<.001$ ). The only variables associated with MFS in univariate analysis were loss of  
192 the *BAP1* locus and gain of 8q. These two variables independently contributed to  
193 MFS in multivariate analysis (Table 1).

194

195 We defined four groups depending on the *BAP1* locus (lost/not lost) and 8q  
196 (gained/not gained) statuses. Prognoses of the *BAP1* locus lost/8q normal and *BAP1*  
197 locus not lost/8q gained were similar so we merged these two groups, as in our  
198 previous classification (eFigure 2 in the supplement)<sup>10</sup>. By analogy with our previous  
199 work, we defined three prognosis groups as follows: (i) a group at low risk of  
200 metastasis without loss of the *BAP1* locus or 8q gain (9 cases), (ii) an intermediate  
201 risk group with tumors carrying either loss of the *BAP1* locus (7 cases) or 8q gain (15  
202 cases) and (iii) a high risk group with loss of the *BAP1* locus and 8q gain (12 cases).  
203 MFS at 60 months were 100%, 70% (95% CI, 50.5 to 96.9) and 13% (95% CI, 2.1 to  
204 73.7) for the low-, intermediate- and high-risk groups, respectively (Figure 4;  $p<.001$ ).

205 OS at 60 months were 100%, 81% (95% CI, 63.3 to 100) and 47% (95% CI, 23.3 to  
206 93.6) for the low-, intermediate- and high-risk groups, respectively ( $p < .001$ ).

207

208 **Discussion**

209 In this work, we explored a relatively large series of UMs with partial deletion of  
210 chromosome 3 and showed that loss of the *BAP1* locus is likely to explain the poor  
211 prognosis of M3 UM. This result was obtained by two different approaches  
212 investigating indirectly the prognostic value of the most frequently deleted regions of  
213 chromosome 3 and then directly assessing the prognostic value of the loss of the  
214 *BAP1* locus in this series. The first consequence is to provide a potentially more  
215 accurate estimation of the prognosis of UMs presenting a partial deletion of  
216 chromosome 3. Our classification suggested efficiency in predicting metastatic  
217 outcome, identifying a group with a very good MFS with no recurrence and a group  
218 with a high risk of 92% of recurrences with a median follow-up of more than 5 years.  
219 Survival rates were close to what we observed in a previous series of UMs  
220 presenting either a M3 or a disomy 3, associated or not with 8q gain<sup>10</sup>. This  
221 hypothesis has yet to be verified in subsequent studies because direct comparison  
222 could not be done here.

223

224 Other teams are using different genomic technologies to assess UM prognosis.  
225 Fluorescence *in situ* hybridization (FISH) is widely used but it may miss the loss of  
226 the *BAP1* locus if the probe is not centered on this gene, as observed in several  
227 publications<sup>2,21-24</sup>. Furthermore, FISH is often performed without chromosome 8q  
228 assessment leading to suboptimal prognosis estimation. Multiplex ligation-dependent  
229 probe amplification (MLPA) assay covering the *BAP1* locus is a good alternative to  
230 characterize recurrent genomic imbalances in UM but MLPA, as well as FISH and  
231 array-CGH, only evaluate copy number and, consequently does not identify  
232 isodisomic cases<sup>25,26</sup>. GEP is a transcriptomic prognosis assay that is widely used in

233 United States<sup>7</sup>. This assay distinguishes two subsets of UMs either at low or high risk  
234 of metastasis by assessing the expression of 12 genes, including four that are  
235 located on the short arm of chromosome 3 (*EIF1B*, *LMCD1*, *ROBO1*, *SATB1*) and  
236 one on the 3q (*FXR1*). Underexpression of these genes, possibly due to M3, is  
237 associated with poor prognosis. A more accurate prediction by GEP is possible by  
238 adding the expression of *PRAME*, a gene located on an instable region of  
239 chromosome 22 exposed to duplication, which was correlated to the 8q status in the  
240 pivotal paper<sup>6</sup>. To our knowledge, GEP has never been specifically tested in a large  
241 series of UMs with partial chromosome 3 deletions. Furthermore, GEP has never  
242 been compared to the combined M3/8q signature in a large cohort, impeding any  
243 conclusion on the superiority of one modality on the other. BAP1  
244 immunohistochemistry is an alternative way to assess the prognosis of UMs<sup>27,28</sup>.  
245 However, immunohistochemistry for BAP1 does not correlate in all cases to the  
246 BAP1 mutational status in UM, and is therefore not a perfect surrogate<sup>27</sup>.  
247  
248 In the present series, partial deletions of chromosome 3 were found in 4% of cases,  
249 which is comparable with some previous series<sup>29-31</sup> but lower than others<sup>17,32,33</sup>.  
250 Recruitment bias may explain part of this discrepancy but it is most probably  
251 explained by the variety of technologies, as well as the different classifications that  
252 were used. Comparison of all these studies is therefore limited. Similarly, the  
253 prognosis of these tumors was not clear as a discrepancy was observed with some  
254 series associating partial loss with good prognosis<sup>17,29,32</sup> while others associated it  
255 with intermediate or poor prognosis<sup>25,33,34</sup>. These differences may be explained by  
256 the absence of distinction depending on the loss of the *BAP1* locus compared to  
257 other losses.

258

259 One explanation for the low MFS associated with the loss of this locus may be that  
260 the loss of one *BAP1* allele contributes to the inactivation of this gene and  
261 subsequent aggressiveness of the tumor. However, the minimal region of deletion we  
262 found associated with the lowest MFS in our series (3p22.1-p14.2) includes 291  
263 genes. Even though this region encompasses *BAP1*, it cannot be excluded that other  
264 important genes are present there and that haploinsufficiency of these genes affects  
265 tumorigenesis. The two alleles of a TSG are commonly inactivated in the two-hit  
266 model by a combination of different mechanisms, including total or partial loss of a  
267 chromosome, deleterious point mutations, short insertions/deletions, large-scale  
268 insertions/deletions and promoter methylations<sup>35</sup>. It is highly intriguing that, *BAP1*  
269 inactivation is so frequently associated with monosomy 3 in UM, contrary to renal  
270 clear-cell carcinomas and mesotheliomas, which rather carry losses of the short arm  
271 of chromosome 3 only or deleterious mutations of both alleles<sup>16</sup>. Furthermore,  
272 haploinsufficiency of other genes on chromosome 3, possibly on its long arm, may  
273 play a role on UM tumorigenesis. This hypothesis may be of particular interest and  
274 should be put in perspective with the recent discovery of *MBD4* (3q21.3) recurrent,  
275 inactivating mutations in UM<sup>36-38</sup>.

276

277 There is, for now, no standard treatment in the metastatic setting, but new drugs are  
278 being developed in UM<sup>39</sup>. When an efficient treatment will be available, the following  
279 step will consist in testing this treatment in the adjuvant setting in high-risk patients<sup>40</sup>.  
280 Accurate prognosis evaluation is essential for such trials and assays able to assess  
281 the status of the *BAP1* locus and 8q status may then be required. Next-generation  
282 sequencing appears to be the best option in the near future as it not only assesses



283 copy number, heterozygosity and mutational statuses of UMs at low cost and with a  
284 lower amount of DNA, but also allow to follow circulating tumor DNA<sup>41-43</sup>. Moving  
285 towards the implementation of such technologies in our daily practice will allow ocular  
286 oncology to enter the modern age of precision medicine while reducing costs and  
287 refining UM prognosis.

288

## 289 **Limitations**

290 The conclusions of this work are limited by its retrospective nature, but prospective  
291 series are unrealistic given the rarity of such tumors. Instead, the present work  
292 provides evidence to refine the current UM genomic classification, which may help  
293 ophthalmologists to better predict the metastatic evolution of their patients. Before  
294 generalization, other series from different centers are required. Furthermore, one  
295 could argue that our series, composed of large tumors (median diameter of 16mm  
296 and median thickness of 10mm) is not reflecting the overall population of UM  
297 patients, particularly as larger UMs are known to host a greater frequency of genomic  
298 alterations, including 8q gains<sup>37,44</sup>. Other centers have reported genomic studies on  
299 biopsies of smaller UMs<sup>45</sup>. However, this procedure is not consensual and must not  
300 be undertaken in inexperienced ocular oncology centers because of potential surgical  
301 complications. Multicenter collaborative studies of small UM genomics are required to  
302 address the question of partial chromosome 3 loss frequency at this stage of primary  
303 UM development. Another limitation of this study is that the array-CGH technology is  
304 not adapted to detect chromosome 3 isodisomy, an infrequent alteration in UM,  
305 probably associated with poor prognosis. SNP-array can resolve this issue but, in the  
306 future, next-generation sequencing will probably be the privileged technology to  
307 circumvent this issue. More importantly, although the *BAP1* locus hypothesis is a

308 logical hypothesis, we cannot definitely affirm that *BAP1* is indeed the target of such  
309 deletions. Chromosome 3 is dense in cancer genes and the *BAP1* region, for  
310 instance, encompasses the tumor-suppressor gene *PBRM1*, which was recently  
311 found mutated in rare UMs. To confirm the *BAP1* locus hypothesis and the  
312 classification, validation series are required, ideally together with further work  
313 sequencing *BAP1* to confirm the presence of a second hit.

314

### 315 **Conclusions**

316 These findings suggest that partial deletion of chromosome 3 encompassing the  
317 *BAP1* locus is associated with poor prognosis. Consequently, a new cytogenetic  
318 classification of UMs is proposed, based on the status of the *BAP1* locus instead of  
319 chromosome 3. The very frequent loss of the whole chromosome 3 in UMs raises the  
320 possibility of other genes associated with UM tumorigenesis on this chromosome.

321

322

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347

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349 **References**

- 350 1. Mahendraraj K, Lau CS, Lee I, Chamberlain RS. Trends in incidence, survival,  
351 and management of uveal melanoma: a population-based study of 7,516 patients  
352 from the Surveillance, Epidemiology, and End Results database (1973-2012). *Clin*  
353 *Ophthalmol.* 2016;10:2113-2119.
- 354 2. Desjardins L, Levy-Gabriel C, Lumbroso-Lerouic L, et al. [Prognostic factors  
355 for malignant uveal melanoma. Retrospective study on 2,241 patients and recent  
356 contribution of monosomy-3 research]. *Journal francais d'ophtalmologie.* Sep  
357 2006;29(7):741-749.
- 358 3. Singh AD, Turell ME, Topham AK. Uveal melanoma: trends in incidence,  
359 treatment, and survival. *Ophthalmology.* Sep 2011;118(9):1881-1885.
- 360 4. Kujala E, Makitie T, Kivela T. Very long-term prognosis of patients with  
361 malignant uveal melanoma. *Investigative ophthalmology & visual science.* Nov  
362 2003;44(11):4651-4659.
- 363 5. Brierley JD, Gospodarowicz MK, Wittekind C. *The TNM Classification of*  
364 *Malignant Tumours. 8th edition.*2016.
- 365 6. Field MG, Decatur CL, Kurtenbach S, et al. PRAME as an Independent  
366 Biomarker for Metastasis in Uveal Melanoma. *Clinical cancer research : an official*  
367 *journal of the American Association for Cancer Research.* Mar 1 2016;22(5):1234-  
368 1242.
- 369 7. Onken MD, Worley LA, Ehlers JP, Harbour JW. Gene expression profiling in  
370 uveal melanoma reveals two molecular classes and predicts metastatic death.  
371 *Cancer research.* Oct 15 2004;64(20):7205-7209.

- 372 8. Prescher G, Bornfeld N, Becher R. Nonrandom chromosomal abnormalities in  
373 primary uveal melanoma. *Journal of the National Cancer Institute*. Nov 21  
374 1990;82(22):1765-1769.
- 375 9. Prescher G, Bornfeld N, Hirche H, Horsthemke B, Jockel KH, Becher R.  
376 Prognostic implications of monosomy 3 in uveal melanoma. *Lancet*. May 4  
377 1996;347(9010):1222-1225.
- 378 10. Cassoux N, Rodrigues MJ, Plancher C, et al. Genome-wide profiling is a  
379 clinically relevant and affordable prognostic test in posterior uveal melanoma. *The*  
380 *British journal of ophthalmology*. Jun 2014;98(6):769-774.
- 381 11. Abdel-Rahman MH, Pilarski R, Cebulla CM, et al. Germline BAP1 mutation  
382 predisposes to uveal melanoma, lung adenocarcinoma, meningioma, and other  
383 cancers. *Journal of medical genetics*. Dec 2011;48(12):856-859.
- 384 12. Robertson AG, Shih J, Yau C, et al. Integrative Analysis Identifies Four  
385 Molecular and Clinical Subsets in Uveal Melanoma. *Cancer Cell*. Jan 8  
386 2018;33(1):151.
- 387 13. Field MG, Durante MA, Anbunathan H, et al. Punctuated evolution of  
388 canonical genomic aberrations in uveal melanoma. *Nature communications*. Jan 9  
389 2018;9(1):116.
- 390 14. Rai K, Pilarski R, Boru G, et al. Germline BAP1 alterations in familial uveal  
391 melanoma. *Genes, Chromosomes & Cancer*. Feb 2017;56(2):168-174.
- 392 15. Harbour JW, Onken MD, Roberson ED, et al. Frequent mutation of BAP1 in  
393 metastasizing uveal melanomas. *Science*. Dec 3 2010;330(6009):1410-1413.
- 394 16. Wiesner T, Obenaus AC, Murali R, et al. Germline mutations in BAP1  
395 predispose to melanocytic tumors. *Nature genetics*. Aug 28 2011;43(10):1018-1021.

- 396 17. Abdel-Rahman MH, Christopher BN, Faramawi MF, et al. Frequency,  
397 molecular pathology and potential clinical significance of partial chromosome 3  
398 aberrations in uveal melanoma. *Modern pathology : an official journal of the United*  
399 *States and Canadian Academy of Pathology, Inc.* Jul 2011;24(7):954-962.
- 400 18. Trolet J, Hupe P, Huon I, et al. Genomic profiling and identification of high-risk  
401 uveal melanoma by array CGH analysis of primary tumors and liver metastases.  
402 *Investigative ophthalmology & visual science.* Jun 2009;50(6):2572-2580.
- 403 19. Furney SJ, Pedersen M, Gentien D, et al. SF3B1 mutations are associated  
404 with alternative splicing in uveal melanoma. *Cancer discovery.* Oct 2013;3(10):1122-  
405 1129.
- 406 20. Johansson P, Aoude LG, Wadt K, et al. Deep sequencing of uveal melanoma  
407 identifies a recurrent mutation in PLCB4. *Oncotarget.* Jan 26 2016;7(4):4624-4631.
- 408 21. Mensink HW, Vaarwater J, de Keizer RJ, et al. Chromosomal aberrations in  
409 iris melanomas. *The British journal of ophthalmology.* Mar 2011;95(3):424-428.
- 410 22. Worley LA, Onken MD, Person E, et al. Transcriptomic versus chromosomal  
411 prognostic markers and clinical outcome in uveal melanoma. *Clinical cancer research*  
412 *: an official journal of the American Association for Cancer Research.* Mar 1  
413 2007;13(5):1466-1471.
- 414 23. van Gils W, Lodder EM, Mensink HW, et al. Gene expression profiling in uveal  
415 melanoma: two regions on 3p related to prognosis. *Investigative ophthalmology &*  
416 *visual science.* Oct 2008;49(10):4254-4262.
- 417 24. Singh AD, Aronow ME, Sun Y, et al. Chromosome 3 status in uveal  
418 melanoma: a comparison of fluorescence in situ hybridization and single-nucleotide  
419 polymorphism array. *Investigative ophthalmology & visual science.* Jun 5  
420 2012;53(7):3331-3339.

- 421 25. Damato B, Dopierala J, Klaasen A, van Dijk M, Sibbring J, Coupland SE.  
422 Multiplex ligation-dependent probe amplification of uveal melanoma: correlation with  
423 metastatic death. *Investigative ophthalmology & visual science*. Jul 2009;50(7):3048-  
424 3055.
- 425 26. Larsen AC, Holst L, Kaczkowski B, et al. MicroRNA expression analysis and  
426 Multiplex ligation-dependent probe amplification in metastatic and non-metastatic  
427 uveal melanoma. *Acta ophthalmologica*. Sep 2014;92(6):541-549.
- 428 27. Kalirai H, Dodson A, Faqir S, Damato BE, Coupland SE. Lack of BAP1 protein  
429 expression in uveal melanoma is associated with increased metastatic risk and has  
430 utility in routine prognostic testing. *British journal of cancer*. Sep 23  
431 2014;111(7):1373-1380.
- 432 28. van Essen TH, van Pelt SI, Versluis M, et al. Prognostic parameters in uveal  
433 melanoma and their association with BAP1 expression. *Br J Ophthalmol*. Aug 21  
434 2014.
- 435 29. Thomas S, Putter C, Weber S, Bornfeld N, Lohmann DR, Zeschnigk M.  
436 Prognostic significance of chromosome 3 alterations determined by microsatellite  
437 analysis in uveal melanoma: a long-term follow-up study. *British Journal of Cancer*.  
438 Mar 13 2012;106(6):1171-1176.
- 439 30. Tschentscher F, Prescher G, Horsman DE, et al. Partial deletions of the long  
440 and short arm of chromosome 3 point to two tumor suppressor genes in uveal  
441 melanoma. *Cancer research*. Apr 15 2001;61(8):3439-3442.
- 442 31. Cross NA, Rennie IG, Murray AK, Sisley K. The identification of chromosome  
443 abnormalities associated with the invasive phenotype of uveal melanoma in vitro. *Clin*  
444 *Exp Metastasis*. 2005;22(2):107-113.



- 445 32. Shields CL, Ganguly A, Bianciotto CG, Turaka K, Tavallali A, Shields JA.  
446 Prognosis of uveal melanoma in 500 cases using genetic testing of fine-needle  
447 aspiration biopsy specimens. *Ophthalmology*. Feb 2011;118(2):396-401.
- 448 33. Damato B, Dopierala JA, Coupland SE. Genotypic profiling of 452 choroidal  
449 melanomas with multiplex ligation-dependent probe amplification. *Clinical cancer  
450 research : an official journal of the American Association for Cancer Research*. Dec  
451 15 2010;16(24):6083-6092.
- 452 34. Ewens KG, Kanetsky PA, Richards-Yutz J, et al. Genomic profile of 320 uveal  
453 melanoma cases: chromosome 8p-loss and metastatic outcome. *Invest Ophthalmol  
454 Vis Sci*. Aug 23 2013;54(8):5721-5729.
- 455 35. Knudson AG. Two genetic hits (more or less) to cancer. *Nat Rev Cancer*. Nov  
456 2001;1(2):157-162.
- 457 36. Rodrigues M, Mobuchon L, Houy A, et al. Outlier response to anti-PD1 in  
458 uveal melanoma reveals germline MBD4 mutations in hypermutated tumors. *Nature  
459 communications*. May 14 2018;9(1):1866.
- 460 37. Rodrigues M, Mobuchon L, Houy A, et al. Evolutionary routes in metastatic  
461 uveal melanomas depend on MBD4 alterations. *Clin Cancer Res*. Jun 21 2019.
- 462 38. Johansson PA, Stark A, Palmer JM, et al. Prolonged stable disease in a uveal  
463 melanoma patient with germline MBD4 nonsense mutation treated with  
464 pembrolizumab and ipilimumab. *Immunogenetics*. May 2019;71(5-6):433-436.
- 465 39. Carvajal RD, Schwartz GK, Tezel T, Marr B, Francis JH, Nathan PD.  
466 Metastatic disease from uveal melanoma: treatment options and future prospects.  
467 *The British journal of ophthalmology*. Jan 2017;101(1):38-44.
- 468 40. Piperno-Neumann S, Rodrigues MJ, Servois V, et al. A randomized  
469 multicenter phase 3 trial of adjuvant fotemustine versus surveillance in high risk uveal

470 melanoma (UM) patients (FOTEADJ). *Journal of Clinical Oncology*.  
471 2017;35(15\_suppl):9502-9502.

472 41. Smit KN, van Poppel NM, Vaarwater J, et al. Combined mutation and copy-  
473 number variation detection by targeted next-generation sequencing in uveal  
474 melanoma. *Mod Pathol*. May 2018;31(5):763-771.

475 42. Afshar AR, Damato BE, Stewart JM, et al. Next-Generation Sequencing of  
476 Uveal Melanoma for Detection of Genetic Alterations Predicting Metastasis. *Transl*  
477 *Vis Sci Technol*. Mar 2019;8(2):18.

478 43. Matet A, Ait Rais K, Malaise D, et al. Comparative Cytogenetic Abnormalities  
479 in Paired Choroidal Melanoma Samples Obtained Before and After Proton Beam  
480 Irradiation by Transscleral Fine-Needle Aspiration Biopsy and Endoresection.  
481 *Cancers (Basel)*. Aug 14 2019;11(8).

482 44. Shain AH, Bagger MM, Yu R, et al. The genetic evolution of metastatic uveal  
483 melanoma. *Nature Genetics*. Jul 2019;51(7):1123-1130.

484 45. Angi M, Kalirai H, Taktak A, et al. Prognostic biopsy of choroidal melanoma:  
485 an optimised surgical and laboratory approach. *The British journal of ophthalmology*.  
486 Aug 2017;101(8):1143-1146.

487

488

489 **Tables**

490 **Table 1. Univariate and multivariate analyses of risk factors for metastasis.**

491 HR (95%CI): hazard ratio (95% confidence interval); mm: millimeters; n: number of  
 492 cases.

<b>Univariate analysis</b>				
		<b>n</b>	<b>HR (95%CI)</b>	<b>p-value</b>
<b>Age</b>	< 60 years-old	23	1	.17
	≥ 60 years-old	20	0.49 (0.17-1.4)	
<b>Gender</b>	male	21	1	.14
	female	22	0.46 (0.16-1.33)	
<b>Diameter</b>	≤ 15 mm	17	1	.31
	> 15 mm	26	1.72 (0.6-4.95)	
<b>Thickness</b>	≤ 10 mm	22	1	.43
	> 10 mm	21	1.49 (0.55-4)	
<b>Tumor location</b>	on the equator	28	1	.07
	anterior to the equator	4	2.55 (0.69-9.36)	
	posterior to the equator	10	0.36 (0.08-1.62)	
<b>Retinal detachment</b>	No	4	1	.06
	Yes	39	0.32 (0.09-1.11)	
<b>Histology</b>	spindle cells	10	1	1.00
	epithelioid/mixed	13	1 (0.28-3.55)	
<b>BAP1 locus deletion</b>	No	24	1	.001
	Yes	19	5.91 (1.89-18.54)	
<b>8q gain</b>	No	16	1	.007
	Yes	27	6.02 (1.36-26.61)	

<b>Multivariate analysis</b>				
		<b>n</b>	<b>HR (95%CI)</b>	<b>p-value</b>
<b>BAP1 locus deletion</b>	No	24		.001
	Yes	19	6.65 (2.09 ; 21.18)	
<b>8q gain</b>	No	16		.01
	Yes	27	6.88 (1.53 ; 30.86)	

493

494 **Figures**

495 **Figure 1. Copy number profiles in metastatic *versus* non-metastatic cases.**

496 Frequencies of losses at a given position are shown at the bottom. Light gray: non-

497 metastatic cases (Met-; n=30); dark gray: metastatic cases (Met+; n=13).

498

499 **Figure 2. Copy number profiles in BAP1del cases *versus* BAP1nl.** Frequencies

500 of deletion at a given position are shown at the bottom. Light gray: BAP1nl cases

501 (n=19); dark gray: BAP1del cases (n=24).

502

503 **Figure 3. Metastasis-free and overall survivals according to the loss of the**

504 ***BAP1* locus.** Metastasis-free survival (left) and overall survival (right) curves in UMs

505 with a partial loss of chromosome 3 encompassing the *BAP1* locus or not. BAP1del:

506 deletion of the *BAP1* locus; BAP1nl: absence of loss of the *BAP1* locus.

507

508 **Figure 4. Metastasis-free and overall survivals according to the three different**

509 **prognosis groups.** Metastasis-free survival (left) and overall survival (right) curves

510 in UMs with a partial loss of chromosome 3 according to the three different prognosis

511 groups.

512

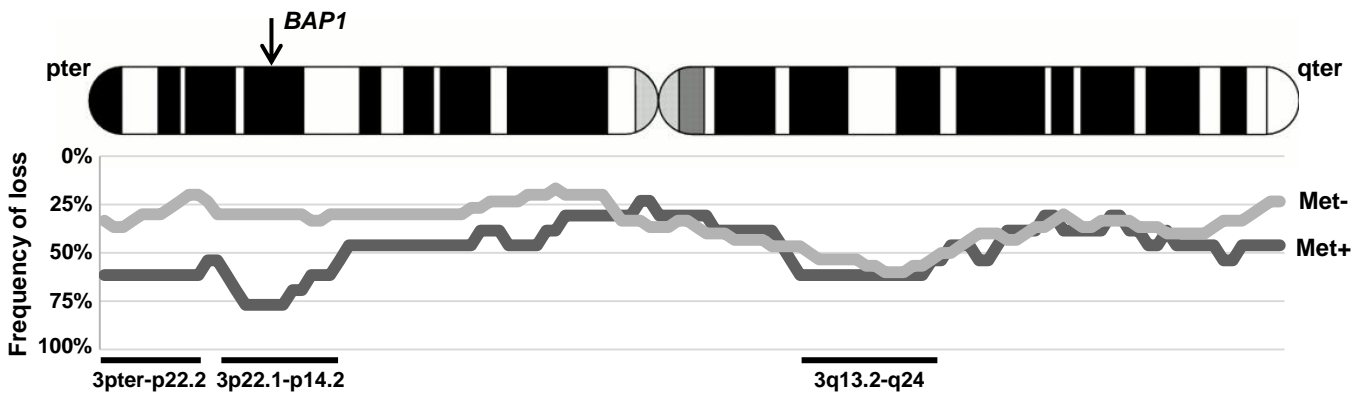


Figure 1. Rodrigues et al.

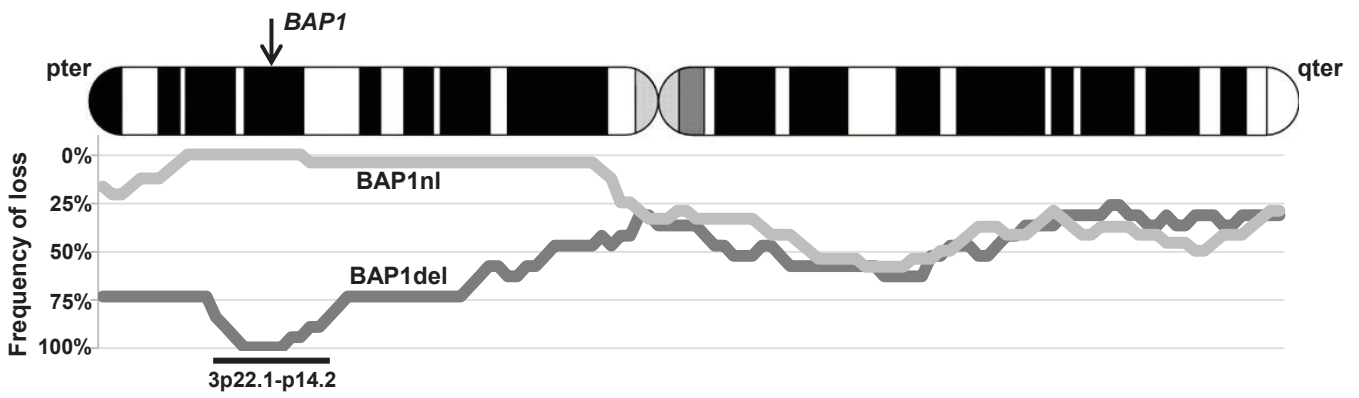
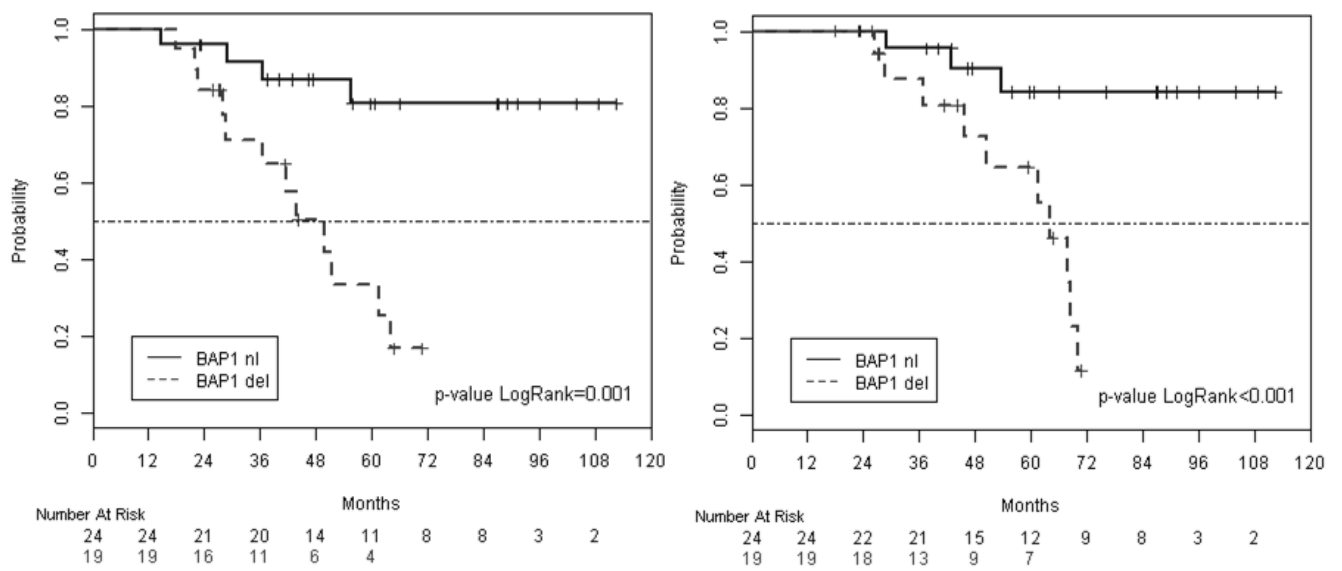
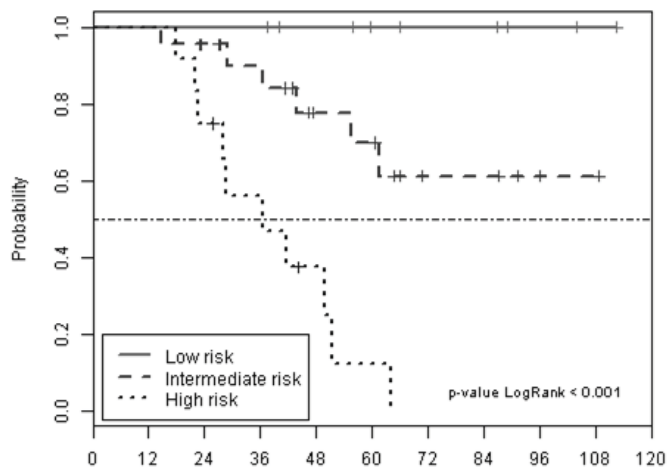


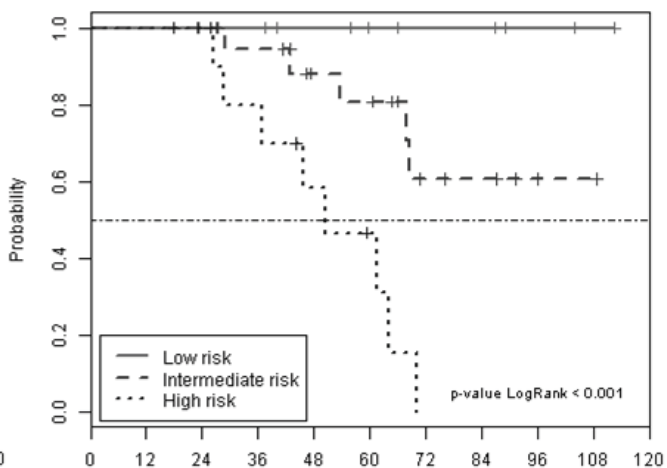
Figure 2. Rodrigues et al.



**Figure 3. Rodrigues et al.**



Number At Risk		Months									
		0	12	24	36	48	60	72	84	96	108
9	9	9	9	9	7	5	4	4	2	1	
22	22	19	16	10	9	4	4	1	1		
12	12	9	6	3	1						



Number At Risk		Months									
		0	12	24	36	48	60	72	84	96	108
9	9	9	9	7	5	4	4	2	1		
22	22	20	17	12	11	5	4	1	1		
12	12	11	8	5	3						

Figure 4. Rodrigues et al.