Association of Partial Chromosome 3 Deletion in Uveal Melanomas With Metastasis-Free Survival

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Manuel Rodrigues[1,2*], M.D. Ph.D., Khadija Ait-Rais[3], Flore Salviat[4], M.D., Nathalie Algret[4], Fatoumata Simaga[3], Raymond Barnhill[5,6], M.D., Sophie Gardrat[1,5], M.D., Vincent Servois[7], M.D., Pascale Mariani[8], M.D., Sophie Piperno-Neumann[2], M.D., Sergio Roman-Roman[9], Pharm.D. Ph.D., Olivier Delattre[1,3], M.D. Ph.D., Nathalie Cassoux[6,10], M.D. Ph.D., Alexia Savignoni[4], M.D. Ph.D., Marc-Henri Stern[1,3], M.D. Ph.D., Gaëlle Pierron[3], Ph.D.

1 - Unit 830 « Cancer, heterogeneity, instability and plasticity » INSERM, Institut Curie, PSL Research University, Paris, 75248, France

2 - Department of Medical Oncology, Institut Curie, PSL Research University, Paris, 75248, France

3- Department of Genetics, Institut Curie, PSL Research University, Paris, 75248, France

4 - Department of Biometry, Institut Curie, PSL Research University, Paris, 75248, France

5- Department of Biopathology, Institut Curie, PSL Research University, Paris, 75248, France

6- Faculty of Medicine, University of Paris Descartes, Paris, 75006, France

7- Department of Medical Imaging, Institut Curie, PSL Research University, Paris, 75248, France

8- Department of Surgical Oncology, Institut Curie, PSL Research University, Paris, 75248, France

9- Department of Translational Research, Institut Curie, PSL Research University, Paris, 75248, France

10- Department of Ocular Oncology, Institut Curie, PSL Research University, Paris, 75248, France

***Correspondence to**: Dr. Manuel Rodrigues, Institut Curie 26 rue d'Ulm, 75248 Paris Cedex 05. email: manuel.rodrigues@curie.fr Tel: +33144324672 Fax: +33153104041

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Key Points

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

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

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

Abstract

Importance

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

Objective

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

Design

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

Setting

Monocentric study in a referral center.

Participants

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

Main Outcomes and Measures

Metastasis-free survival and overall survival at 60 months.

Results

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

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

Conclusions and Relevance

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

Introduction

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

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

The most common hypothesis to explain the poor prognosis of M3 tumors is the presence of one or more tumor suppressor genes (TSG) on chromosome 3. *BAP1* (*BRCA1 associated protein-1*), a TSG located on the 3p21.1 cytoband, is now established as a main actor of UM malignant transformation as it is frequently mutated in M3 tumors and germline mutations are associated with UM predisposition[11-16]. However, all or most *BAP1*-mutated UMs intriguingly present a M3 (or a loss of heterozygosity of the whole chromosome 3 due 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[17]. The goals of the present study were to explore these UMs with partial deletions of chromosome 3, as assessed by comparative genomic hybridization (array-CGH), in order to assess their prognosis and to determine the minimal region of deletion associated with poor prognosis.

Materials and methods

Patients

This study was approved by our institutional ethics committee. Written informed consent for the use of tissues 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 our institution and followed up by our physicians. Clinical diagnosis of uveal melanoma was based on the presence of typical clinical findings as previously described[10]. Local treatment consisted of proton beam radiotherapy, iodine 125 brachytherapy or enucleation, depending on the size and location of tumors. Tumor samples were obtained by enucleation, endoresection or fine-needle aspiration at the time of clip or plaque positioning. Liver ultrasound, liver magnetic resonance imaging or body computed tomography were performed at diagnosis and every 6 months afterwards. Diagnosis of metastasis was systematically confirmed by a biopsy.

Genomic analysis

Tumor DNA was extracted and processed as previously described[10]. Array-CGH was performed on three different platforms according to the period when the test was performed: bacterial artificial chromosome arrays as previously described[18], NimbleGen 4×72 K arrays (Roche NimbleGen, Madison, Wisconsin, USA) and Agilent 180K CGH/LOH custom chip (Santa Clara, California, USA). Array-CGH were interpreted by three of the authors (MR, KAR, GP). Partial deletion of chromosome 3 was defined as the loss of at least one 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.

Statistical analysis

Clinical, pathological and genomic data at diagnosis and follow-up events (local and distant recurrences, second cancers, death from UM or from any other cause) were collected. The French Death Registry was consulted for patients lost to follow-up. The 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. The 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 significant level of 5%. In order to identify variables associated with MFS, a Cox regression analysis of candidate prognostic factors was performed using a forward stepwise selection procedure. The added value of each variable to the Cox model was determined using a likelihood ratio test with a significant level of 5%. Statistical analysis was performed using R software V3.3 (http://www.r-project.org/).

Results

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

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

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

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

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

Discussion

In this work, we explored a relatively large series of UMs with partial deletion of chromosome 3 and showed that loss of the *BAP1* locus is likely to explain the poor prognosis of M3 UM. This result was obtained by two 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 first consequence is to provide a potentially more accurate estimation of the prognosis of UMs presenting a partial deletion of chromosome 3. Our classification suggested efficiency in predicting metastatic outcome, identifying a group with a very good MFS with no recurrence and a group with a high risk of 92% of recurrences with a median follow-up of more than 5 years. Survival rates were close to what we observed in a previous series of UMs presenting either a M3 or a disomy 3, associated or not with 8q gain[10]. 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 publications[2,21-24]. Furthermore, FISH is often performed without chromosome 8q assessment leading to suboptimal prognosis estimation. Multiplex ligation-dependent probe amplification (MLPA) assay covering the *BAP1* locus is a good alternative to characterize recurrent genomic imbalances in UM but MLPA, as well as FISH and array-CGH, only evaluate copy number and, consequently does not identify isodisomic cases[25,26]. GEP is a transcriptomic prognosis assay that is widely used in

United States[7]. This assay distinguishes two subsets of UMs either at low or high risk of metastasis by assessing the expression of 12 genes, including four that are located on the short arm of chromosome 3 (*EIF1B, LMCD1, ROBO1, SATB1*) and one on the 3q (*FXR1*). Underexpression of these genes, possibly due to M3, is associated with poor prognosis. A more accurate prediction by GEP is possible by adding the expression of *PRAME*, a gene located on an instable region of chromosome 22 exposed to duplication, which was correlated to the 8q status in the pivotal paper[6]. To our knowledge, GEP has never been specifically tested in a large series of UMs with partial chromosome 3 deletions. Furthermore, GEP has never been compared to the combined M3/8q signature in a large cohort, impeding any conclusion on the superiority of one modality on the other. BAP1 immunohistochemistry is an alternative way to assess the prognosis of UMs[27,28]. However, immunohistochemistry for BAP1 does not correlate in all cases to the BAP1 mutational status in UM, and is therefore not a perfect surrogate[27].

In the present series, partial deletions of chromosome 3 were found in 4% of cases, which is comparable with some previous series[29-31] but lower than others[17,32,33]. Recruitment bias may explain part of this discrepancy but it is most probably explained by the variety of technologies, as well as the different classifications that were used. Comparison of all these studies is therefore limited. Similarly, the prognosis of these tumors was not clear as a discrepancy was observed with some series associating partial loss with good prognosis[17,29,32] while others associated it with intermediate or poor prognosis[25,33,34]. These differences may be explained by the absence of distinction depending on the loss of the *BAP1* locus compared to other losses.

One explanation for the low MFS associated with the loss of this locus may be that the loss of one *BAP1* allele contributes to the inactivation of this gene and subsequent aggressiveness of the tumor. However, the minimal region of deletion we found associated with the lowest MFS in our series (3p22.1-p14.2) includes 291 genes. Even though this region encompasses *BAP1*, it cannot be excluded that other important genes are present there and that haploinsufficiency of these genes affects tumorigenesis. The two alleles of a TSG are commonly inactivated in the two-hit model by a combination of different mechanisms, including total or partial loss of a chromosome, deleterious point mutations, short insertions/deletions, large-scale insertions/deletions and promoter methylations[35]. It is highly intriguing that, *BAP1* inactivation is so frequently associated with monosomy 3 in UM, contrary to renal clear-cell carcinomas and mesotheliomas, which rather carry losses of the short arm of chromosome 3 only or deleterious mutations of both alleles[16]. Furthermore, haploinsufficiency of other genes on chromosome 3, possibly on its long arm, may play a role on 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[36-38].

There is, for now, no standard treatment in the metastatic setting, but new drugs are being developed in UM[39]. When an efficient treatment will be available, the following step will consist in testing this treatment in the adjuvant setting in high-risk patients[40]. 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 as it not only assesses

copy number, heterozygosity and mutational statuses of UMs at low cost and with a lower amount of DNA, but also allow to follow circulating tumor DNA[41-43]. Moving towards the implementation of such technologies in our daily practice will allow ocular oncology 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, the present work provides evidence to refine the current UM genomic classification, which may help ophthalmologists to better predict the metastatic evolution of their patients. Before generalization, other series from different centers are required. Furthermore, one could argue that our series, composed of large tumors (median diameter of 16mm and median thickness of 10mm) is not reflecting the overall population of UM patients, particularly as larger UMs are known to host a greater frequency of genomic alterations, including 8q gains[37,44]. Other centers have reported genomic studies on biopsies of smaller UMs[45]. However, this procedure is not consensual and must not be undertaken in inexperienced ocular oncology 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, probably associated with poor prognosis. SNP-array can resolve this issue but, in the future, next-generation sequencing will probably be the privileged technology to circumvent this issue. More importantly, although the *BAP1* locus hypothesis is a

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

Conclusions

These findings suggest that partial deletion of chromosome 3 encompassing the *BAP1 locus* is associated with poor prognosis. Consequently, a new cytogenetic classification of UMs is proposed, based on the status of the *BAP1* locus instead of chromosome 3. The very 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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Author Contributions: M. Rodrigues had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Rodrigues, Savignoni, Stern, Pierron
Acquisition, analysis, or interpretation of data: Rodrigues, Ait-Rais, Salviat, Algret, Simaga, Barnhill, Gardrat, Servois, Mariani, Piperno-Neumann, Roman-Roman, Delattre, Cassoux, Savignoni, Stern, Pierron.
Drafting of the manuscript: Rodrigues, Savignoni, Stern, Pierron.
Critical revision of the manuscript for important intellectual content: Rodrigues, Ait-Rais, Salviat, Algret, Simaga, Barnhill, Gardrat, Servois, Mariani, Piperno-Neumann, Roman-Roman, Delattre, Cassoux, Savignoni, Stern, Pierron
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Tables

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

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

Univariate analysis

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

Multivariate analysis

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

Figures

Figure 1. Copy number profiles in metastatic *versus* non-metastatic cases. Frequencies of losses at a given position are shown at the bottom. Light gray: non-metastatic cases (Met-; n=30); dark gray: metastatic cases (Met+; n=13).

Figure 2. Copy number profiles in BAP1del cases *versus* BAP1nl. Frequencies of deletion at a given position are shown at the bottom. Light gray: BAP1nl cases (n=19); dark gray: BAP1del cases (n=24).

Figure 3. Metastasis-free and overall survivals according to the loss of the *BAP1* locus. Metastasis-free survival (left) and overall survival (right) curves in UMs with a partial loss of chromosome 3 encompassing the *BAP1* locus or not. BAP1del: deletion of the *BAP1* locus; BAP1nl: absence of loss of the *BAP1* locus.

Figure 4. Metastasis-free and overall survivals according to the three different prognosis groups. Metastasis-free survival (left) and overall survival (right) curves in UMs with a partial loss of chromosome 3 according to the three different prognosis groups.

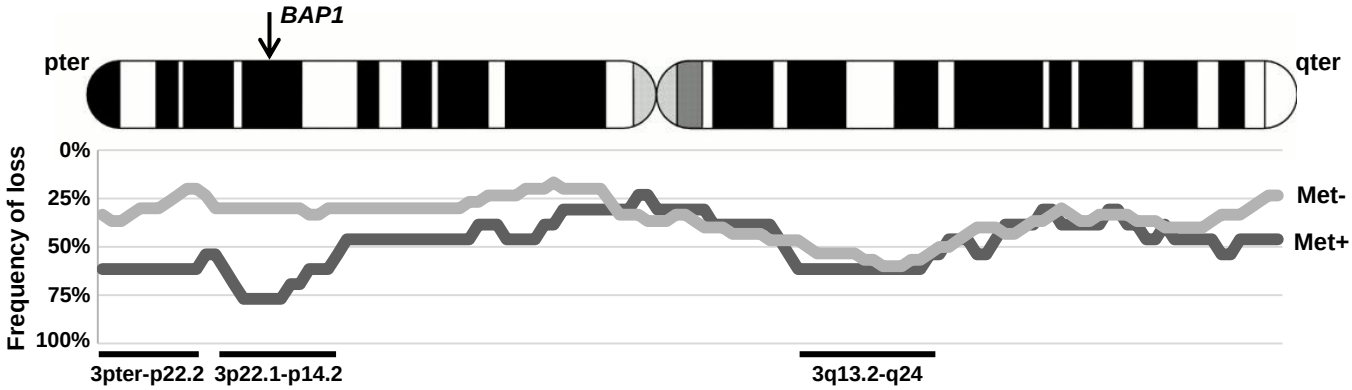

Figure 1. Rodrigues et al.

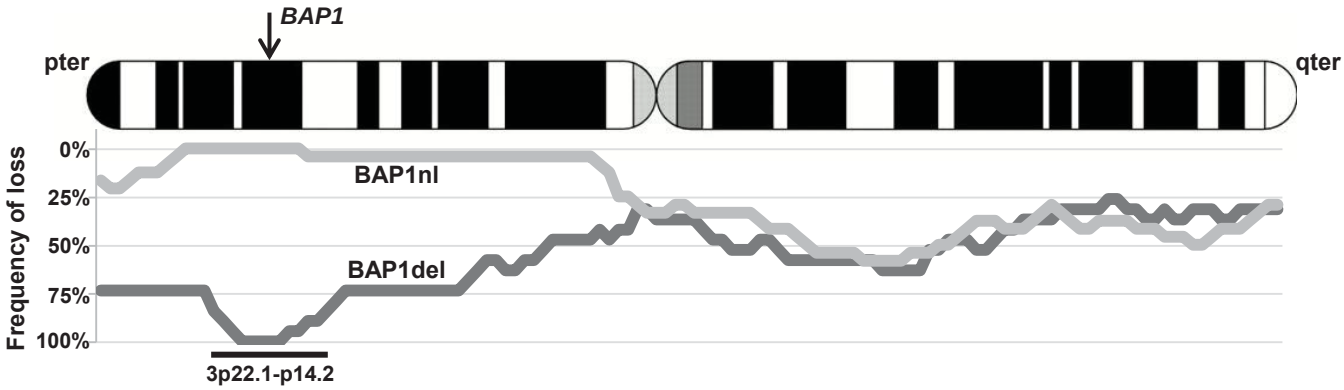

Figure 2. Rodrigues et al.

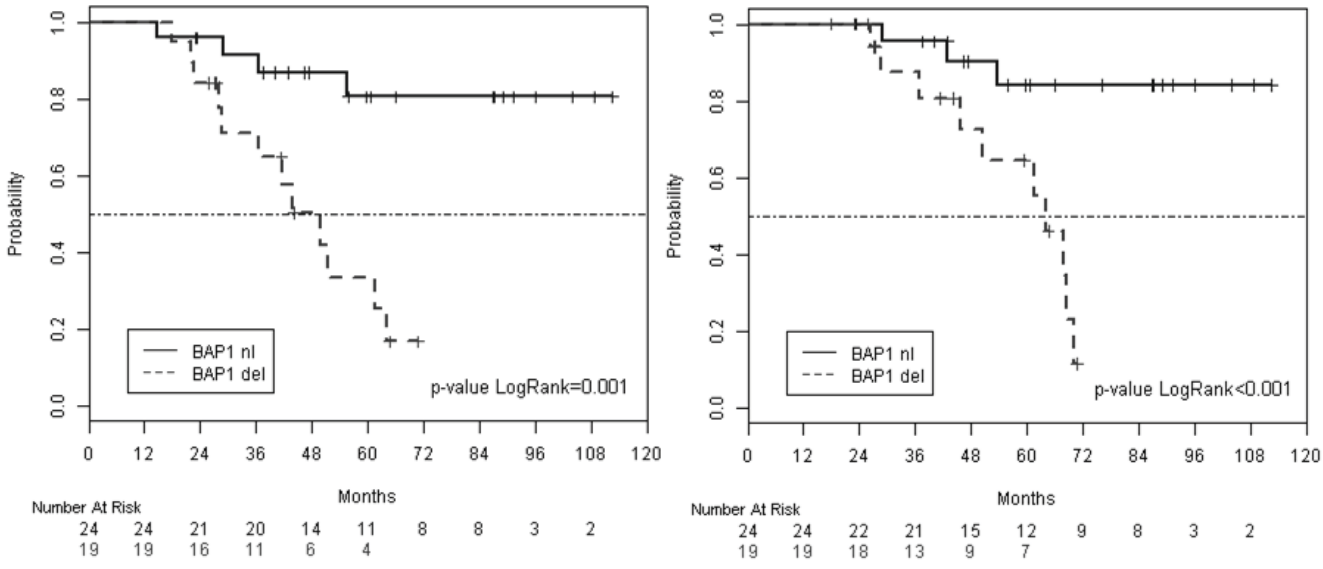

Figure 3. Rodrigues et al.

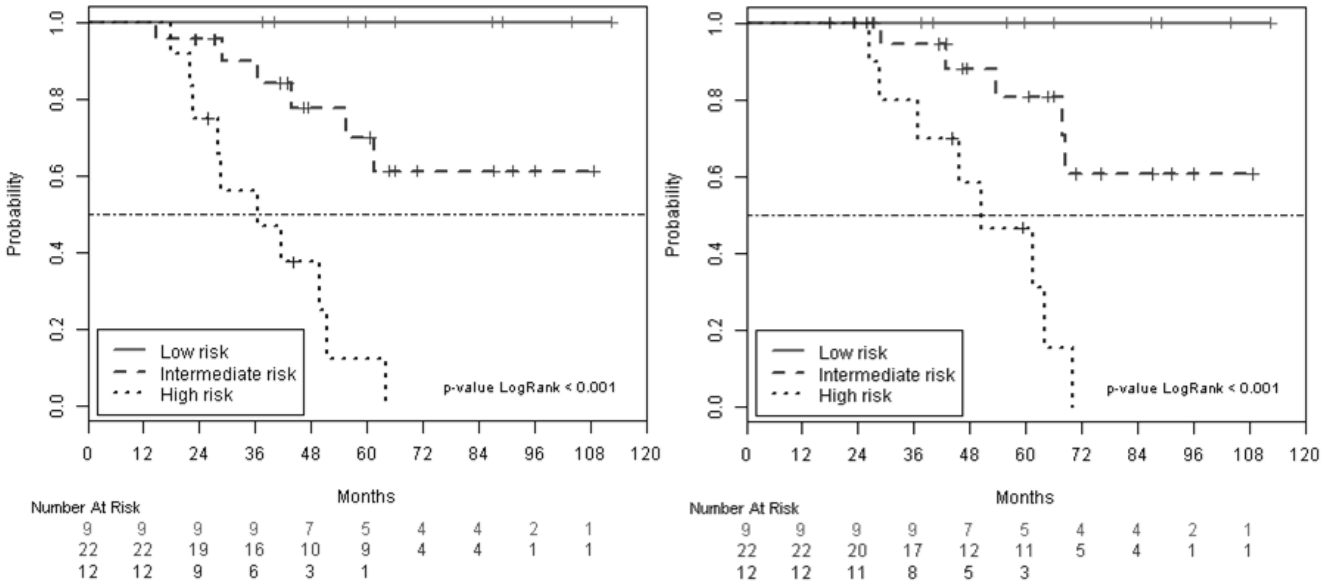

Figure 4. Rodrigues et al.