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Genetic Status Affects Disease-Specific Mortality But Not the Incidence of Local Recurrence in Patients with Uveal Melanoma

Mette Bagger, MD, PhD,[1] *Charlotte Espensen, PhD,*[1,2] *Kristina Rasmussen, MD,*[1] *Mehmet Dogrusöz, MD, PhD,*[3] *Martine J. Jager, MD, PhD,*[3] *Ane Appelt, MSc, PhD,*[4] *Jens F. Kiilgaard, MD, PhD*[1]

Purpose: Increased disease-specific mortality has been observed among patients with local recurrence (LR) from uveal melanoma (UM), but the underlying mechanism is unknown. The purpose of this study was to determine if copy number alterations of chromosomes 3 and/or 8q, at the time of diagnosis, increase the incidence of LR and if disease-specific mortality among patients with LR depends on the chromosome status of the primary tumor.

Design: Retrospective cohort study.

Participants: The study included 239 consecutive patients with primary UM (choroidal or ciliary body) treated with Ruthenium-106 (Ru-106) brachytherapy from January 2009 to December 2019 at a single national referral center.

Methods: Cox regression modeling and Kaplan−Meier analyses were used to assess the effect of the status of chromosomes 3 and 8q on the incidence of LR and disease-specific mortality after the event of LR. Multistate models were used to illustrate the probabilities over time of patients being alive and disease-free, alive with LR, dead from UM metastases, or dead from other causes split on the status of chromosomes 3 and 8q.

Main Outcome Measures: Incidence of LR and disease-specific mortality.

Results: Local recurrence was observed in 42 patients (16%). Overall incidence of LR was not affected by aberrations of chromosomes 3 and/or 8q ($P = 0.87$). Although LR occurred earlier in patients with aberrations of chromosomes 3 and/or 8q compared with patients with a normal copy number of chromosomes 3 and 8q, the median time from primary diagnosis to LR was 1.6 years (interquartile range [IQR], 1.0−2.0) and 3.2 years (IQR, 2.1−5.0), respectively. Cox regression found LR to be an independent risk factor for disease-specific mortality (hazard ratio [HR], 2.7; 95% confidence interval [CI], 1.5−5.0) among all patients, but multistate models demonstrated a low risk of disease-specific death among patients with normal chromosomes 3 and 8q status, even after an LR.

Conclusions: Copy number alterations of chromosome 3 and/or 8q in the primary UM did not increase the overall incidence of LR. However, the development of an LR enhanced the risk of disease-specific mortality among patients with copy number alterations of chromosomes 3 and/or 8q. Even after an LR, disease-specific mortality remained low among patients with normal copy numbers of chromosomes 3 and 8q.

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Eye-conserving treatment (brachytherapy or proton beam therapy) is preferred in patients with uveal melanoma (UM) because of the ability to spare healthy tissues and preserve as much vision as possible.[1] Eye-conserving procedures have been considered safe treatment options since the Collaborative Ocular Melanoma Study (COMS) failed to identify a significant difference in overall survival when brachytherapy was compared with conventional enucleation in medium-sized tumors.[2] However, studies have subsequently raised concern about an increased disease-specific mortality among patients with local recurrence (LR) of UM after eye-conserving therapies.[3−5] A large multicenter study identified a 6-fold risk of disease-specific mortality after LR, but it remains unclear whether local recurrent tumor cells serve as a direct cause for further systemic dissemination or simply represent the presence of a more aggressive underlying tumor biology where the tumor is both destined to recur locally and metastasize.[6] The hypothesis of a more aggressive tumor biology is supported by a prior genetic study on primary tumors and their matched metastases, which showed that the metastatic tumor cells are seeded early, even before the diagnosis of the primary tumor.[7]

Chromosome status is known to be a highly significant predictor of disease-specific mortality in patients with UM.[8] Specifically, somatic alterations of chromosomes 3 and 8q are strongly linked to a high risk of metastatic disease.[9−12] In addition, loss of chromosomes 6q and 1p is also

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associated with a poor prognosis, whereas gain of 6p tends to predict a more favorable survival outcome.[13] Prior survival analyses among patients with LR have not included the effect of adverse chromosomal alterations in the primary tumor, where only tumor size category, ciliary body involvement, and extraocular extension were taken into account.[6]

In this retrospective single-center study, we aim to determine if somatic alterations of chromosomes 3 and 8q in the primary tumor increase the risk of LR and if this could explain the increased disease-specific mortality among patients who experience LR. We examined this in a consecutive cohort of patients with UM treated with Ruthenium-106 (Ru-106) brachytherapy during a 10-year period.

Methods

Patient Material

Consecutive patients diagnosed with primary UM (choroidal or ciliary body) and treated with Ru-106 brachytherapy from January 2009 to December 2019 at the ocular tumor division at the Copenhagen University Hospital were included in the study. Approximately 65% of all posterior UM cases in Denmark are managed at this national referral center. Patients were referred to brachytherapy if they had locally confined disease and if the tumor dimensions were within the limits treatable with Ru-106 brachytherapy (8 mm in height, 20 mm in largest basal dimension). All patients were treated with a planned prescribed apical dose of 100 Gy. Assessments for local control were carried out regularly after the primary treatment (every third month for the first year, every sixth month the second year, annually up to 5 years, and thereafter at 7 and 10 years) until the end of the study (May 20, 2022) or death.

All patients were offered a transvitreal retinochoroidal biopsy that was performed during the same session as the placement of the Ru-106 plaque.[14] The diagnosis of choroidal or ciliary body melanoma was confirmed by histopathologic examination of the specimen. Additionally, the biopsy was sent for copy number evaluation of chromosomes 1p, 3, 6, and 8.

The diagnostic criteria for an LR were increased tumor height (at least 25% of tumor height) measured by ultrasound B-scan on 2 consecutive visits (central growth), increased basal tumor diameter (visualized by thoroughly comparing tumor borders and landmarks on consecutive wide-field retinographies) (marginal growth), or development of a new noncontiguous tumor documented on wide-field retinographies (new location). In 2 cases, a pigmented lesion was detected on the sclera, and a biopsy confirmed an extraocular lesion of the UM (extrascleral extension). Ultimately, histopathological descriptions of the secondary enucleated eyes were reviewed to ensure the presence of viable tumor in the specimen. The evaluation of disease-specific mortality included review of the following data when available: autopsy reports, histopathological description of metastatic specimens, clinical charts with description of suspected metastatic lesions (imaging), and evaluation of other co-occurring malignancies in each patient. Our approach has previously been described in detail, and the diagnostic criteria adhere to the COMS study recommendations for assessment of metastatic status at death.[15,16]

Cancer staging and tumor size categories were classified using the American Joint Committe on Cancer (AJCC) Tumor Node Metastasis (TNM) Classification scheme 8th edition.[17] Baseline patient and tumor characteristics, chromosome status, and clinical outcome were registered and stored in a local Access database (Microsoft Access 2010; Microsoft Corp.). The study was conducted in accordance with the tenets of the World Medical Association's Declaration of Helsinki. All patients were offered a biopsy for genetic testing and were informed of known and potential risks. Oral informed consent was obtained from all patients before treatment. The Regional Research Ethical Committee in Copenhagen waived the need for approval of this retrospective study (ref. H-4-2014-FSP). The collection of clinical data was approved by the Danish Data Protection Agency (ref. 2016-41-4897) and the Danish Health Authority (ref. 3-3013-980/1/).

Genetic Analysis

Fluorescence in situ hybridization analysis was carried out using a telomeric probe for chromosome 1p (Vysis TelVysion 1p) and centromeric probes for chromosomes 3 (CEP3 D3Z1), 6 (CEP6 D6Z1), and 8 (CEP8) (probes from Abbott Molecular, Inc.; www.abbottmolecular.com [in the public domain]). The analysis was performed in accordance with the manufacturer's recommended procedures. At least 100 cells from each specimen were evaluated when possible, and abnormalities were reported when more than 10% of the cells showed cytogenetic changes.[18] A total of 64 cases were analyzed with fluorescence in situ hybridization alone. Supplementary multiplex ligation-dependent probe amplification analysis evaluating copy number alterations on chromosomes 1p, 3, 6, and 8 (SALSA MLPA P027 Uveal melanoma; MRC-Holland, Amsterdam, The Netherlands) was performed prospectively on samples from all patients treated since 2012 and retrospectively in patients with available tumor tissue from snap-frozen biopsies from 2009 to 2011 (n = 22 samples).[19] To attain adequate power in the survival analysis, we only included alterations on chromosomes 3 and 8, because the prognostic effect of these chromosomes is by far the most well documented.[13] Thus, patients with UM were defined as high risk with respect to metastatic disease if they had an aberrant copy number of chromosomes 3 and 8q and low risk if they had a normal copy number. Ultrasonography B-scan, retinal images, and clinical charts were reviewed for all recurrent tumors, and the recurrence patterns were classified as "marginal growth," "growth in thickness," "both marginal growth and growth in thickness," "new location," and "extra scleral growth."

Data Analysis and Modeling

Disease-specific survivals for all patients and for the subgroup of patients who experienced LR (i.e., outcome after recurrence) were calculated using Kaplan−Meier estimators, stratifying for chromosome status. The median time from initial treatment to LR was calculated for patients with and without alterations of chromosomes 3 and 8q. The difference between the time to LR in the 2 groups was tested with Moods Median Test.

To illustrate the estimated probability over time of patients being alive and disease-free, alive with recurrence, dead from UM metastases, or dead from other causes, we used a multistate model.[20,21] This allowed transitions from alive and disease-free to one of the 3 other states and from alive after recurrence to dead from UM metastases or dead from other causes. The probability of being in one of the possible states at a specific time was estimated using the Aalen−Johansen estimator on cumulative hazards from Cox regression stratified on the different transitions. We estimated and plotted outcomes separately depending on chromosome status at the time of diagnosis to illustrate the effect of alterations on chromosomes 3 and 8q. Furthermore, the effect of chromosome status on the transition probability from alive and disease-free to alive after LR was tested using Cox regression.

The impact of risk factors on disease-specific mortality was examined using multivariate Cox regression modeling, taking LR and prespecified relevant baseline clinical factors into account

Table 1. Baseline Characteristics of Study Participants (n=260)

	Baseline Characteristics		
	All Patients n = 260	Patients without LR n = 218	Patients with LR n = 42
Patient characteristics			
Age (yrs) median (IQR, range)	64 (54–71, 20–91)	63 (53–70, 20–91)	65 (59–74, 30–83)
Gender			
Female	122 (47%)	103 (47%)	19 (45%)
Male	138 (53%)	115 (53%)	23 (55%)
Follow-up (yrs) median (IQR, range)	6.0 (3.5–8.9, 0.2–13.3)	4.9 (2.7–7.9, 0.2–12.4)	6.5 (4.0–9.5, 1.3–13.3)
Tumor characteristics			
Tumor height (mm) median (IQR, range)	3.8 (2.8–5.5, 1.2–11.7)	3.7 (2.8–5.1, 1.2–11.7)	4.3 (3.3–6.3, 1.9–11.1)
Tumor LBD (mm) median (IQR, range)	11.0 (8.8–13.0, 4.4–23.0)	11.0 (8.8–13.0, 4.9–23.0)	11.0 (8.9–14.5, 4.4–20.0)
AJCC tumor size			
1	94 (36%)	81 (37%)	13 (31%)
2	119 (46%)	101 (46%)	18 (43%)
3	39 (15%)	31 (14%)	8 (19%)
4	8 (3%)	5 (2%)	3 (7%)
AJCC stage			
I	86 (33%)	75 (34%)	11 (26%)
II	155 (60%)	129 (59%)	26 (62%)
III	19 (7%)	14 (6%)	5 (12%)
Chromosome 3			
Normal	112 (43%)	92 (42%)	20 (48%)
Abnormal	126 (48%)	107 (49%)	19 (45%)
NA	22 (9%)	19 (9%)	3 (7%)
Chromosome 8q			
Normal	127 (49%)	107 (49%)	20 (48%)
Abnormal	108 (42%)	89 (41%)	19 (50%)
NA	25 (9%)	22 (10%)	3 (7%)
Chromosomes 3 and 8q			
Normal (both)	86 (33%)	70 (32%)	16 (38%)
Abnormal (3 or 8q)	149 (57%)	126 (58%)	23 (55%)
NA	25 (10%)	22 (10%)	3 (7%)

AJCC = American Joint Committee on Cancer; IQR = interquartile range; LBD = largest base dimension; LR = local recurrence; NA = not available.

(AJCC stage and patient age). In a sensitivity analysis, we also performed the multivariate Cox regression model using tumor height and largest base dimension instead of AJCC stage. The effect of LR was handled as a time-dependent covariate to properly assess any impact of LR on risk of dying from UM metastases.[22]

For disease-specific mortality analyses and LR, the time variable was defined from initiation of primary Ru-106 treatment until death or study cutoff date. The inverse Kaplan–Meier estimate was used to determine the median potential follow-up time.[23] The proportional hazards assumption for the Cox regression models was tested using Schoenfeld residuals. We used Oncoprint to demonstrate the copy number alterations of all 4 chromosomes in relation to LR pattern and tumor height.[24] All analyses were conducted in R (version 3.4.1) using RStudio (version 1.0.153).

Results

In total, 261 patients underwent Ru-106 brachytherapy during the study period and were considered for analysis. One patient had distant metastases at diagnosis (AJCC stage IV) and was excluded from the analysis. Baseline characteristics of the remaining 260 patients are listed in Table 1. In 257 cases, a tumor biopsy was obtained (4 patients refused a tumor biopsy). Among the 257 biopsy samples, 2 samples were not sent for genetic testing, and 16 samples failed to produce a conclusive genetic result. Thus, the genetic status of chromosomes 3 and 8q was available in 239 patients (92%).

Data sampling was performed on May 20, 2022. The median potential follow-up time was 6.9 years (interquartile range [IQR], 4.0–9.9). Only 1 patient was lost to follow-up due to migration.

A total of 42 of 260 patients with UM (16%) experienced LR, and the 3- and 5-year cumulative incidences for LR were 11% (95% confidence interval [CI], 85–93) and 15% (95% CI, 80–88), respectively. Two patients were re-treated with brachytherapy and enucleation, respectively, due to initial plaque misplacement, but no tumor growth was observed, and they were not counted as LR.

Of the 42 patients with an LR, 16 patients (38%) had normal chromosomes 3 and 8q, 23 patients (55%) had abnormal chromosomes 3 and 8, and the chromosome status was unavailable in 3 patients (7%). This was similar to the situation in the nonlocal tumor recurrence group, with 32%, 58%, and 10%, respectively. The median time from initial treatment to LR was 3.2 years (IQR, 2.1–5.0 years) and 1.6 years (IQR, 1.0–2.0) for patients with and without aberrations of chromosome 3 or 8q, respectively. Thus, LR occurred significantly earlier in patients with aberrations of chromosome 3 or 8q ($P = 0.002$). The effect of chromosome status on the transition probability from alive and disease-free to alive after LR was tested using Cox regression models and was not found to be significant ($P = 0.87$). Other parameters such as tumor size or stage did not differ between patients with and without a recurrence.

Clinical outcome in relation to chromosome status and LR is summarized in Table 2. During the study period, a total of 79 patients (30%) died. Of these, 46 deaths were disease-specific. The 3- and 5-year disease-specific survivals for the study group

Table 2. Clinical Outcome Characteristics of Study Participants (n = 260)

Clinical Outcome Characteristics			
	All Patients n = 260	*Patients without LR n = 218*	*Patients with LR n = 42*
Clinical outcome			
Death all causes (n, %)	79 (30%)	58 (27%)	21 (50%)
Normal chromosomes 3 and 8q	12	6	6
Only abnormal chromosome 3	10	9	1
Only abnormal chromosome 8q	9	7	2
Abnormal chromosomes 3 and 8q	41	30	11
Chromosome status NA	7	6	1
Death due to UM metastasis (n, %)	46 (18%)	32 (15%)	14 (33%)
Normal chromosomes 3 and 8q	2	1	1
Only abnormal chromosome 3	5	4	1
Only abnormal chromosome 8q	3	3	0
Abnormal chromosomes 3 and 8q	33	22	11
Chromosome status NA	3	2	1

LR = local recurrence; NA = not available; UM = uveal melanoma.

were 92% (95% CI, 89−96) and 86% (95% CI, 82−91), respectively.

Table 2 demonstrates that disease-specific mortality is increased in the LR group. Disease-specific death was observed in 2 patients with a normal copy number of chromosomes 3 and 8q. In one of these cases, a blood sample identified a germline *BAP1* mutation, and in the other case, multiplex ligation-dependent probe amplification analysis showed gain of chromosome 6p and loss of chromosome 6q. The copy number variations of the patients are shown in relation to LR type and disease-specific death in Figure 1. There was no association between the type of recurrence and the genetic status of chromosomes 3 and 8q.

Disease-specific survival estimates stratified by the status of chromosomes 3 and 8q for all patients (timeline: from primary treatment until death due to UM or end of study) and after LR (timeline: from treatment of LR until death due to UM or end of study) are illustrated in Figure 2A and B, respectively.

The probabilities of patients being alive and disease-free, alive after recurrence, dead from UM metastases, or dead from other causes as a function of time are illustrated in Figure 3A and B for patients with normal chromosomes 3 and 8q and abnormal chromosomes 3 and 8q, respectively. During the first 2 years, a trend toward an increased incidence of LR was observed among patients with abnormal chromosomes 3 and 8q. After 2 years, a considerable number of the patients with abnormal chromosomes 3 and 8q have died of UM metastases, and thus a reduction in the proportion of patients alive with LR was observed. All but 1 patient with normal chromosomes 3 and 8q were alive or died of other causes after the event of LR, which tended to occur later compared with patients with an aberrant chromosomal status.

Cox regression analyses examined the effect of chromosomes 3 and 8q alterations on disease-specific mortality, with LR included as a time-dependent covariate. The risk of disease-specific death differed considerably between patients with normal and abnormal status of chromosomes 3 and 8q (hazard ratio [HR], 13.9; 95% CI, 3.5−55.4). Local recurrence increased the disease-specific mortality, although to a lesser extent, with an HR of 2.7 (95% CI, 1.5−5.0) (Table 3). We were not able to test an interaction effect between LR and chromosome status (i.e., whether the disease-specific mortality after LR was increased only in patients with an abnormal chromosome status) due to lack of power.

We repeated the Cox regression analyses using largest basal diameter and tumor height instead of AJCC stage, but HRs of chromosome status (HR, 12.2; 95% CI, 3.0−48.9) and LR (HR, 2.7; 95% CI, 1.4−5.2) remained similar. In addition, largest base dimension was also a significant factor (HR, 1.2; 95% CI, 1.1−1.4) for disease-specific mortality, but tumor height was not (HR, 0.9; 95% CI, 0.8−1.1).

Discussion

The overall incidence of LR in patients with UM treated with ruthenium brachytherapy was unaffected by the status of chromosomes 3 and 8q in the primary tumor. In contrast, disease-specific mortality was highly dependent on the genetic status of chromosomes 3 and 8q. Of note, the Cox regression analyses identified LR as an independent risk factor for increased disease-specific mortality. However, Kaplan−Meier survival analysis demonstrated an extremely low disease-specific mortality among patients with no aberrations of chromosomes 3 and 8q, even after LR had occurred (Fig 2B). In addition, the 2 patients with a normal copy number of chromosomes 3 and 8q, who died of metastatic disease, were not regarded as low risk in retrospective, because 1 patient had a germline BAP1 mutation and the other demonstrated loss of 6q, which is associated with a poor prognosis.[13] Thus, although LR increased the risk of metastatic death further among patients with aberrant chromosomes 3 and 8q, the same effect was not seen among patients with normal copy numbers of chromosomes 3 and 8q. Unfortunately, the low number of events in the group with normal chromosomes 3 and 8q did not allow for an individual Cox regression analysis.

This interpretation was also supported by multistate survival modeling (Fig 3), where we were able to illustrate the markedly different disease courses for patients with and without abnormal chromosomes 3 and 8q. The multistate model takes competing risks into account and allows for modeling of the different stages of disease: disease-free survival, survival after LR, disease-specific death, and death due to other causes. The model

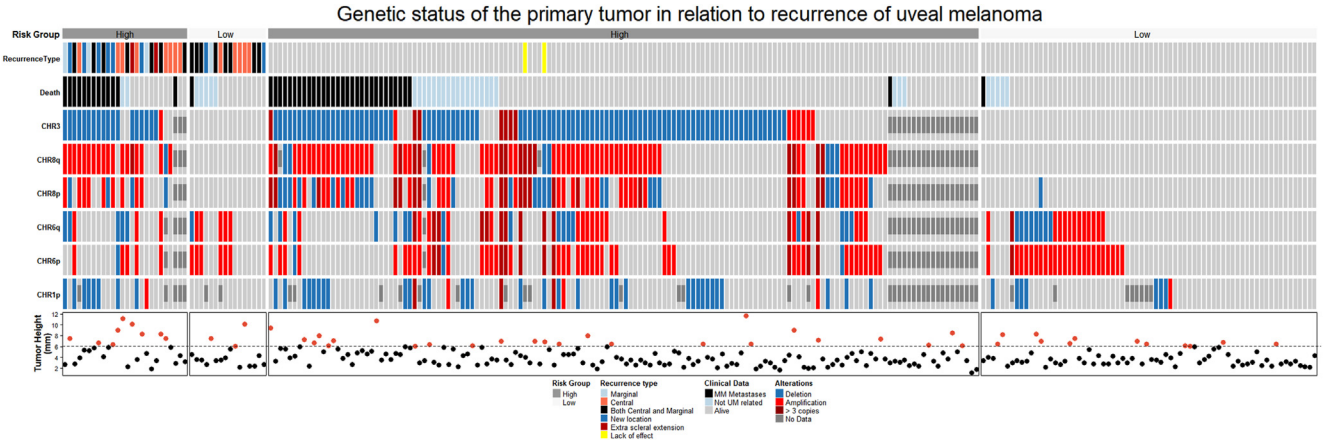

Figure 1. Oncoprint representation of an integrated annotation of copy number alterations of the 4 tested chromosomes 3, 8, 6, and 1p with respect to defined risk group, local recurrence (LR) type, and death. The bar below shows tumor height, with tumors larger than 6 mm represented with a **red dot**. Risk group was defined in accordance with the Copenhagen uveal melanoma (UM) criteria as high risk if the tumor had copy number alterations of chromosomes 3 and 8q and low risk if the tumor had normal copy number of chromosomes 3 and 8q. There is no evident association between LR type/pattern and risk group.

showed no difference in the overall incidence of LR between the 2 groups; however, disease-specific death was significantly higher in the group with copy number variations of chromosomes 3 and 8q. Additionally, the model illustrated that the incidence of LR among patients with normal chromosomes 3 and 8q occurred gradually during follow-up, whereas LR was seen as an early event among patients with copy number alterations of chromosomes 3 and 8q. This could be explained in part by the fact that the follow-up for patients with abnormal chromosomes 3 and 8q was limited because a significant subset experienced disease-specific death during the first 2 years (Fig 3). However, it could also suggest different underlying mechanisms of LR based on genetic status, which was

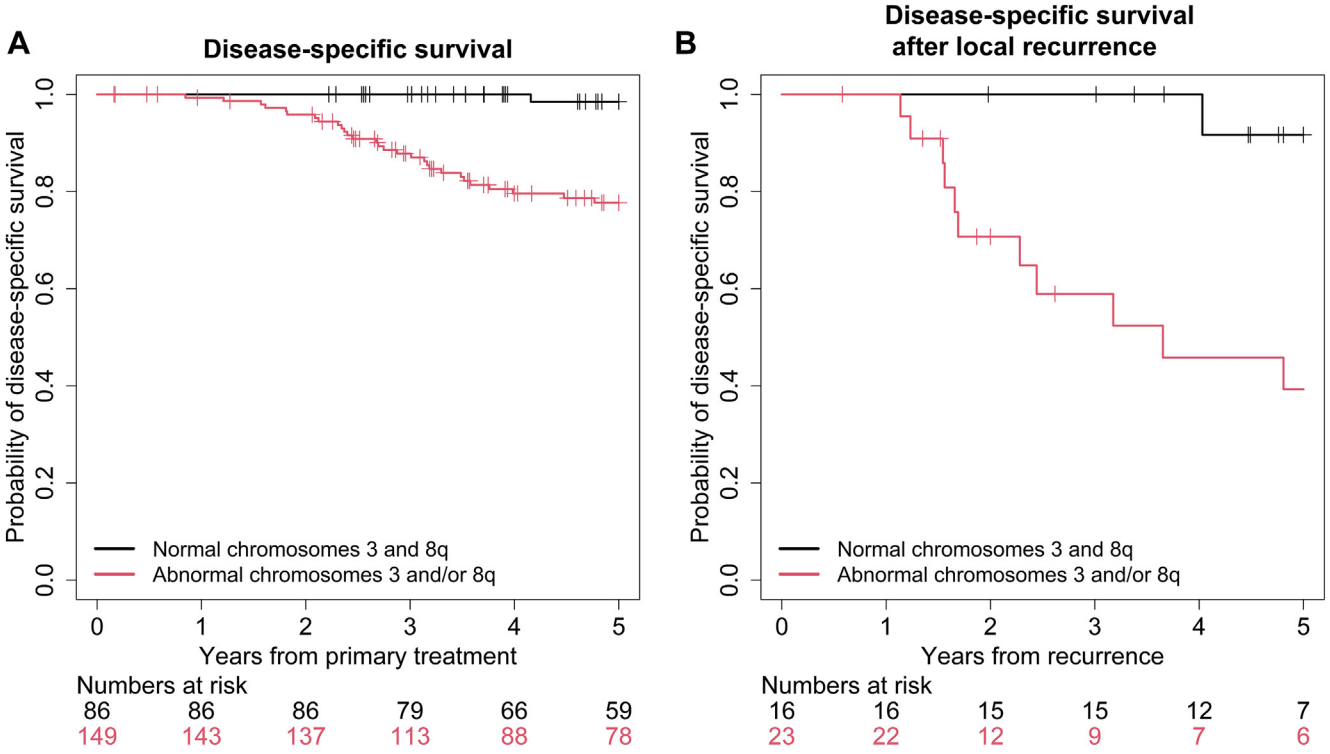

Figure 2. Kaplan–Meier estimates of disease-specific survival stratified by the status of chromosomes 3 and 8q. **A,** All patients with time from diagnosis to disease-specific death used as timeline. **B,** The subgroup of patients who experienced local recurrence (LR) with time from LR to disease-specific death used as timeline.

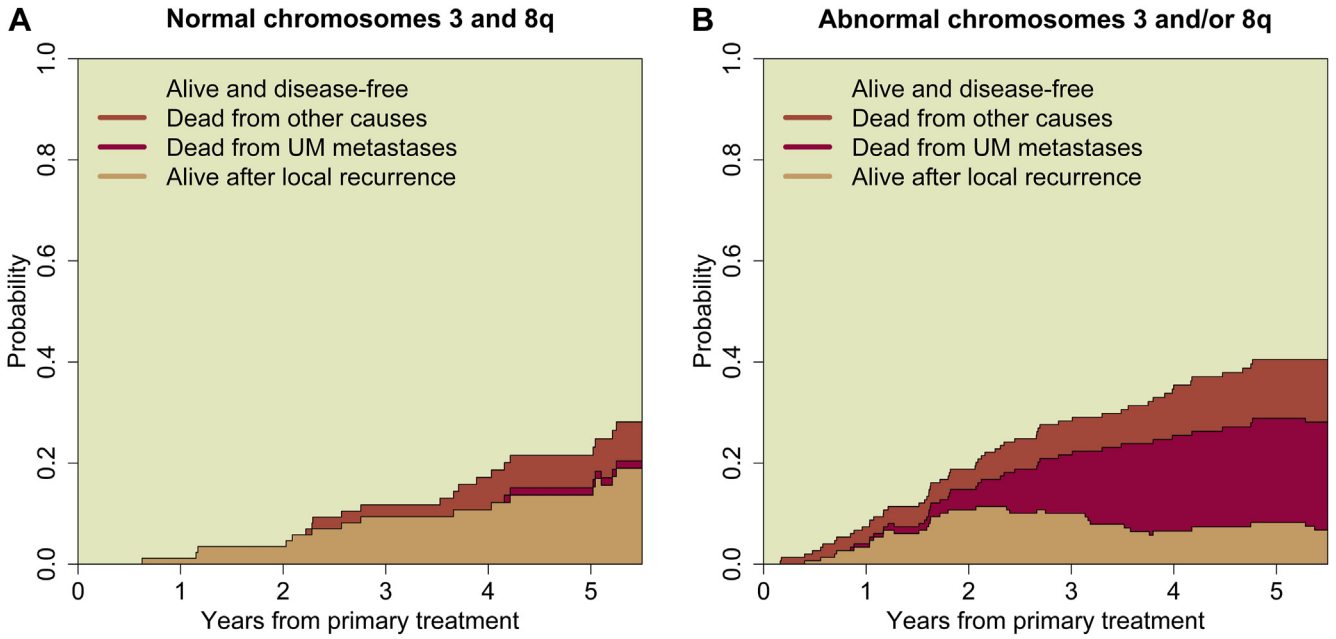

Figure 3. Multistage models of **(A)** patients with normal chromosomes 3 and 8q and **(B)** patients with abnormal chromosomes 3 and 8q show the probabilities of patients being alive and disease-free, alive after recurrence, dead from uveal melanoma (UM) metastases, or dead from other causes as a function of time. The 2 models demonstrate a markedly different disease courses depending on genetic status of the tumor.

supported by the significantly shorter median time to LR among patients with aberrations of chromosomes 3 and 8q.

Echegaray et al[25] described 3 different LR patterns: marginal, diffuse, and extraocular. The marginal recurrence pattern, which could represent misplacement of the plaque, showed a high Ki-67 proliferation index only in the tumor areas that were not sufficiently irradiated. In contrast, the diffuse type showed high Ki-67 proliferation throughout the tumor, suggesting resistance to the radiation. Harbour et al[26] found that diffuse recurrence patters were more strongly associated with metastatic risk compared with marginal recurrence patterns. These findings indicate different mechanisms of LR; marginal recurrences might be explained by failure to adequately treat tumor cells along the margins, whereas diffuse recurrence might represent a distinct clinicopathologic entity of radio resistance and thus a more aggressive tumor biology. This supports the findings that LR is associated with increased disease-specific mortality but only in patients with abnormal chromosomes 3 and 8q.[25] We identified 4 different clinical LR patterns in this cohort, but we did not have sufficient power to perform statistical analyses on the effect of recurrence patterns on disease-specific mortality. However, crude numbers indicated a random distribution of LR patterns regarding the genetic status of chromosomes 3 and 8q and disease-specific mortality (Fig 1). We were not able to identify the histopathological patterns of LR because immunohistochemical staining was not possible in this retrospective study. There are likely other important genetic alterations in the primary UM cells that enhance radio resistance of the tumor and increase the likelihood of LR. Previous studies performed on UM cell lines have associated overexpression of phosphorylated DNA-protein kinase and ataxia telangiectasia mutated protein with radio resistance.[27,28] However, exploration of additional genes was beyond the scope of this study, in which the main aim was to evaluate how "high-risk" genetic alterations in the primary tumor affects LR. Tumor tissue from recurrent

Table 3. Cox Regression Models

	Adjusting for LR, Age, and AJCC Stage		Adjusting for LR, Age, AJCC Stage, and Chromosome Status	
Variable	*HR (95% CI)*	P *Value*	*HR (95% CI)*	P *Value*
LR	2.8 (1.6–4.9)	0.0005	2.7 (1.5–5.0)	0.001
Age*	1.3 (1.1–1.6)	0.01	1.3 (1.0–1.6)	0.04
AJCC stage II (relative to I)	4.9 (1.8–13.3)	0.002	6.1 (1.9–19.2)	0.002
AJCC stage III (relative to I)	10.1 (3.0–33.7)	0.0002	9.8 (2.4–39.1)	0.001
Abnormal chromosomes	-	-	13.9 (3.5–55.4)	0.0002

AJCC = American Joint Committee on Cancer; CI = confidence interval; HR = hazard ratio; LR = local recurrence.
*Ten-year increase.

tumor cells was unfortunately not available for genetic testing in this study; therefore, we were not able to evaluate whether recurrent tumor cells from patients with a normal copy number of chromosomes 3 and 8q underwent transformation to a more aggressive genetic profile. The continuous low disease-specific mortality among patients with LR and normal copy number of chromosomes 3 and 8q argues against genetic transformation, but this needs to be tested in future studies.

If increased disease-specific mortality is caused by seeding from recurrent tumor cells, one would expect superior survival for patients primarily enucleated due to instant tumor control, in the absence of extrascleral growth, but the prospective randomized COMS study that compared brachytherapy (Iodine-125) with enucleation failed to identify any clinically or statistically meaningful differences in survival between the 2 groups up to 12 years after primary treatment.[2] In addition, it has been proposed using mathematical modeling, that UM metastases arise from metastatic tumor cells seeded before diagnosis of the primary tumor.[29,30] This hypothesis is supported by a prior genetic study on primary tumors and their matched metastases performed by our group.[7]

The strength of this study is the quality of the dataset of consecutive patients treated with Ru-106 brachytherapy with only 1 patient lost to follow-up, with routine genetic data acquired by biopsy. We observed a high cumulative 5-year incidence of LR (15%) compared with some previous studies, but this is in line with the reported LR rates after brachytherapy (from 0%−18%).[6,31] Ultimately, the survival rate of the patients in this cohort did not differ from previous reported survival rates for patients treated with brachytherapy despite a high rate of LR.[32]

A recent study by Dogrusöz et al[33] showed that disease-specific mortality for patients with UM who underwent primary enucleation and had survived the first 5 years after the treatment was related to old age, the presence of monosomy 3, 8q gain, and a large original tumor diameter. This fits with our current results that indicate that patients with a normal copy number of chromosomes 3 and 8q continue to have a favorable survival, even after the event of an LR. However, because of limited follow-up, it cannot be ruled out that LR can cause late metastases in patients with normal chromosome status.

Conclusions

The overall incidence of LR did not depend on chromosome status of the primary tumor; however, LR occurred earlier in patients with aberrations of chromosomes 3 and 8q. Local recurrence was an independent risk factor for metastatic disease, but only among patients with aberrations of chromosomes 3 and 8q, whereas the event of LR did not appear to influence the good survival of patients with normal status of chromosomes 3 and 8q.

Footnotes and Disclosures

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[1] Department of Ophthalmology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark.

[2] Department of Oncology (Section of Radiotherapy), Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark.

[3] Department of Ophthalmology, Leiden University Medical Centre, Leiden, the Netherlands.

[4] Leeds Institute of Medical Research at St James's, University of Leeds, and Leeds Cancer Centre, St. James's University Hospital, Leeds, United Kingdom.

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HUMAN SUBJECTS: Human subjects were included in this study. The study was conducted in accordance with the tenets of the World Medical Association's Declaration of Helsinki. All patients were offered a biopsy for genetic testing and were informed of known and potential risks. Oral informed consent was obtained from all patients before treatment. The Regional Research Ethical Committee in Copenhagen waived the need for approval of this retrospective study (ref. H-4-2014-FSP). The collection of clinical data was approved by the Danish Data Protection Agency (ref. 2016-41-4897) and the Danish Health Authority (ref. 3-3013-980/1/).

No animal subjects were included in this study.

Author Contributions:

Conception and design: Bagger, Espensen, Dogrusöz, Jager, Appelt, Kiilgaard

Data collection: Bagger, Espensen, Rasmussen, Kiilgaard

Analysis and interpretation: Bagger, Espensen, Dogrusöz, Jager, Appelt, Kiilgaard

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Overall responsibility: Bagger, Espensen, Jager, Appelt, Kiilgaard

Abbreviations and Acronyms:
AJCC = American Joint Committee on Cancer; **CI** = confidence interval; **COMS** = Collaborative Ocular Melanoma Study; **HR** = hazard ratio; **IQR** = interquartile range; **LR** = local recurrence; **Ru-106** = Ruthenium-106; **UM** = uveal melanoma.

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Correspondence:
Mette Bagger, MD, PhD, Department of Ophthalmology, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, Copenhagen, Denmark 2100. E-mail: mette.marie.bagger@regionh.dk.

References

1. Jager MJ, Shields CL, Cebulla CM, et al. Uveal melanoma. *Nat Rev Dis Prim*. 2020;6:18−20.
2. Collaborative Ocular Melanoma Study (COMS) Group. The COMS Randomized Trial of Iodine 125 Brachytherapy for Choroidal Melanoma. *Arch Ophthalmol*. 2006;124: 1684−1693.
3. Caujolle J-P, Paoli V, Chamorey E, et al. Local recurrence after uveal melanoma proton beam therapy: recurrence types and prognostic consequences. *Int J Radiat Oncol*. 2013;85: 1218−1224.
4. Jampol LM, Moy CS, Murray TG, et al. The COMS randomized trial of iodine 125 brachytherapy for choroidal melanoma: IV. Local treatment failure and enucleation in the first 5 years after brachytherapy. COMS report no. 19. *Ophthalmology*. 2002;109:2197−2206.
5. Gragoudas ES, Lane AM, Munzenrider J, et al. Long-term risk of local failure after proton therapy for choroidal/ciliary body melanoma. *Trans Am Ophthalmol Soc*. 2002;100:43−49.
6. Gallie BL, Simpson ER, Saakyan S, et al. Local recurrence significantly increases the risk of metastatic uveal melanoma. *Ophthalmology*. 2016;123:86−91.
7. Shain AH, Bagger MM, Yu R, et al. The genetic evolution of metastatic uveal melanoma. *Nat Genet*. 2019;51:1123−1130.
8. Robertson AG, Shih J, Yau C, et al. Integrative analysis identifies four molecular and clinical subsets in uveal melanoma. *Cancer Cell*. 2017;32:204−220.e15.
9. Damato B, Dopierala JA, Coupland SE. Genotypic profiling of 452 choroidal melanomas with multiplex ligation-dependent probe amplification. *Clin Cancer Res*. 2010;16:6083−6092.
10. Prescher G, Bornfeld N, Hirche H, et al. Prognostic implications of monosomy 3 in uveal melanoma. *Lancet*. 1996;347: 1222−1225.
11. Cassoux N, Rodrigues MJ, Plancher C, et al. Genome-wide profiling is a clinically relevant and affordable prognostic test in posterior uveal melanoma. *Br J Ophthalmol*. 2014;98: 769−774.
12. Versluis M, de Lange MJ, van Pelt SI, et al. Digital PCR validates 8q dosage as prognostic tool in uveal melanoma. *PLoS One*. 2015;10:e0116371.
13. Dogrusöz M, Jager MJ. Genetic prognostication in uveal melanoma. *Acta Ophthalmol*. 2018;96:331−347.
14. Bagger M, Tebering JF, Kiilgaard JF. The ocular consequences and applicability of minimally invasive 25-gauge transvitreal retinochoroidal biopsy. *Ophthalmology*. 2013;120:2565−2572.
15. Bagger MM. Intraocular biopsy of uveal melanoma Risk assessment and identification of genetic prognostic markers. *Acta Ophthalmol*. 2018;96:1−28.
16. Jampol LM, Moy CS, Murray TG, et al. The COMS randomized trial of iodine 125 brachytherapy for choroidal melanoma. *Ophthalmology*. 2002;109:2197−2206.
17. American Joint Committee on Cancer. *AJCC Cancer Staging Manual*. Eighth Edition. Heidelberg, Germany: Springer; 2017:805−817.
18. Bagger M, Andersen MT, Andersen KK, et al. The prognostic effect of American Joint Committee on Cancer Staging and Genetic Status in Patients With Choroidal and Ciliary Body Melanoma. *Invest Ophthalmol Vis Sci*. 2015;56:438−444.
19. Bagger M, Andersen MT, Andersen KK, et al. The prognostic effect of American Joint Committee on Cancer staging and genetic status in patients with choroidal and ciliary body melanoma. *Invest Ophthalmol Vis Sci*. 2014;56:438−444.
20. de Wreede L, Fiocco M, Putter H. mstate : An R Package for the Analysis of Multi-state Models. *J Stat Softw*. 2011;38:1−30.
21. Putter H, Fiocco M, Geskus RB. Tutorial in biostatistics: competing risks and multi-state models. *Stat Med*. 2007;26: 2389−2430.
22. Diva U, Banerjee S, Dey DK, et al. Using time dependent covariates and time dependent coefficients in the Cox model. *Red*. 2007;33:1−8.
23. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. *Control Clin Trials*. 1996;17:343−346.
24. Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics*. 2016;32:2847−2849.
25. Echegaray JJ, Plesec T, Bellerive C, Singh AD. Histopathologic patterns of recurrent choroidal melanoma following I-125 plaque brachytherapy. *Ocul Oncol Pathol*. 2020;6:50−57.
26. Harbour JW, Char DH, Kroll S, et al. Metastatic risk for distinct patterns of postirradiation local recurrence of posterior uveal melanoma. *Ophthalmology*. 1997;104:1785−1793.
27. Hussain RN, Coupland SE, Khzouz J, et al. Inhibition of ATM increases the radiosensitivity of uveal melanoma cells to photons and protons. *Cancers (Basel)*. 2020;12:1−12.
28. Zhang B, Wu H, Hao J, et al. Inhibition of DNA-PKcs activity re-sensitizes uveal melanoma cells to radio- and chemotherapy. *Biochem Biophys Res Commun*. 2020;522:639−646.
29. Eskelin S, Pyrhönen S, Summanen P, et al. Tumor doubling times in metastatic malignant melanoma of the uvea: tumor progression before and after treatment. *Ophthalmology*. 2000;107:1443−1449.
30. Singh AD. Uveal melanoma: implications of tumor doubling time. *Ophthalmology*. 2001;108:829−830.
31. Chang MY, McCannel TA. Local treatment failure after globe-conserving therapy for choroidal melanoma. *Br J Ophthalmol*. 2013;97:804−811.
32. Singh AD, Turell ME, Topham AK. Uveal melanoma: trends in incidence, treatment, and survival. *Ophthalmology*. 2011;118:1881−1885.
33. Dogrusöz M, Brouwer NJ, de Geus SJR, et al. Prognostic factors five years after enucleation for uveal melanoma. *Investig Ophthalmol Vis Sci*. 2020;61:1−8.