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The Prognostic Effect of American Joint Committee on Cancer Staging and Genetic Status in Patients with Choroidal and Ciliary Body Melanoma

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Abstract

Purpose: To evaluate the prognostic effect of a combination of American Joint Committee on Cancer (AJCC) staging (7th ed.) and genetic status in patients with posterior uveal melanoma.

Methods: A consecutive cohort of 153 patients with posterior uveal melanoma treated at Copenhagen University Hospital from January 1, 2009 through December 31, 2012 was followed until October 2014. Survival, AJCC stage and cytogenetic data were registered. AJCC stage was available for all patients, and cytogenetic information of chromosomes 3 and 8 was available for 139 patients. The individual and joint prognostic effect of AJCC staging and cytogenetic changes were evaluated by cumulative incidence curves and Cox proportional hazard models.

Results: An overall 5 year survival rate of 62% (95% CI: 0.50–0.73) was observed. A normal genetic status of chromosomes 3 and 8, as found in 42 patients (30%) minimized the additional prognostic effect of AJCC staging. The frequency of tumors with normal genetic status decreased with increasing AJCC stage. Both AJCC stage III (hazard ratio (HR): 11.0, 95% CI: 1.4–85.6) and abnormal copy number of chromosomes 3 (HR: 6.3, 95% CI: 1.4–28.3) and 8 (HR: 2.8, 95% CI: 1.03–7.8) were identified as significant predictors of a poor prognosis in the multivariate Cox regression analysis.

Conclusion: Identification of a normal genetic status of chromosomes 3 and 8 minimized the prognostic effect of AJCC staging, while a combination of genetic status and AJCC staging provided the most accurate prediction of survival in patients with an abnormal chromosomal status.

Introduction

Despite radical treatment of the primary tumor, close to 50% of patients with choroidal and ciliary body melanoma die from metastatic disease.[1] Accurate prediction of prognosis is therefore important in relation to patient counseling and planning of follow-up. Intensified follow-up for early detection of metastatic disease in high-risk patients could allow for life-sustaining treatment options such as liver resection.[2]

Clinical, pathological and genetic factors are continually evaluated to identify patients with a high metastatic potential. Increasing tumor size has been shown to be one of the most important clinical characteristics.[3] Tumor staging by the American Joint Committee on Cancer (AJCC)[4] evaluates tumor size along with other clinical features to identify a poor prognosis, including involvement of the ciliary body, extra scleral growth and tumor dissemination at diagnosis. The AJCC staging system (7th ed.) has been proved to be a good predictor of survival in patients with posterior uveal melanoma.[5,6] A poor prognosis is also associated with specific genetic markers such as acquired loss of chromosome 3, gain of chromosome 8 and to some extent loss of chromosome 1p.[7-10] Loss of chromosome 6q has also been associated with poor survival,[9] while gain of chromosome 6p seems to predict a favorable prognosis.[11,12]

Genetic mutations in uveal melanoma-related genes such as BAP1, GNAQ, GNA11, SF3B1 and EIF1AX have recently been identified and shown to provide additional prognostic accuracy.[13] The assessment of genetic status by Gene Expression Profiling (GEP) divides tumors into two groups representing a low risk (class 1) and a high risk (class 2) of metastatic disease.[14,15] It has been proposed that GEP could be used to predict prognosis without the evaluation of clinical parameters.[14]

The aim of this study was to evaluate the individual and combined prognostic effect of clinical tumor characteristics captured in the AJCC staging system 7th ed. and genetic status determined as the presence or absence of abnormalities in chromosomes 3 and 8 in a consecutive cohort of patients with choroidal and ciliary body melanoma.

Methods

All patients from the eastern part of Denmark who were treated for ciliary and choroidal melanoma in the period from January 1, 2009 to December 31, 2012 were included in the study. All 153 patients were treated and followed by the Ocular Tumor Division at the Copenhagen University Hospital. Approximately 65% of all posterior uveal melanoma cases in Denmark are managed at this national referral center. The study group represents a crude estimated incidence rate of 1.03 per 100 000 person years (the population of the catchment area is approximately 3 700 000).

Tumor tissue was obtained in 148 patients by a transvitreal retinochoroidal (TVRC) biopsy as described previously.[16] The diagnosis of ciliary and choroidal melanoma was confirmed by histopathological examination of the specimen. The biopsy was sent for genetic testing in 146 cases. Biopsy was declined by the patient in three cases and omitted due to poor visibility in two patients (one due to a dense cataract and one due to the tumor). The diagnosis of these five patients was confirmed with histopathological examination after enucleation in three cases and relied on clinical examination, ultrasonography B-scan and magnetic resonance imaging (MRI) in two cases. The design of the study was a single-center consecutive retrospective cohort study, and registration with an International Committee of Medical Journal Editors clinical trial database was

therefore not needed. The Regional Research Ethical Committee in Copenhagen waived the need for approval of this retrospective study. The study was conducted in accordance with the tenets of the World Medical Association's Declaration of Helsinki. All patients were offered biopsy for genetic testing and were informed of known and potential risks. Oral informed consent was obtained from all patients prior to treatment.

Fluorescence in situ hybridization (FISH) analysis was carried out using a telomeric probe for chromosome 1p (Vysis TelVysion 1p) and centromeric probes for chromosome 3 (CEP3 D3Z1), 6 (CEP6 D6Z1) and 8 (CEP8) (All probes from Abbott Molecular Inc, Des Plaines, IL, USA, www.abbottmolecular.com). 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. A cut off of 5% would have classified an additional four patients as having an abnormal chromosomal status. None of the four patients had developed metastatic disease by the study endpoint.

Supplementary multiplex ligation-dependent probe amplification (MLPA) analysis (SALSA MLPA P027 Uveal melanoma, MRC-Holland, Amsterdam, the Netherlands) was performed on samples from all patients treated in 2012 and retrospectively in patients with available tumor tissue from snap frozen biopsies 2009–2011. In cases of disagreement between FISH and MLPA results, the greatest aberration from disomy was registered. The chromosomal abnormalities were either loss, gain, or both loss and gain, of a whole or partial chromosome. Tumor stages were classified in accordance with the AJCC tumor node metastasis (TNM) classification scheme[4]. Patient charts were reviewed and information on survival, AJCC stage and genetic status of chromosomes 1, 3, 6 and 8 were collected and entered into a database (Microsoft access 2010, Microsoft, Redmond, WA, USA).

Data sampling was performed on October 1, 2014, thus allowing for a minimum of 21 months of follow-up. Only one patient was lost to follow-up, due to emigration. All patients were offered a physical examination, liver function tests, chest X-ray and liver ultrasonography at 3, 6, 12, 18, 24, 30, 36, 48, 60, 84 and 120 months post treatment. If metastases were suspected an MRI or computer tomography (CT) scan were performed. If positive, additional positron emission tomography (PET)-CT was performed. A liver biopsy was taken for immunohistochemical and histopathological examination if the metastatic spread was limited to the liver. Otherwise a biopsy from the most accessible site was done. Our center was notified directly when a patient died. Physical examination notes concerning the patient's final illness and any laboratory procedures or diagnostic tests were evaluated. Descriptions of pathological specimens from metastatic melanoma or other metastatic malignancies were collected from The Danish National Pathology Registry, which is a database containing detailed nationwide records of all pathology specimens analyzed in Denmark since 1997.

Statistical analyses

Descriptive statistics were reported as mean and standard deviation when normally distributed and as median, range and interquartile range when the data were skewed. Kaplan–Meier survival curves were computed for chromosomal status and AJCC staging separately and compared by log-rank test and log-rank test for trend for ordinal variables. A combination of genetic status and AJCC staging was furthermore illustrated with cumulative incidence curves of melanoma-related death, which accounted for death by other causes as a competing risk. The relation between chromosomal status and AJCC stage was evaluated with the Chi-squared test. Only AJCC stages I–III were included in the analysis due to the limited number of patients in AJCC stage IV (two patients).

The relative risks for uveal melanoma by genetic status and AJCC staging were estimated using Cox regression models of the events of death by censoring for end of follow-up, or loss to follow-up, whichever came first. Death by other causes was censored as well in the evaluation of disease-specific survival. We used time from treatment for uveal melanoma as the time scale and conducted both unadjusted and adjusted analyses. Stage IV was not included, since the two patients in this category had already developed metastatic disease. Furthermore, in the adjusted analysis we accounted for age and sex. Age was included as a continuous variable and tested for linearity using higher order polynomials. Age was only borderline significant in the multivariate Cox regression analysis and thus was not included in the final model.

Violation of the proportional hazard assumption was tested for all covariates using the test based on weighed residuals proposed by Grambsch et al.[17] Effect estimates were reported as hazard ratios with 95% confidence intervals. All statistical tests were two-sided and based on the likelihood ratio test. A significance level of 5% was applied. The statistical software packages SAS 9.3 (SAS Institute Inc., Cary, NC, USA), R,[18] and Sigmaplot 12.5 (Systat Software Inc., San Jose, CA, USA) were used for all analyses.

Results

A total of 153 patients were followed through the observation period or until death (44 patients, 29%). Causes of death included metastatic spread of posterior uveal melanoma in 30 patients (68%), metastatic spread of other synchronous cancers in seven patients (16%) and other causes in seven patients (16%). Metastatic spread of uveal melanoma was confirmed by histopathological examination of liver biopsy in 27 of the 30 cases. The diagnosis of synchronous cancers was confirmed by histopathological examination in all cases and metastatic spread was confirmed by histopathological examination in six cases. In the seventh case, the clinical and radiological findings were indicative of metastatic breast cancer as a cause of death, however, no biopsy was obtained from the multiple metastatic lesions and their origin could therefore not be histologically verified. One patient was lost to follow-up 7.2 months after treatment. Median follow-up time was 3.1 years (range: 0.2–5.7; interquartile range: 2.2–4.1). Patient and tumor characteristics at baseline are described in Table 1. Overall survival rates were 78% (95% CI: 71–85%) after 3 years and 62% (95% CI: 50–73%) after 5 years.

Information on AJCC staging was available for all patients. Genetic information from either FISH or MLPA was available for chromosome 3 in 141 cases, chromosome 8 in 139 cases, chromosome 6 in 138 cases and chromosome 1 in 115 cases (Table 1). In five cases (3.4%) no chromosomal status could be obtained from the biopsy. Both FISH and MLPA test results for chromosome 3, 6

and 8 were available in 64 patients. Identical test results between the two methods were found in 79.6% for chromosome 3 and in only 67.2% for chromosome 8. If the tests only distinguished between normal and abnormal chromosomal status, these fractions increased to 84.4% and 76.6%, respectively. In 7.8% of cases a partial loss of chromosome 3 was missed by the centromeric FISH probe, while hyperploidy of chromosome 3 was detected by FISH and missed by MLPA in 7.8% of cases. In 15.6% of cases a partial gain of the 8q arm was missed by the centromeric FISH probe while FISH detected gain or loss in 7.8% of cases, which was missed by the MLPA analysis. Chromosomes 3 and 8 were both normal in 42 of 139 patients (30.2%) and chromosomes 1, 3, 6 and 8 were normal in 19 of the 114 patients (16.7%) for whom information on all four tested chromosomes was available. The proportion of patients with a normal chromosomal status decreased with increasing AJCC stage or AJCC tumor size; chromosomes 3 and 8 were normal in 39.3% of patients with a stage I tumor, 33.3% of patients with a stage II tumor, 12.0% of patients with a stage III tumor and in 0 patients with a stage IV tumor (Fig. 1). There was no significant association between normal genetic status and AJCC stages I–III ($p = 0.069$) or tumor size T1-T4 ($p=0.13$).

Chromosomal aberrations of chromosomes 1, 3, 6 and 8 were pooled and classified as either normal or abnormal. Univariate analyses evaluating loss and gain showed a significantly increased risk of melanoma-related death by both loss (HR: 8.2, 95% CI: 1.9–35.0, $p = 0.004$) and gain (HR: 5.1, 95% CI: 1.9–13.8, $p = 0.001$) of chromosome 8 and loss of chromosome 3 (HR: 11.5, 95% CI: 2.7–48.8, $p = 0.0009$). Gain of chromosome 3 demonstrated a non-significant increased risk compared to a normal copy number (HR: 3.1, 95% CI: 0.3–34.5, $p = 0.35$). Furthermore, there was no significant difference between the survival distributions of loss of chromosome 3 and gain of chromosome 8 compared to any abnormality of chromosomes 3 and 8 (Fig. 2). Any abnormality of chromosomes 3 ($p < 0.001$) or 8 ($p < 0.001$) was significantly associated with poor prognosis, while abnormalities of chromosome 1 ($p = 0.51$) and 6 ($p = 0.38$) were not significantly associated with poor prognosis using a log-rank test.

Survival of patients in each of the four AJCC stages differed significantly ($p < 0.0001$). Prognostic accuracy was increased when patients were stratified for both chromosomal abnormalities and AJCC staging as demonstrated by cumulative incidence curves in Figure 3. Only patients with a known genetic status of chromosomes 3 and 8 (139 patients) were included in the analyses excluding one melanoma-specific death and two deaths by other causes (Table 2). AJCC stage IV (2 melanoma-specific deaths) was also excluded from the Cox regression analyses, leaving 27 melanoma-specific events and 39 all-cause events for study. Melanoma-related deaths were observed in one (2.4 %) of 42 patients with a normal genetic status of chromosomes 3 and 8, and in 28 (28.9 %) of 97 patients with any abnormality of chromosomes 3 and 8. Significant associations with melanoma-related death were demonstrated in Cox univariate analyses for age (HR: 1.03, 95% CI: 1.002–1.06, $p = 0.04$), AJCC stage III tumors (HR: 20, 95% CI: 2.6–153.9, $p = 0.004$), and aberrations of chromosome 3 (HR: 11, 95% CI: 2.5–45.1, $p = 0.001$) and chromosome 8 (HR: 5.4 95% CI: 2.01–14.3, $p = 0.0008$) (Table 3). In Cox multivariate analysis, AJCC stage III (HR: 11.1, 95% CI: 1.4–86.3, $p = 0.02$) and abnormal status of chromosomes 3 (HR: 6.3, 95% CI: 1.4–28.3, $p = 0.02$) and 8 (HR: 2.8, 95% CI: 1.03–7.8, $p = 0.043$) remained separate significant predictors of melanoma-related death.

Discussion

The AJCC staging system and genetic status for chromosomes 3 and 8 are both valuable tools when counseling patients with ciliary and choroidal melanoma on metastasis-free survival. Interestingly, we found that normal genetic status minimized the prognostic value of AJCC staging (Fig. 3B), while AJCC staging provided further stratification of tumors with chromosomal abnormalities for a more accurate prediction of survival (Fig. 3C). To our knowledge the reduced prognostic effect of AJCC staging among patients with a normal genetic status has not previously been described, although it has been implied for GEP, where all class one tumors are considered low risk regardless of clinical tumor characteristics.[19] The importance of combining clinicopathological characteristics and genetic factors, as shown for genetically abnormal tumors in our consecutive cohort, has previously been suggested by Kivelä et al.[19] and demonstrated in a case-control study of 116 uveal melanoma patients by Ewens et al.[13] However, the non-consecutive design and artificial sampling of their study did not allow an evaluation of metastatic risk beyond two years. The population-based design of our study allowed us to evaluate the time from primary treatment to metastatic death with Cox multivariate analyses which identified both AJCC stage III and abnormal genetic status of chromosome 3 as strong individual factors of a poor prognosis. Our study presents a well-defined cohort from a Scandinavian population and the overall five-year survival rate of 62% was in accordance with previous Scandinavian studies.[20-22] The consecutive series of patients from a single center rules out bias of more complicated or advanced cases that could be a problem in larger series from tertiary referral centers. In addition, the central registry in Denmark allows for a thorough follow-up. Indeed, only one patient was lost to follow-up at the time of data sampling. It has previously been shown that death by metastatic melanoma is underestimated in non-audited registry data.[1] We therefore evaluated all histopathological descriptions and clinical records regarding the final illness of all diseased patients in our cohort. Furthermore, histopathological diagnosis of metastases arising from other cancers was confirmed in all cases but one. In this case, the cause of death relied on a review of clinical charts.

Our findings regarding the predictive value of chromosomes 3, 8 and AJCC stage (Table 3) correlated well with results from another study by Ewens et al. evaluating prognostic factors in a cohort of 320 cases.[9] However, we were unable to demonstrate the same significant prognostic effect of chromosome 1p loss, even though, the frequency of chromosome 1p loss detected in our study (23.5 %) was similar to the frequency described by Ewens et. al (18.8%).[9] Limited statistical power in our study may explain this difference, as genetic status of chromosome 1 was only available in 114 patients. The cohort study by Ewens et al. also showed a significant correlation between male gender and poor survival, which was not reproduced in our data. As a larger proportion of males died from other causes in our cohort, they were censored and subsequently no longer at risk for metastatic disease. This could explain why we found that females had a statistically non-significant 43% greater risk of melanoma-related death than males. Deaths by other causes constituted 32% of all observed deaths in our study, and this likely contributed to bias in the effect of age and gender on survival. To address this problem we evaluated both endpoints e.g. melanoma-related death and all-cause mortality in the statistical analyses (Table 3). Furthermore the individual and combined prognostic effect of genetic status and AJCC staging was evaluated by cumulative incidence rates of melanoma-related death which accounted for death by other causes as a competing risk. It has previously been demonstrated that cumulative incidence

estimates of melanoma related mortality are more accurate than Kaplan-Meier estimates in the presence of competing risks.[1]

The small sample size was an important limitation of our study. We were therefore unable to provide information on survival in the different subgroups of the AJCC stages. We did, however, demonstrate a significant ($p < 0.0001$) and similar stratification of survival between the four AJCC stages (Figure 3A) as previously described.[5] In addition, the small sample size did not enable us to differentiate between loss and gain of chromosomes 3 and 8. However, both loss and gain of chromosome 8 was associated with a significant elevated risk of melanoma related death in our study. This is in accordance with previous studies where loss of the p-arm of chromosome 8 has been associated with a poor prognosis.[9] As our results relied mostly on FISH using a centromeric probe for chromosome 8, we were, however, not able to determine the specific location of the aberrations. Gain of chromosome 3 was detected by FISH analysis in 13 tumors (9.2%), which all demonstrated a complex genotype with more than two copies of at least two of the four tested chromosomal regions. Gain of chromosome 3 has previously been demonstrated in 2–4% of cases.[23,24] In our study we found a trend towards an increased risk of melanoma-related death in patients with gain of chromosome 3, though it was not significant which might be caused by the limited number of patients. A restricted evaluation of survival based only on loss of chromosome 3 would have excluded these 13 (9.2%) patients, subsequently making counseling and planning of follow-up for these patients difficult from a clinical point of view. In fact, of all patients with abnormal genetic status of chromosomes 3 and 8 (n = 59), 20 patients did not present the genetic combination of both loss of chromosome 3 and gain of chromosome 8 but showed either gain of chromosome 3 or loss of chromosome 8. In our study these patients presented a similar poor survival as the patients demonstrating tumors with loss of chromosome 3 and gain of chromosome 8 (Figure 2). Consequently any aberration of both chromosomes 3 and 8 identified a group of patients with a high incidence of melanoma-related mortality, while an abnormal status of only one of the two chromosomes identified a ‘middle’ group with a moderately elevated incidence of melanoma-related mortality (Figure 3D). Furthermore, when compared to a study population with similar composition in regard to tumor size (mean largest basal diameter = 12 mm, mean tumor height = 5.8 mm), our study demonstrated equivalent distinction between mortality rates of normal and abnormal chromosomal status as found with GEP for class 1 and class 2 (2.4% vs. 4.6% and 28.9% vs. 28.6%, respectively). [25]

The prognostic evaluation of chromosome 6 in our study was limited. A frequent occurrence of simultaneous gain of chromosome 6p and loss of 6q was detected by MLPA. The probe for FISH analysis of chromosome 6 covered the centromeric region only and could therefore miss an abnormality of either the 6p or 6q arm. Gain of chromosome 6p has previously been associated with a favorable prognosis. However as most of the genetic data in our study was based on FISH analysis, we were not able to assess the individual effect of chromosome 6p loss.

The overall agreement between FISH and MLPA was in accordance with previous studies looking at FISH as a primary test method.[26,27] In addition, we found that MLPA and FISH provided further information for chromosome 3 in 7.8 % and 7.8 % of cases, respectively, which suggest that a combination of tests could beneficial.

The frequency of a normal genetic status in the tumors decreased with increasing tumor size and thereby increasing AJCC stage (Figure 1). The correlation between increasing tumor size and aberration of chromosomes 3 and 8 has previously been shown.[28] It has also been shown that

tumors can be heterogeneic and genetic aberrations could be missed with a biopsy especially in large tumors.[29] However, a normal genetic status was only identified in three patients with a stage III tumor and none of these patients had developed metastatic disease during follow-up (Figure 3B).

AJCC staging is a validated prognostic tool for patients with choroidal and ciliary body melanoma, but information on genetic status provided additional information on survival. While normal chromosomal status predicted a favorable survival, a combination of both AJCC staging and chromosomal status gave the most accurate prediction of melanoma-related death.

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Table 1 baseline characteristics

Parameter		**%***
No. of ptt	153	
No. of biopsies	148	
Age at diagnosis (years)		
Mean (± SD)	62.0	(± 14.4)
Median (range; interquartile range)	63.0	(24–91; 52.0–73.5)
Gender, (no. of ptt (%))		
Male	75	(49.0)
Female	78	(51.0)
Tumor size (median, range; interquartile range)		
Largest basal diameter (mm)	11.9	(3–25; 10.0–15.49)
Tumor Height (mm)	5.0	(1–22; 3.5–7.5)
Tumor size (mean (± SD))		
Largest basal diameter (mm)	12.7	(± 4.2)
Tumor Height (mm)	5.9	(± 3.4)
AJCC stages, no. of ptt (%)		
Stage I	32	(20.9)
Stage IIA	63	(41.2)
Stage IIB	28	(18.3)
Stage IIIA	18	(11.8)
Stage IIIB	10	(6.5)
Stage IIIC	0	(0.0)
Stage IV	2	(1.3)
Chromosomal status		
Chromosome 1	115	
Normal	81	(70.4)
Loss	27	(23.5)
Gain	7	(6.1)
Chromosome 3	141	
Normal	56	(39.7)
Loss	72	(51.1)
Gain	13	(9.2)
Chromosome 6	138	
Normal	87	(63.0)
Loss	9	(6.5)
Gain	42	(30.4)
Chromosome 8	139	
Normal	66	(47.5)
Loss	9	(6.5)
Gain	64	(46.0)

* Data are expressed as percentages unless otherwise indicated. SD = Standard deviation, No. = number, ptt = patients, AJCC = American Joint Committee on Cancer staging (7th ed.)

Table 2 The distribution of observed deaths in relation to AJCC stage and chromosomal status.

Chromosomal status	AJCC Stage I	AJCC Stage II	AJCC Stage III	AJCC Stage IV
Normal	**11**	**28**	**3**	**0**
MM-related death	0	1	0	0
Death by other causes	0	2	0	0
Abnormal Chr. 3 OR 8	**9**	**22**	**7**	**0**
MM-related death	0	3	2	0
Death by other causes	1	4	0	0
Abnormal Chr. 3 AND 8	**8**	**34**	**15**	**2**
MM-related death	1	10	10	2
Death by other causes	1	3	1	0
No information on Chr. 3 or 8	**4**	**7**	**3**	**0**
MM-related death	0	0	1	0
Death by other causes	0	1	1	0
Total	**32**	**91**	**28**	**2**
MM-related death	1	14	13	2
Death by other causes	2	10	2	0

Chr. = chromosomes, MM = Metastatic melanoma

Table 3 Univariate and multivariate Cox regression analysis for the association of AJCC stage and genetic status with uveal melanoma related mortality and all-cause mortality

	Univariate analysis						Multivariate analysis (AJCC stage, Chr. 3 and Chr. 8)					
	Cause-specific mortality			All-cause mortality			Cause-specific mortality			All-cause mortality		
Variables	HR	(95% CI)	p-value	HR	(95% CI)	p-value	HR	(95% CI)	p-value	HR	(95% CI)	p-value
Sex												
male	Ref.			Ref.			-			-		
female	1.43	(0.67–3.085)	0.36	1.048	(0.56–1.97)	0.88	-			-		
Age (yrs)	**1.034**	**(1.004–1.07)**	**0.03**	**1.034**	**(1.008–1.06)**	**0.01**	-			-		
AJCC* st.												
I	Ref.			Ref.			Ref.			Ref.		
II	6.07	(0.79–46.39)	0.082	3.31	(0.99–11.1)	0.051	4.83	(0.63–37.30)	0.13	2.91	(0.86–9.86)	0.086
III	**17.55**	**(2.28–135.13)**	**0.0059**	**6.35**	**(1.81–22.33)**	**0.004**	**11.04**	**(1.42–85.63)**	**0.022**	**4.62**	**(1.26–15.84)**	**0.021**
Chr. abn.												
Chr. 1	1.33	(0.58–3.04)	0.27	1.46	(0.74–2.88)	0.27	-			-		
Chr. 3	**10.43**	**(2.47–44.06)**	**0.0014**	**5.60**	**(2.19–14.33)**	**0.0003**	**6.31**	**(1.41–28.27)**	**0.016**	**4.14**	**(1.54–11.09)**	**0.0048**
Chr. 6	0.68	(0.29–1.61)	0.38	0.85	(0.43–1.69)	0.65	-	-		-		
Chr. 8	**5.37**	**(2.013–14.33)**	**0.0008**	**2.98**	**(1.47–6.03)**	**0.0023**	**2.83**	**(1.033–7.77)**	**0.043**	**1.70**	**(0.81–3.55)**	**0.16**

HR = Hazard ratio, CI = confidence interval, yrs=years, AJCC st.= American Joint Committee on Cancer stage, Chr.abn.= chromosomal abnormalities, chr. = chromosome, ref = reference. *AJCC stage IV was not included in the analyses because this patient group had already developed metastatic disease at time of diagnosis

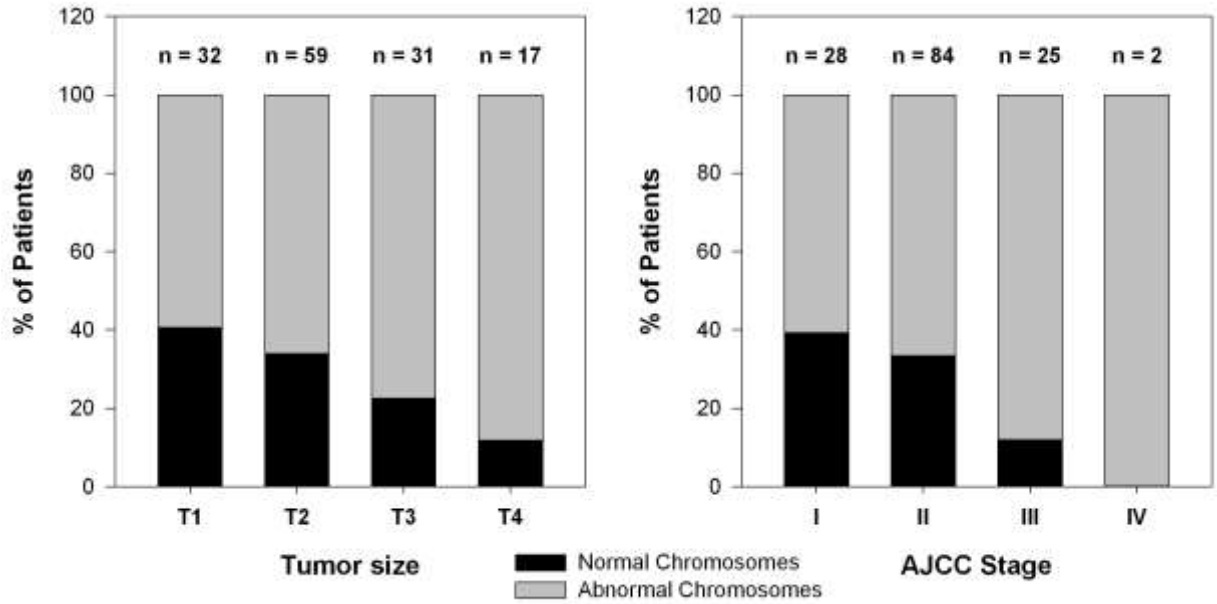

Figure 1 The proportion of normal genetic status of chromosomes 3 and 8 (black box) in relation to AJCC tumor size and AJCC stage.

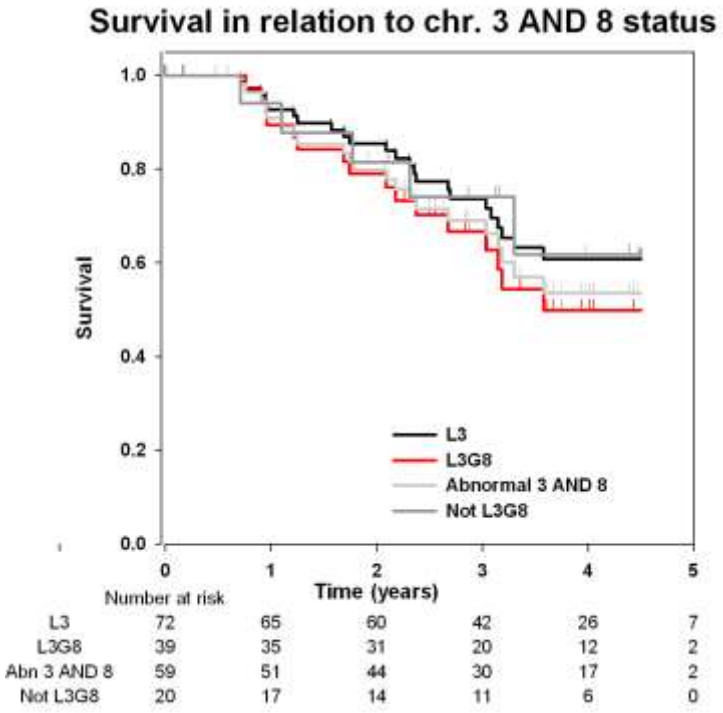

Figure 2 Melanoma-related survival distribution of all patients with aberrations of chromosomes 3 AND 8 (Abnormal 3 AND 8; light grey line), subdivisions of patients with the combination of loss of chromosome 3 AND gain of chromosome 8 (L3G8; red line) and patients with either gain of chromosome 3 in addition to gain of chromosome 8 or loss of chromosome 3 and loss of chromosome 8 (Not L3G8; dark grey line). The survival distribution of loss of chromosome 3 regardless of chromosome 8 status is also shown (L3; black line).

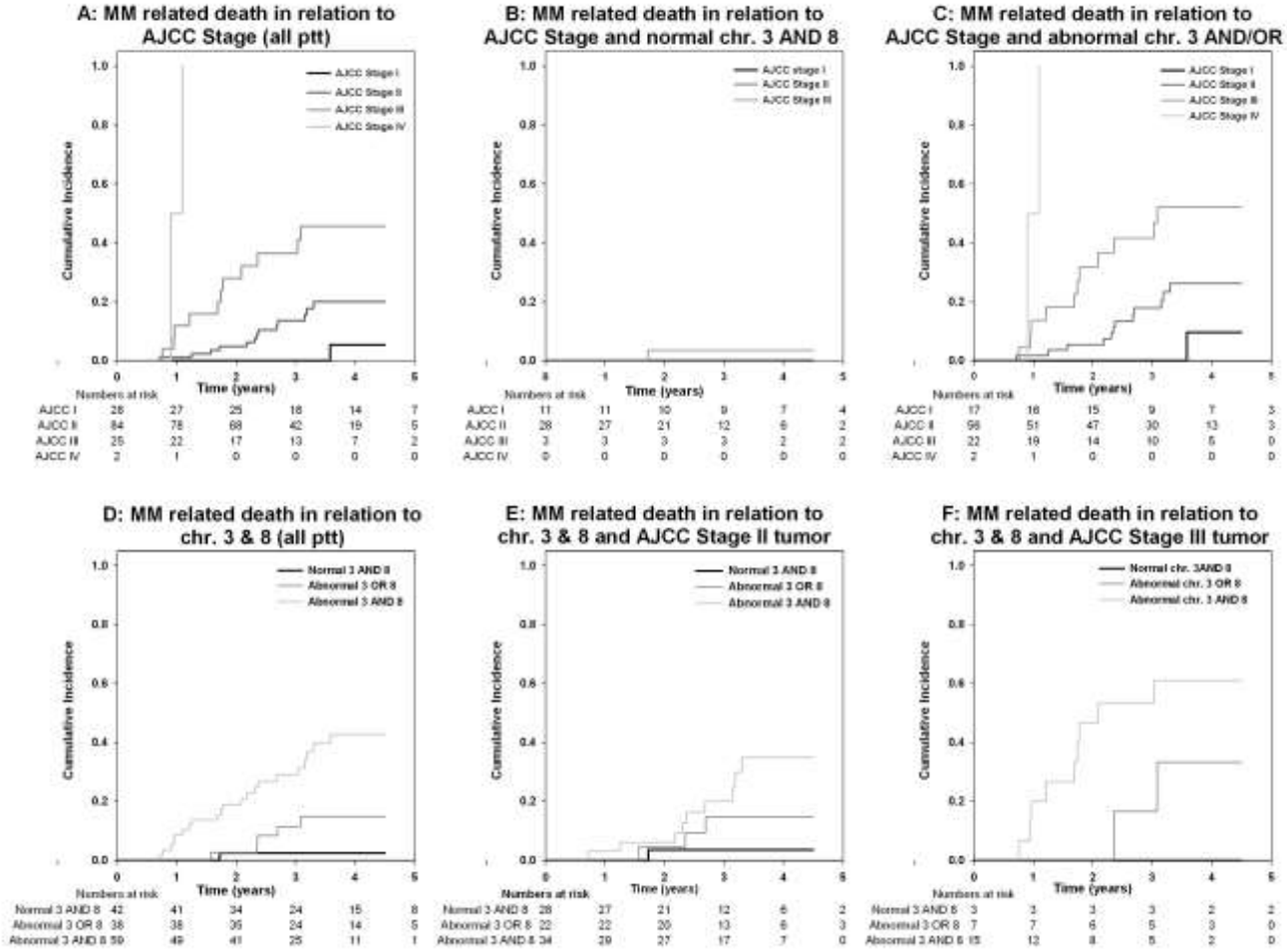

Figure 3 Cumulative incidence of melanoma-related which accounts for death by other causes as a competing risk. First row: Risk of melanoma-related death according to AJCC stage **A)** In all patients. **B)** In patients with normal chromosomal status of 3 AND 8. **C)** In patients with abnormal chromosomal status of 3 AND/OR 8. Second row: Risk of melanoma-related death according to genetic status of chromosomes 3 and 8 **D)** In all patients. **E)** In patients with an AJCC stage II tumor. **F)** In patients with an AJCC stage III tumor. All curves were terminated at 4.5 years due to the limited patient numbers at risk beyond this point. Patient number at risk is shown for all groups below the graphs. Ptt = patients, chr. = chromosome