1   Title:

2   **Survival Analysis Following Enucleation for Uveal Melanoma**

3

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21

22   *Running Title:* Survival following enucleation for uveal melanoma

23 **Abstract**

24 Objectives

25 To determine survival outcomes following enucleation for uveal melanoma. To compare

26 these outcomes with the 8th edition AJCC classification and determine the influence of

27 cytogenetics, using Fluorescent in situ Hybridisation (FISH), on survival. To determine

28 whether failure to gain sufficient sample for cytogenetics using Fine Needle Aspiration

29 Biopsy (FNAB) correlates with survival.

30

31 Subjects/Methods

32 All patients undergoing primary enucleation for uveal melanoma at Moorfields Eye Hospital

33 between 2012 and 2015 were included. Clinical, pathological, cytological and survival data

34 were analysed for all patients.

35

36 Results

37 155 subjects were included. Mean age at enucleation was 65.9 years (SD 14.13). 88 (56.8%)

38 patients died at a mean of 3(SD 1.9) years following enucleation. Of these, 52 (33.5%) died

39 from metastatic melanoma, 16 (10.3%) from other causes and 20 (12.9%) causes of death

40 were unknown. Cumulative incidence analysis demonstrated AJCC grade, chromosome 8q

41 gain and monosomy 3 all predict metastatic mortality. The greatest 5-year mortality rate

42 (62%, SD10.1%) was in those with both chromosome abnormalities and AJCC stage III

43 (Stage IV patients excluded due to low numbers). Largest basal diameter and chromosome

44 status, both independently (p=0.02 and p<0.001) predicted metastatic mortality on

45 multivariable regression analysis. Those who had an insufficient sample of cells gained

46 during FNAB (n=16) had no different prognosis.

47

48 Conclusions

49 This study confirms, in this population, the poor survival of patients enucleated for uveal

50 melanomas. It confirms the prognostic utility of adding AJCC grade to cytogenetic

51 information. It demonstrates that lack of sample in patients undergoing FNAB is not related

52 to prognosis.

53

54 **Introduction**
55
56 Uveal melanoma is a relatively rare tumour occurring in 6 per million people per year in

57 England[1]. Metastases develop in almost 50% of patients[2], usually to the liver. The efficacy of

58 current treatments for metastatic uveal melanoma are limited and mortality within the first

59 year is common[2].

60

61 Factors predictive of metastasis are multiple and have been described at length previously[3].

62 They include: anatomical factors, such as tumour size, extraocular extension and ciliary body

63 involvement; histopathological factors such as the presence of epithelioid cells, closed

64 connective tissue loops and high mitotic count; and genetic aberrations, such as chromosome-

65 3 loss, chromosome 8q gain, *BAP1* loss of function mutations and a class 2 gene expression

66 profile.

67

68 Patients find it helpful to be given an idea of their life expectancy at the time of diagnosis[4].

69 Prognostication may enable some practitioners to adjust the intensity of surveillance for

70 metastasis according to each patient's estimated mortality. The standard prognostic tool is the

71 American Joint Committee on Cancer (AJCC) Tumour Node Metastasis (TNM)

72 classification[5]. This is now in its eighth edition and has been validated and modified from a

73 series of over 7000 uveal melanoma patients provided by the European Ophthalmic

74 Oncology Group[6].

75

76 A limitation of the AJCC classification is that it uses only anatomic predictors, without taking

77 into account genetic and histopathological risk factors. Several studies suggest including

78 these laboratory findings, particularly cytogenetic information, can improve the accuracy of

79 prognostication.[7-10]. The Liverpool Uveal Melanoma Prognosticator Online (LUMPO), now

80   in its third iteration, combines anatomic findings with genetic and histopathologic data. An

81   international validation study of LUMPO has validated the use of this prognostic tool in

82   uveal melanoma with data from seven international ocular oncology centres[11].

83

84   Moorfields Eye Hospital is one of four Ocular Oncology centres in the UK receiving referrals

85   from a large population in the South of England. Since 2012, we have routinely performed

86   FISH (fluorescence in situ hybridization) cytogenetic analysis on all consenting patients

87   undergoing primary enucleation for choroidal melanoma.

88

89   In this paper, we compare survival outcomes following primary enucleation for choroidal

90   melanoma with the standard 8th edition AJCC classification based on TNM and determine the

91   influence of cytogenetic FISH results, and other known prognostic markers on this cohort of

92   patients. We also sought to investigate whether failure to obtain enough sample for FISH

93   analysis using fine needle aspiration biopsy (FNAB) indicates a better prognosis as has been

94   suggested previously.[12] In theory, smaller tumours with cohesive spindle cells, indicating

95   better prognosis, may be less likely to yield sufficient cells for cytogenetic analysis.

96   **Methods**
97
98   This is a single centre case series study. Subjects were identified from the enucleation

99   database of the Department of Pathology, University College London Institute of

100 Ophthalmology. All primary enucleation cases performed by the department between 1st

101 January 2012 and 31st December 2014 were included.

102

103 With prior consent from patients, cells for cytogenetic analysis were gained from enucleation

104 specimens following eye removal using trans-scleral fine needle aspiration biopsy (FNAB).

105 FISH analysis was carried out using centromeric and subtelomeric probes for chromosome 3

106 (D3S4559, D3Z1, Cytocell Ltd, Cambridge, United Kingdom) and centromeric and MYC

107 probes for chromosome 8 (D8Z2, MYC, Abbott Molecular Inc., Des Plaines, IL, USA). At

108 least 100 cells from each enucleation specimen were evaluated when possible, and

109 abnormalities were reported when more than 10% of cells showed cytogenetic changes.

110

111 Clinical records were reviewed for demographic data, including age and sex. Pathology

112 findings were reviewed for data on tumour size, mitotic count, the presence or absence of

113 ciliary body involvement (defined as including the pars plana), epithelioid cells, extravascular

114 matrix loops and extraocular extension.

115

116 The United Kingdom National Health Service keeps Summary Care Records for the entire

117 population (The NHS Digital Spine). These Summary Care Records can be accessed digitally

118 by registered health professionals. These records were searched on 13th May 2020 to identify

119 whether patients in this study were alive or dead and the date of death of the deceased. The

120 General Practitioners (family doctors) of all the deceased patients were contacted to find out

121 the cause of death. If the General Practitioners were not able to provide us with this

122 information we attempted to contact next of kins of the deceased patients.

123

124 *Statistical analysis*

125 For the analysis, the statistical software package R (version 3.6.3) was used ([www.r-](www.r-project.org)

126 [project.org](www.r-project.org)). Participant characteristics were summarised using percentages, means and

127 standard deviations (SD). Pearson's chi-squared, Fisher's exact test and the Kruskal-Wallis

128 test were performed to evaluate the inter-correlations between baseline characteristics.

129

130   Missing and non-missing cases were compared using sensitivity analysis to assess the

131   robustness of the missing at random assumption. Schoenfeld's residuals were plotted against

132   failure time for each covariate to assess the proportional hazards assumption. Violations in

133   the proportional hazard's assumption were handled via stratification or time dependent

134   covariate methods. To enhance statistical power and ensure stable model estimation, AJCC

135   stages I and IV were discounted from analyses due to low numbers (n=1 and n=7) and also

136   due to relatively low numbers in each subgroup, stages IIA and IIB were grouped to form

137   stage II and stages IIIA, IIIB and IIIC grouped as stage III.

138

139   Cumulative incidence functions (CIFs) were plotted to show the estimated marginal

140   probability of each cause of death post treatment accompanied by the numbers at risk. Gray's

141   test for equality of CIFs was performed to evaluate statistical significance. Cumulative

142   incidence rates (95% CI) of death due to melanoma were computed at 5 years of follow-up.

143   Largest basal diameter (LBD) and mitotic count were categorised for graphical visualisation;

144   however, when taken forward into the multivariable models these variables remained

145   continuous to increase power and limit loss of information.

146

147   Subdistribution-hazard ratios with 95% CI's were estimated using the Fine and Gray

148   regression model in both univariate and multivariable analysis[13]. For ease of interpretation

149   additional analyses were performed using the Cox regression model. In this model, effect

150   estimates were reported as hazard ratios (HRs) with 95% CIs.

151

152   In the multivariable analyses, a backward stepwise procedure with entry selection criterion

153   set at a nominal p-value of 10% and elimination criterion at 5% were employed to select the

154   final model. Forward-selection was also performed with the same entry and stay criterions

155   and models were compared. In both model selection routines, confounders such as age and

156   gender were forced in regardless of statistical significance, unless either variable had a

157   negative effect on the model accuracy. The relative effect of incorporating variables into the

158   model was assessed based on model apparent and bootstrap adjusted C-statistics with 95%

159   CIs, as well as Akaike information criterion (AIC), allowing a rank order of relative

160   importance to be produced. Time-dependent Receiver Operating Characteristic (ROC) and

161   Brier scores were also checked, as per Blanche *et al* 2019[14]. Unadjusted p-values are

162   provided unless indicated otherwise.

163

164   This study was approved by the Institutional Review Board at Moorfields Eye Hospital

165   (CA20/ONC/606). The study adhered to the tenets of the Declaration of Helsinki.

166   **Results**
167
168   From 1st January 2012 to 31st December 2014, 159 primary enucleations were performed for

169   uveal melanoma at Moorfields Eye Hospital. There were four patients excluded from the

170   analysis because of the lack of either survival data or pathology/cytopathology results,

171   leaving a total of 155 cases. Table 1 summarises the population characteristics of the cohort.

172   There were 90/155 (58%) males and 65/155 (42%) females. The average age at enucleation

173   was 65.9 years (SD 14.13). 88 (56.8%) patients died at a mean of 3 (SD 1.9) years following

174   enucleation. 52 (33.5%) patients died from metastatic melanoma, 16 (10.3%) from other

175   causes and 20 (12.9%) causes of death were unknown. The 20 unknown deaths were

176   excluded from further statistical analysis leaving 135 patients in the final sample for analysis.

177   Demographic and tumour characteristics of individuals with known and unknown causes of

178   death were compared and no statistically significant differences were noted (Table S1).

179

180   A total of 29.6% of tumours were graded as AJCC stage IIB and 35% as stage IIIA..

181   Tumours missing data on genotype were compared to those where the data was not missing,

182   and no statistically significant differences in demographics or other tumour characteristics

183   were noted (Table S2).

184

185   As shown in table 1, mean age and basal diameter were higher in those who died during the

186   follow-up period ($p<0.001$). There was a higher proportion of patients with tumours showing

187   both monosomy 3 (M3) and chromosome 8q gain who died during the study period ($p=0.005$

188   and $p=0.002$; chi-squared test). Table 2 shows the p-values for the correlations between all

189   tumour characteristics at baseline together with the statistical tests performed to investigate

190   these correlations. AJCC stage and presence of M3 and 8q gain, had a significant association

191   ($p= 0.025$). AJCC stage and presence of just M3 had a trend towards significance ($p=0.057$),

192   whereas there was little to no association with presence of just chromosome 8q gain

193   ($p=0.209$). There was strong evidence for an association between M3 and chromosome 8q

194   gain ($p<0.001$).

195

196   *Cumulative Incidence Analysis*

197   Cumulative incidence curves are shown in figure 1. These demonstrate graphically the

198   prognostic risk factors that statistically significantly predict metastatic mortality (AJCC

199   grade, chromosome 8q gain, monosomy 3, tumour diameter, ciliary body involvement and

200   mitotic rate). For example, figures 1B-D show the cumulative incidence curves by presence

201   or absence of chromosome 8q gain and/or monosomy 3 (M3). The presence of 8q gain or M3

202   is associated with a higher overall incidence in melanoma related death ($p=0.001$; $p=0.002$

203   for chromosome 8q gain and M3 respectively). Taking the respective categories of no M3 or

204   gains in 8q, and both gains in 8q and M3, the incidence of death from melanoma is highest in

205   those who have both aberrations (p<0.001;figure 2D). Table S3 presents the cumulative

206   incidence curve results numerically. The table shows that those with the highest 5-year

207   mortality rate (62% SD; 10.1%) are those with both chromosome abnormalities and AJCC

208   stage III.

209

210   Cumulative rates of melanoma-related deaths for AJCC stage II and III patients are shown in

211   figures 2a and 2b respectively. These figures, for comparison, have superimposed the

212   cumulative incidence curves from the European Ophthalmic Oncology group's 2013 study of

213   7369 patients, the data of which was used to create the 7th edition of the AJCC[6]. To illustrate

214   how adding information about chromosome status to the AJCC data enhances prognostic

215   ability, curves for monosomy 3 and 8q gain patients are also shown.

216

217   Shown in figure 3 are cumulative incidence curves showing survival in those patients whose

218   FISH failed due to insufficient sample for at least one chromosome (n=16, 14%) compared to

219   those whose FISH was successful for both chromosomes (n=99, 86%). No statistically

220   significant difference was noted between these groups.

221
222   *Regression analysis*

223   Results from the univariate analyses for the Fine and Gray model are shown in table S4. Only

224   baseline demographics (age and gender), mitosis rate, chromosome status, ciliary body

225   involvement and largest basal diameter passed the nominal threshold for inclusion at 10%.

226   Higher AJCC stage(p=0.007), larger basal diameter (p<0.001), gain in chromosome

227   8q(p<0.001), monosomy 3(p=0.003), mitotic rate (p=0.004), ciliary body involvement

228   (p=0.014) and higher age(p=0.011) were found to be associated with melanoma-related death

229   however sex was not associated with metastatic mortality. Univariate cox regression analysis

230 (cause-specific hazards) was in concordance with the results from the Fine-Gray model (table

231 S5), where hazard ratios and 95% CIs are presented for ease of interpretation.

232

233 Multivariable analysis limited to chromosome status and largest basal diameter are presented

234 in table S6. We studied the combined variable chromosome status (categorised into 2 groups-

235 absence of both chromosome abnormalities vs both chromosome abnormalities present) to

236 offset issues related to multicollinearity between the binary variables ($p<0.001$; chi2-test).

237 We decided to group presence of only one chromosomal defect (e.g. M3 or 8q gain) with

238 none due to similar survival experience at 5-years in this sample (see figure 1 and 2D) and to

239 enhance statistical power for multivariable analysis. Furthermore, because of inadequate

240 sample size in AJCC stage (low numbers in groups other than stage II and III) largest basal

241 diameter was taken forward into to the multivariable analysis only.

242

243 Residual diagnostic plots for the Fine-Gray model are shown in figures S1-S4. Calibration curves for

244 the Fine-Gray model are shown in figure S5. As shown in table S7, taking age and gender in a

245 "base" model, adding largest basal diameter produced better model discrimination than

246 chromosome status; however, taken together these gave the highest AUC(95% CI) (bootstrap

247 adjusted ROC: 72(67.9,83.7), Time-dependent AUC (AUC_t): 77.7(69.3, 86.2)) and smallest

248 prediction error (Brier score; 16.3(11.3,21.3)). This two marker-model, despite having more

249 parameters, also had the lowest AIC(281.37). For AIC, smaller values indicate better model

250 fit. Brier score combines discrimination and calibration. Smaller values indicate higher

251 predictive accuracy.

252

Discussion

This study specifically focuses on survival in patients with large, advanced tumours who have not received previous treatment. The results demonstrate that in our particular population of patients, survival following enucleation for large uveal melanoma is poor. Fifty seven percent of our cohort of patients enucleated between 2012 and 2014 had died by May 2020. Only 18% of these patients were known to have died from other causes. The remaining 82% either died from metastatic melanoma or had unknown causes of death.

As shown in figure 2, our survival results based on AJCC criteria are comparable to the European Ophthalmic Oncology Group's 2013 study that validated the AJCC criteria[6]. Over and above this, using both cumulative incidence analysis and multivariable regression analysis, we corroborate the findings of previous studies that have demonstrated the utility of combining the additional information from cytogenetics with tumour size/AJCC grade[7-10]. In our patients, adding information about chromosome status to information about tumour size, more accurately predicts mortality than AJCC data alone (see Figure 2). Overall, patients with the worst prognosis are those with tumours with diameter of 16 mm or more with both monosomy of chromosome 3 and chromosome 8q gains. In these patients the 5-year mortality rate measures 61%.

Since 2012, we have routinely performed FISH (fluorescence in situ hybridization) cytogenetic analysis on all consenting patients undergoing primary enucleation for advanced uveal melanoma. Although several other techniques for genetic analysis exist, with this study we have demonstrated that FISH remains a useful tool. Benefits of FISH over these other methods include the fact that it is able to assess for heterogeneity in tumours and that it can also be used to detect the percentage of cells with monosomy 3 and 8q amplification, which

278   has been shown previously to correlate with patient survival[15]. By using two probes for

279   chromosome 3 (a centromeric and sub-telomeric probe) we are able to detect partial deletions

280   of chromosome 3, which used to be a weakness of FISH as compared to MLPA. We

281   demonstrate that tumours providing insufficient sample for FISH analysis have a similar

282   prognosis to those who have successful FISH, although the numbers involved are small

283   (n=16). This result is in contrast to previous theories that insufficient-sample FNAB results

284   are more likely in more cohesive, spindle-cell tumours that are smaller and have a better

285   overall prognosis.[12]

286

287   The strengths and challenges of this study included the ascertainment of survival data on a

288   cohort of enucleated patients with advanced uveal melanoma, all of whom had been

289   discharged from routine Ocular Oncology follow-up but still attended other hospitals for

290   surveillance scans of the liver. Although we had robust data on whether patients were alive or

291   deceased from the NHS Digital Spine, obtaining the cause of death data was more difficult.

292   This meant that 20/88, 22% of patients had unknown causes of death. National collection of

293   survival data for uveal melanoma in the United Kingdom is flawed because in central

294   registries, it is coded as a head and neck cancer rather than eye cancer.

295

296   Chromosome 8 status was known only in 104/155 (67%), and chromosome 3 status only in

297   100/155 (64%). Despite this, we have used robust statistical methods to ensure that the

298   conclusions we have drawn from the study are valid. The main reasons for lack of

299   cytogenetic information in patients were because patients declined the test or the cytogenetic

300   test failed due to insufficient material (16 samples). Performing cytogenetic testing on all

301   patients is a possible way of increasing the amount of cytogenetic information available for

302   further studies. Rather than a fine needle aspirate, a scleral flap approach or punch biopsy

303   may permit a greater yield. Newer molecular techniques may also yield better results. Next

304   generation sequencing (NGS) in choroidal melanoma analysis[16], may provide further

305   avenues of research as to whether NGS provides the same, or better ability to add to AJCC

306   prognostic ability as FISH. In this study we relied on pathology measurements of tumour size

307   due to inconsistencies in the reporting of ultrasound and clinical measurements. It should be

308   acknowledged that pathology measurements, depending on where the globe is cut, can

309   provide inaccurate measurements in some cases. This, however, is the same with both

310   ultrasound and clinical measurements, which also include an element of subjectivity and can

311   vary between operators.

312

313   In conclusion, this study will help patients and ocular oncology practitioners in the future

314   with prognostication as it has confirmed in our population the results of previous studies

315   demonstrating poor survival in patients enucleated for large uveal melanomas. It has also

316   confirmed results from previous studies that have demonstrated the utility of adding AJCC

317   grade to cytogenetic information in producing more accurate prognostication. In addition, it

318   demonstrates that FISH remains a useful tool and that lack of sample in patients undergoing

319   FNAB is not related to prognosis.

330

331   **Author Contribution Statement**

332   All the authors made substantial contributions to the conception or design of the work; or the

333   acquisition, analysis, or interpretation of data for the work. All the authors were involved in

334   drafting the work or revising it critically for important intellectual content. All the authors

335   were involved in final approval of the version to be published. All the authors agree to be

336   accountable for all aspects of the work in ensuring that questions related to the accuracy or

337   integrity of any part of the work are appropriately investigated and resolved.

References

1. Keenan TDL, Yeates D, Goldacre MJ. Uveal Melanoma in England: Trends Over Time and Geographical Variation. Br J Ophthalmol. 2012 96(11):1415-9.

2. Martine J. Jager, Carol L. Shields, Colleen M. Cebulla, Mohamed H. Abdel-Rahman, Hans E. Grossniklaus, Marc-Henri Stern, Richard D. Carvajal, Rubens N. Belfort, Renbing Jia, Jerry A. Shields, Bertil E. Damato. Uveal Melanoma. *Nature Reviews.* 2020 6(24):1-25

3. Damato B, Eleuteri A, Hussain R, Kalirai H, Thornton S, Taktak A, Heimann H, Coupland SE. Parsimonious Models for Predicting Mortality from Choroidal Melanoma. Invest Ophthalmol Vis Sci. 2020 9;61(4):35

4. Beran T.M., McCannel T.A., Stanton A.L., Straatsma B.R., Burgess B.L. Reactions to and desire for prognostic testing in choroidal melanoma patients. J. Genet. Couns. 2009;18:265–274

5. Amin MB, Edge S, Greene F , et al., eds. *AJCC Cancer Staging Manual*. 8th ed. Cham, Switzerland: Springer; 2017;813– 826.

6. Kujala E, Damato B, Coupland S et al. Staging of Ciliary Body and Choroidal Melanomas Based on Anatomic Extent. Journal of Clinical Oncology. 2013;31:2825-2831

7. Dogrusöz M, Bagger M, van Duinen SG, Kroes WG, Ruivenkamp CA, Böhringer S, Andersen KK, Luyten GP, Kiilgaard JF, Jager MJ. The Prognostic Value of AJCC Staging in Uveal Melanoma Is Enhanced by Adding Chromosome 3 and 8q Status. Invest Ophthalmol Vis Sci. 2017 Feb 1;58(2):833-842

8. Damato B, Duke C, Coupland SE, Hiscott P, Smith PA, Campbell I, Douglas A, Howard P. Cytogenetics of uveal melanoma: a 7-year clinical experience. Ophthalmology. 2007 Oct;114(10):1925-31.

9. Bagger M, Andersen MT, Andersen KK, Heegaard S, Andersen MK, Kiilgaard JF. 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 Dec 23;56(1):438-44

10. Walter SD, Chao DL, Feuer W, Schiffman J, Char DH, Harbour JW. Prognostic Implications of Tumor Diameter in Association With Gene Expression Profile for Uveal Melanoma. JAMA Ophthalmol. 2016 Jul 1;134(7):734-40

11. Cunha Rola A, Taktak A, Eleuteri A, Kalirai H, Heimann H, Hussain R, Bonnett LJ, Hill CJ, Traynor M, Jager MJ, Marinkovic M, Luyten GPM, Dogrusöz M, Kilic E, de Klein A, Smit K, van Poppelen NM, Damato BE, Afshar A, Guthoff RF, Scheef BO, Kakkassery V, Saakyan S, Tsygankov A, Mosci C, Ligorio P, Viaggi S, Le Guin CHD, Bornfeld N, Bechrakis NE, Coupland SE. Multicenter External Validation of the Liverpool Uveal Melanoma Prognosticator Online: An OOG Collaborative Study. Cancers (Basel). 2020 Feb 18;12(2):477

12. Augsburger JJ, Corrêa ZM, Trichopoulos N. Prognostic implications of cytopathologic classification of melanocytic uveal tumors evaluated by fine-needle aspiration biopsy. Arq Bras Oftalmol. 2013 Mar-Apr;76(2):72-9.

13. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. Journal of the American Statistical Association. 1999;94(446):496-509.

14. Blanche P, Kattan MW, Gerds TA. The c-index is not proper for the evaluation of t-year predicted risks. Biostatistics. 2019 Apr 1;20(2):347-357.

15. van den Bosch T, van Beek JG, Vaarwater J, Verdijk RM, Naus NC, Paridaens D, de Klein A, Kiliç E. Higher percentage of FISH-determined monosomy 3 and 8q

amplification in uveal melanoma cells relate to poor patient prognosis. Invest

Ophthalmol Vis Sci. 2012 May 14;53(6):2668-74

16. Afshar AR, Damato BE, Stewart JM, Zablotska LB, Roy R, Olshen AB, Joseph NM,

Bastian BC. Next-Generation Sequencing of Uveal Melanoma for Detection of

Genetic Alterations Predicting Metastasis. Transl Vis Sci Technol. 2019 Apr

17;8(2):18

Table 1. Baseline characteristics of those patients who were alive at final follow up and those who were deceased. Patients deceased from all causes, including unknown, are included in this table.

Patient Characteristic	Alive(n=67)	Deceased (n=88);
Male, n(%)	43(64.2%)	47(53.4%)
Mean age (SD)	60.8(12.5)	71.1(13.5)
Mean largest basal diameter, mm (SD)	13.1(3.71)	15.7(4.88)
Missing, n(%)	0(0%)	3(3.41%)
Mean tumour thickness, mm (SD)	9.3(3.2)	9.9(3.6)
Missing, n(%)	0(0%)	3(3.41%)
AJCC stages, n(%)		
I	1(1.5%)	0(0%)
IIA	12(17.9%)	11(12.5%)
IIB	26(38.8%)	19(21.6%)
IIIA	20(29.9%)	35(39.8%)
IIIB	6(9.0%)	17(19.32%)
IIIC	1(1.5%)	0(0%)
IV	1(1.5%)	6(6.8%)
Missing	0%	0%
Monosomy 3, n(%)		
Absent	34 (50.75%)	21 (23.86%)
Present	17 (25.37%)	39 (44.32%)
Missing	16 (23.88%)	28 (31.82%)
Chromosome 8gain, n(%)		
Absent	27(40.3%)	12(13.6%)
Present	25(37.3%)	51(58.0%)
Missing	15(22.4%)	25(28.4%)
Extraocular extension		
Absent	55 (82.1%)	70 (79.5%)
Present	12(17.9%)	18 (20.4%)
Ciliary body		
Absent	38 (56.7%)	40 (45.5%)
Present	29 (43.3%)	48 (54.5%)
Epithelioid cells		
Absent	44 (65.7%)	57(64.8%)
Present	23 (34.3%)	31(35.2%)
Loops		
Absent	34 (50.7%)	38(43.2%)
Present	24 (35.8%)	43(48.9%)
Missing	9 (13.4%)	7(8.0%)
Mean Mitosis rate (SD)	2.9(2.3)	3.9(3.2)
Missing n(%)	3(0.04)	3(0.03)
Follow-up time mean (SD) median (IQR) (years)	6.8(0.8) 6.7(1.4)	3(1.9) 2.8(2.9)

Table 2. Unadjusted P-values for correlations between tumour characteristics

	LBD	TT	AJCC stages	Monosomy 3	Chromosome 8q	M3 and 8q+	EOE	Cb	Epi	Loops	Mitosis
LBD	x										
TT	*0.018; ρ=0.192*	x									
AJCC stage	***<0.001***	***<0.001***	x								
Monosomy 3	0.414	0.766	0.0574	x							
Chromosome 8gain	0.174	0.310	0.2091	***<0.001***	x						
M3 and 8q+	0.135	0.848	*0.025*	-	-	x					
Extraocular extension	0.301	0.252	[a]-	0.645	1	0.458	x				
Cb	0.112	0.198	***<0.001***	*0.010*	*0.002*	0.005	1	x			
Epi	0.699	0.225	1	0.737	0.156	0.455	0.382	0.367	x		
Loops	0.533	0.660	0.2331	0.266	0.062	0.116	0.286	0.945	0.229	x	
Mitosis	0.131; ρ=0.125	0.193; ρ=0.108	0.982	0.590	0.470	0.335	0.715	0.354	0.626	0.160	x

Abbreviations: LBD, largest basal diameter; TT, tumour thickness; EOE, extraocular extension; Cb, ciliary body involvement; Epi, epithelioid cells; Loops, closed connective tissue loops present;
Spearman's rank correlation with yate's correction, approximate p-values for continuous vs continuous
Kruskal Wallis rank sum test for continuous vs categorical
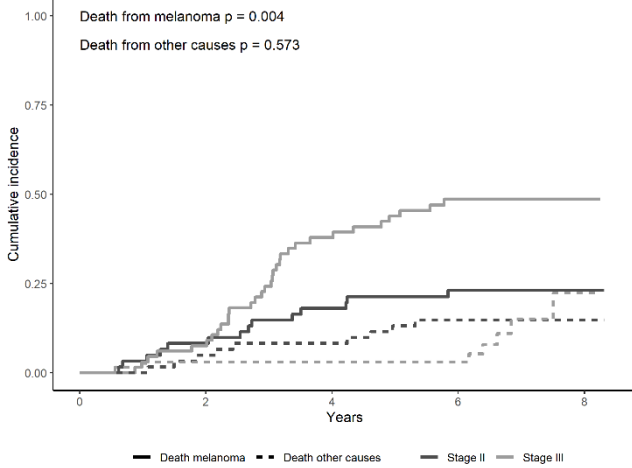Pearson's Chi-squared test for categorical vs categorical
Statistically significant p-values(p<0.05) have been *italicized,* applying the Bonferroni correction to the usual level of acceptable type-1 error (0.05) for 64 tests sets the corrected alpha threshold at **0.001**, statistically significant values in **bold**.
[a] due to low sample size this test was omitted

Figure Legends

Figure 1A-K Cumulative incidence curves by melanoma and competing risk (death by other causes)

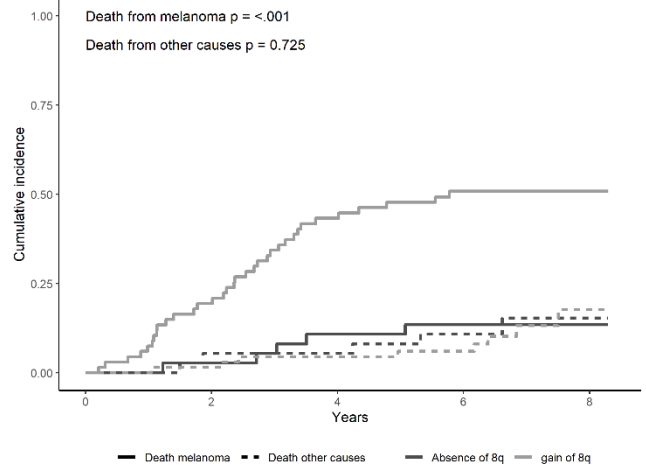for all variables considered in this study.

A) Melanoma death by AJCC status

Death from melanoma p = 0.004
Death from other causes p = 0.573

Death melanoma — Death other causes — Stage II — Stage III

Number at risk

61	53	45	33	5
66	59	39	22	2

B) Melanoma death by Chromosome 8q status

Death from melanoma p = <.001
Death from other causes p = 0.725

Death melanoma — Death other causes — Absence of 8q — gain of 8q

Number at risk

37	34	31	23	2
67	53	35	23	2

C) Melanoma death by M3 status

Death from melanoma p = 0.002
Death from other causes p = 0.996

Death melanoma — Death other causes — Absence of M3 — M3
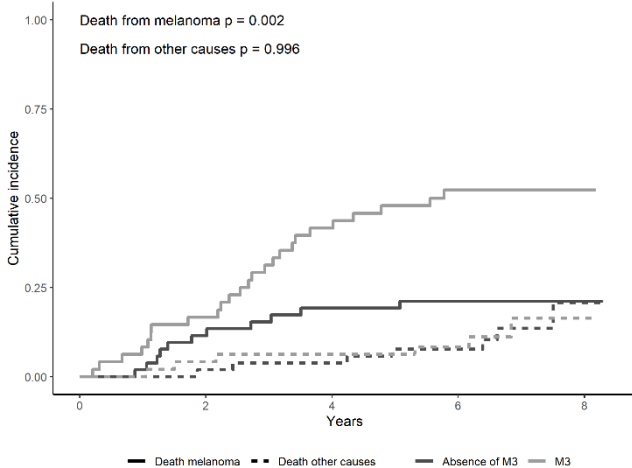
Number at risk

52	45	40	30	3
48	38	25	16	1

D) Chromosome 8q and Chromosome 3 status

Death from melanoma p = <.001
Death from other causes p = 0.541

Death melanoma — Death other causes — No M3 OR No 8q — Presence of M3 AND 8q
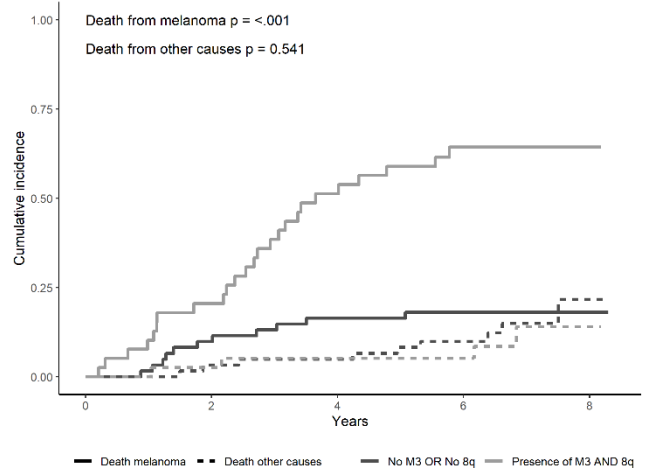
Number at risk

61	53	48	35	3
39	30	17	11	1

E) Melanoma death by Largest ultrasound diameter, mm

Death from melanoma p = 0.012

Death from other causes p = 0.124

Cumulative incidence

Years

Death melanoma — Death other causes — <12 — 12-15 — >15
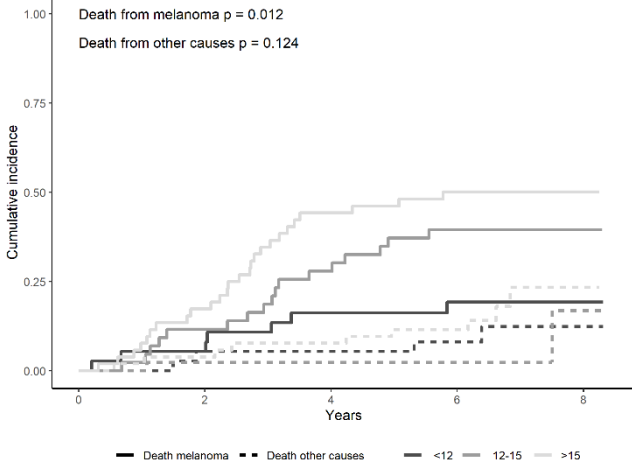
Number at risk

37	33	29	22	3
43	37	30	20	3
52	41	25	15	1

F) Melanoma death by extraocular extension

Death from melanoma p = 0.07

Death from other causes p = 0.219

Cumulative incidence

Years

Absent — Present — Death melanoma — Death other causes
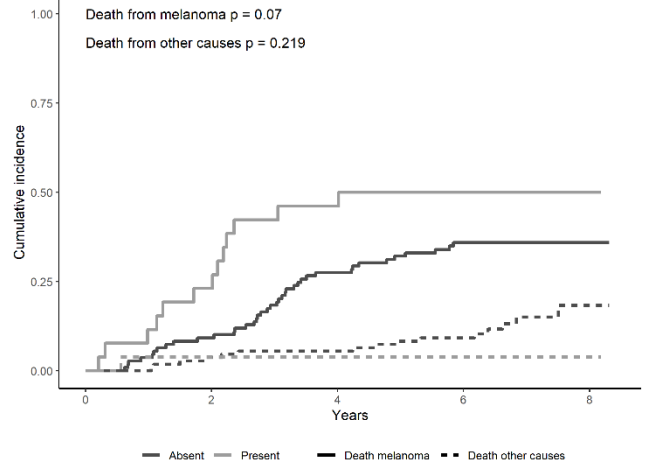
Number at risk

109	95	73	49	6
26	19	13	8	1

G) Melanoma death by cb

Death from melanoma p = 0.014

Death from other causes p = 0.355

Cumulative incidence

Years

Absent — Present — Death melanoma — Death other causes
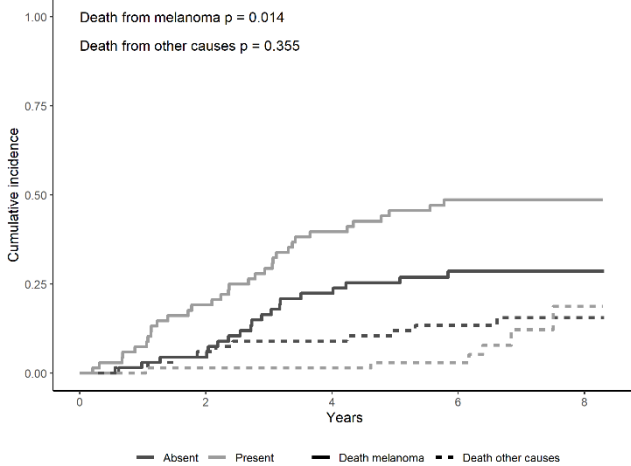
Number at risk

67	60	46	34	5
68	54	40	23	2

H) Melanoma death by epitheliod cells

Death from melanoma p = 0.155

Death from other causes p = 0.613

Cumulative incidence

Years

Death melanoma — Death other causes — Absent — Present
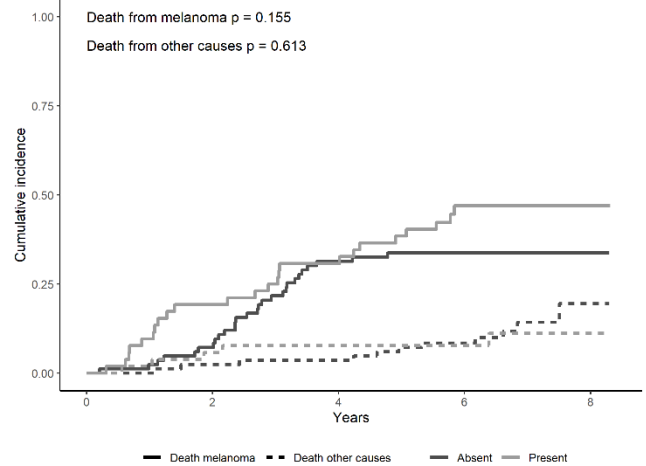
Number at risk

83	75	54	40	4
52	39	32	17	3
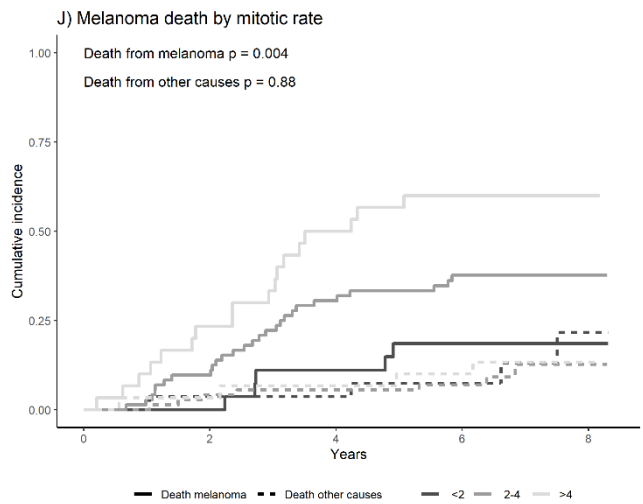
I) Melanoma death by loops

Death from melanoma p = 0.138

Death from other causes p = 0.903

J) Melanoma death by mitotic rate

Death from melanoma p = 0.004

Death from other causes p = 0.88

K) Melanoma death by Tumour thickness, mm

Death from melanoma p = 0.963

Death from other causes p = 0.498
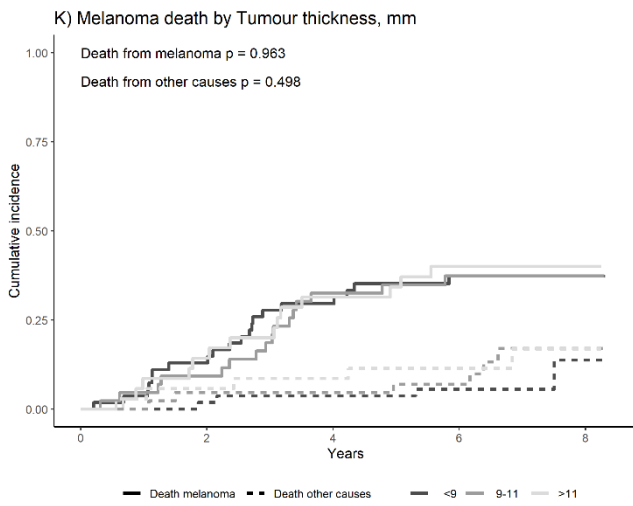
Abbreviations: M3, monosomy 3; cb, ciliary body involvement; loops, closed vascular loops.
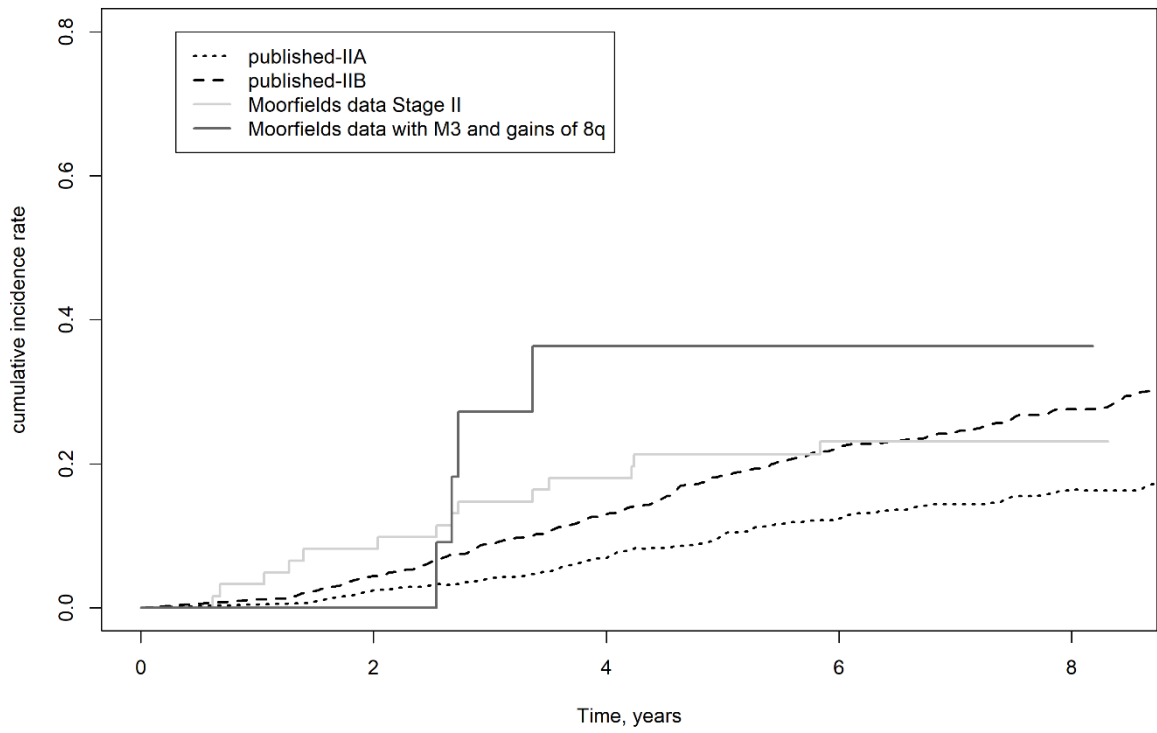
Figure 2A-B Cumulative incidence curves for AJCC stage II (figure 2a) and III (figure 2b) subjects. Plotted on the same graphs are curves for stage II and III patients with monosomy 3 and 8q gain and the curves from the European Ophthalmic Oncology group's 2013 study that formed the basis for the most recent AJCC staging criteria[6].
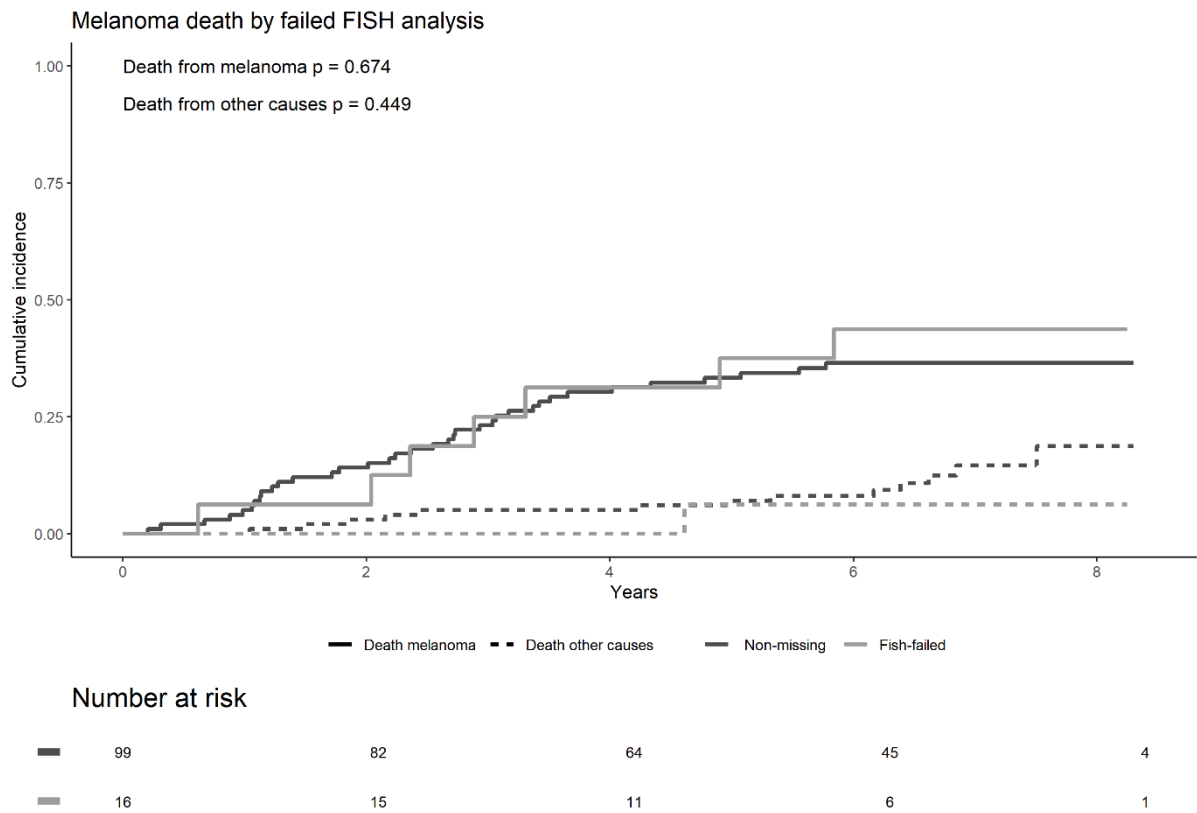
A)
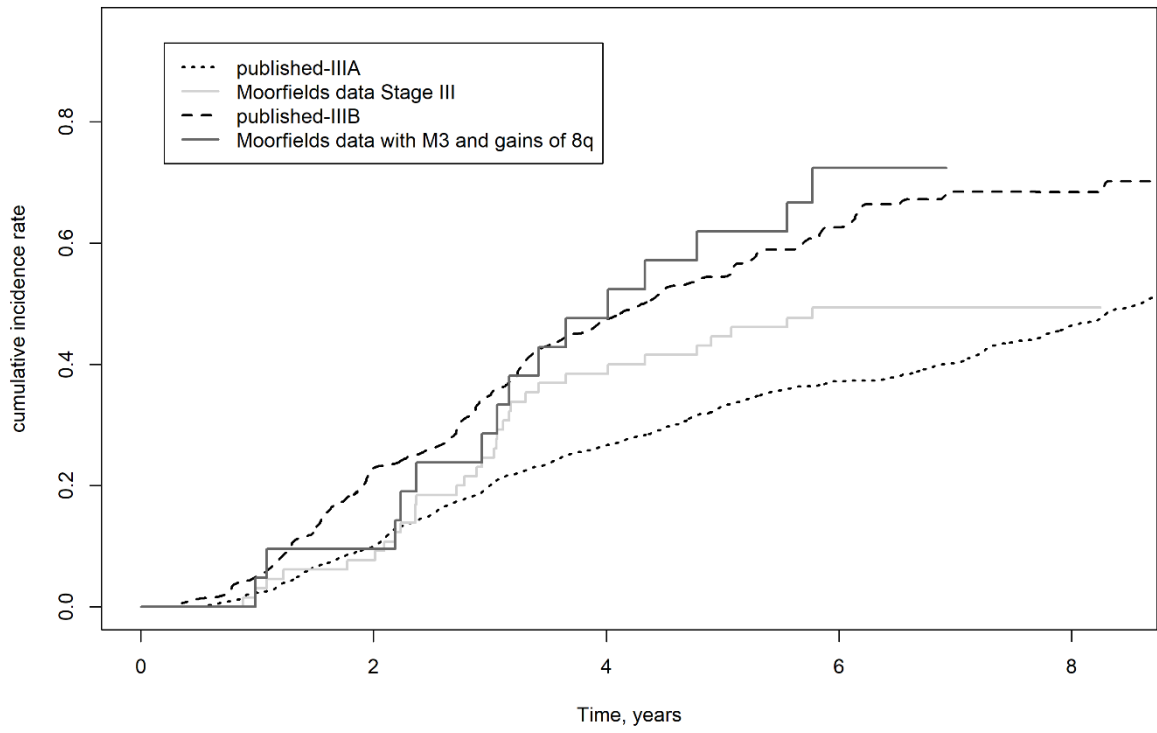
B)

Figure 3 Cumulative incidence curves for those whose FISH failed due to insufficient biopsy sample compared to those whose FISH was successful in producing a result.

Melanoma death by failed FISH analysis

Death from melanoma p = 0.674

Death from other causes p = 0.449

Supplement

Table S1. Observed COD vs Missing COD

	Observed COD	Missing COD	P-value
Patient Characteristic n(%) or mean(SD) and/or Median(IQR)	Dead (n=68); Melanoma=52, other=16	Dead(n=20)	
Male , n(%)	36(52.9%)	11(55%)	1
Mean age (SD)	71(13.9)	71.6(12.3)	0.858
largest basal diameter, unit mean(SD)	16.3(4.9)	13.9(4.5)	0.070
tumour thickness, unit mean(SD)	9.7(3.7)	10.4(3.2)	0.489
AJCC stages, n(%) I IIA IIB IIIA IIIB IIIC IV	0(0%) 9(13.2%) 14(20.6%) 24(35.3%) 15(22.1%) 0% 6(8.8%)	0(0%) 2(10%) 5(25%) 11(55%) 2(10%) 0(0%) 0(0%)	-a
Monosomy 3, n(%) Absent Present	18(36.7%) 31(63.3%)	3(27.3%) 8(72.7%)	-a
Chromosome 8gain, n(%) Absent Present	10(19.2%) 42(80.8%)	2(18.2%) 9(81.8%)	-a
Extraocular extension Absent Present	54(79.4%) 14(20.6%)	16(80%) 4(20%)	-a
Cb Absent Present	29(41.6%) 39(57.4%)	11(55%) 9(45%)	0.472
Epi Absent Present	39(57.4%) 29(42.6%)	18(90%) 2(10%)	*0.016*

Loops **Absent** **Present**	31(50%) 31(50%)	7(36.8%) 12(63.2%)	0.458
Mitosis, **mean(SD)** **Median(IQR)**	4.1(3.5)	3.2(1.6)	0.693
follow-up time **mean (SD)** **median (IQR)**	3(1.8)	3.3(2.2)	0.640

Abbreviations: COD, cause-of-death

[a] Due to small sample size (expected cell count <5) no p-value was computed and fishers exact test was not performed as they tend to be overly conservative

Chi-squared test for categorical variables and Kruskal Wallis for continuous

Statistically significant p-values (p<0.05) have been *italicised*, there was one statistically significant p-value in epithelioid cells, proportion excluded contained 10% epi cells whereas observed COD contained 42.6%

Table S2. Missing vs non-missing cases in 8gain, monosomy 3 and largest basal diameter (final sample included in multivariable analysis)

Patient Characteristic n(%) or mean(SD) or mean(SD) and Median(IQR) and range	Complete cases (n=98)	Missing cases (n=37)	P-value	Non-missing 8gain (n=104)	Missing 8gain (n=31)	p-value	Non-missing mono3 (n=100)	Missing mono3 (n=35)	p-value	Non-missing LBD (n=132)	Missing LBD (n=3)
Male , n(%)	61(62.2%)	18(48.6%)	0.217	64 (61.5%)	15 (48.4%)	0.273	62 (62%)	17(48.6%)	0.235	78(59.1%)	1(33.3%)
Mean age (SD)	65.6(14.3)	66.8(13.9)	0.544	65.1(14.7)	68.6(11.7)	0.332	65.8(14.2)	66.2 (14.1)	0.332	65.7(14.2)	3 observations: 64.73, 78.93, 84.39
Mean largest basal diameter, unit (SD)	14.7(4.8)	14.5(4.1)	0.739	14.9(4.8)	13.8(3.9)	0.910	14.7(4.8)	14.4 (4.1)	0.910	-	-
Mean tumour thickness, unit (SD)	9.3(3.5)	9.9(3.4)	0.353	9.34(3.5)	9.7 (3.5)	0.395	9.3 (3.5)	9.9 (3.4)	0.395	9.5(3.4)	-[b]

AJCC stages, n(%) I IIA IIB IIIA IIIB IIIC IV	1(1%) 17(17.3%) 30(30.6%) 28(28.6%) 14(14.3%) 1(1%) 7(7.1%)	0(0%) 4(10.8%) 10(27.0%) 16(43.2%) 7(18.9%) 0 0	-[a]	1(1.0%) 17(16.4%) 30(28.8%) 31(29.8%) 17(16.4%) 1(1.0%) 7(6.7%)	0(0%) 4(12.9%) 10(32.3%) 13(41.9%) 4(12.9%) 0(0%) 0(0%)	-[a]	1(1.0%) 17(17%) 30(30%) 29(29%) 15(15%) 1(1%) 7(7%)	0(0%) 4(11.4%) 10(28.6%) 15(42.9%) 6(17.1%) 0(0%) 0(0%)	-[a]	1(0.8%) 20(15.2%) 39(29.5%) 43(32.6%) 21(15.9%) 1(0.8%) 7(5.3%)	0 1(33.3%) 1(33.3%) 1(33.3%) 0 0 0
AJCC stage II III	47(52.2%) 43(47.8%)	14(37.8%) 23 (62.2%)	0.201	47(49.0%) 49(51.0%)	14(45.2%) 17(54.8%)	0.872	47(51.1%) 45(48.9%)	14(40%) 21(60%)	0.358	59(47.6%) 65(52.4%)	2(66.7%) 1(33.3%)
Monosomy 3, n(%) Absent Present	51(52.0%) 47(48.0%)	1(50%) 1(50%)	-[a]	51(51.5%) 48(48.5%)	1(100%) 0(0%)	-[a]	- 	- 	-	52(52.5%) 47(47.5%)	0 1(100%)
Chromosome 8gain, n(%) Absent Present	37(37.8%) 61(62.2%)	0 6(100%)	-[a]	- -	- -	-	37(37.4%) 62(62.6%)	0(0%) 5(100%)	-[a]	37(35.9%) 66(64.1%)	0 1(100%)
Extraocular extension Absent Present	82(83.7%) 16(16.3%)	27(73.0%) 10(27.0%)	0.245	86(82.7%) 18(17.3%)	23(74.2%) 8(25.8%)	0.306	83(83%) 17(17%)	26(74.3%) 9 (25.7%)	0.319	107(81.1%) 25(18.9%)	2(66.7%) 1(33.3%)
Cb Absent Present	49(50%) 49(50%)	18(48.6%) 19(51.3%)	1	51(49.0%) 53(51.0%)	16(51.6%) 15(48.4%)	0.840	50(50%) 50(50%)	17(48.6%) 18(51.4%)	1	66(50.0%)	1(33.3%) 2(66.67%)

										66(50.0%)	
Epithelioid cells Absent Present	63(64.3%) 35(35.7%)	20(54.0%) 17(46.0%)	0.373	68(65.4%) 36(34.6%)	15(48.4%) 16(51.6%)	0.097	65(65.0%) 35(35.0%)	18(51.4%) 17(48.6%)	0.164	81(61.4%) 51(38.6%)	2(66.7%) 1(33.3%)
Loops Absent Present	51(58.0%) 37(42.0%)	14(43.8%) 18(56.2%)	0.241	52(55.9%) 41(44.1%)	13(48.2%) 14(51.9%)	0.516	51(56.7%) 39(43.3%)	14(46.7%) 16(53.3%)	0.4	65(55.1%) 53(44.9%)	0(0%) 2(100%)
Mitosis, mean(SD), median(IQR), range	3.5(3.1); 3(2); 0-20	3.6(3);3(2);0-12	0.750	3.5(3.0);3(2);0-20	3.6(3.3);3(2.2), 0-12	0.947	3.4(3.1)	3.7(3.0)	0.659	3.5(3.1)	2 observations: 4, 5
follow-up time mean (SD); median (IQR); range	5(2.4); 5.8(4); 0.2-8.3	4.7(2.4);4.9(3.8);0.6-8.3	0.498	4.9(2.4); 5.8(4); 0.2-8.3	4.8(2.5); 5.6(3.8); 0.6-8.3	0.849	4.9(2.4); 5.8(4); 0.2-8.3	4.7(2.4); 4.9(3.8); 0.6-8.3	0.572	4.9(2.4) 5.8(4); 0.2-8.3	3 observations: 2.19, 4.24,4.61

Abbreviations: Cb, Ciliary body involvement

Due to small sample size in those missing LBD, a formal statistical test comparing complete and missing cases were not carried out.

[a] No statistical test was performed due to small sample size (expected cell count <5), as fishers exact test can be overly conservative, we did not report these either

[b] No observations due to small sample size

Chi-squared test for categorical variables or Kruskal Wallis for continuous variables

1 Table S3. Observed cumulative incidence rates (variance) at 5 years for melanoma related
2 death, by stage, LBD and chromosome status

|		8q gain		M3		Chromosomes (8q gain and M3)	
		Absence (n=37)	Presence (n=67)	Absence (n=52)	Presence (n=48)	Absence (n=61)	Both (n=39)
AJCC stage	**II (n=69)**[a]	0.05(0.002);n=21	0.27(0.01);n=26	0.13(0.004);n=31	0.25(0.01);n=16	0.11(0.003);n=36	0.36(0.02); n=11
	III (n=74)[b]	0.20(0.01);n=15	0.56(0.01);n=34	0.30(0.01);n=20	0.52(0.01);n=25	0.25(0.01);n=24	0.62(0.01); n=21
LBD, mm (n=98)	**<12 (n=37)**[c]	0.00(0.00);n=13	0.24(0.01);n=17	0.06(0.003);n=18	0.25(0.02);n=12	0.05(0.002);n=22	0.38(0.03); n=8
	12-15.99 (n=43)[d]	0.00(0.00);n=13	0.58(0.01);n=19	0.19(0.01);n=16	0.53(0.02);n=15	0.16(0.001);n=19	0.67(0.02); n=12
	>15.99 (n=52)[e]	0.36(0.02);n=11	0.53(0.01);n=30	0.33(0.01);n=18	0.55(0.01);n=20	0.30(0.01);n=20	0.61(0.01); n=18

3 Abbreviations: LBD, largest basal diameter.
4 [a]22 missing chromosome 8 and chromosome 3
5 [b]25 missing chromosome 8 and 29 missing chromosome 3
6 [c]7 missing chromosome 8 and chromosome 3
7 [d]11 missing chromosome 8 and 12 missing chromosome 3
8 [e]11missing chromosome 8 and 14 missing chromosome 3
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30 Figures S1-S4 demonstrate some non-constant curvature in the residual plots for age, gender,
31 ciliary body involvement and presence of both chromosomes, these variables were
32 subsequently modelled with a time interaction at the univariate level, this did not yield an
33 effect that varied with time (p>0.05). Variables that passed the stay criterion after applying a
34 backward selection procedure included both largest basal diameter (p=0.02) and the presence
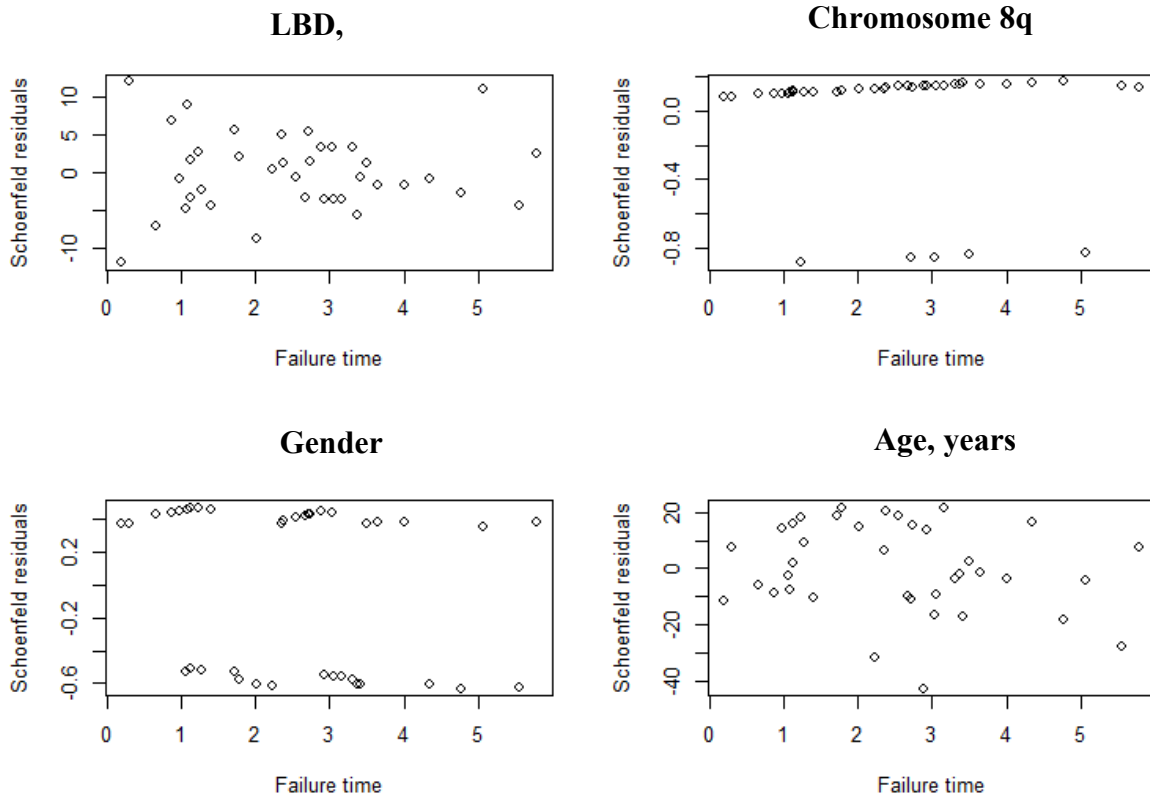35 of both chromosomes (p<0.001).
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38 Figure S1. Residual diagnostic plots for Fine-Gray models at univariate level
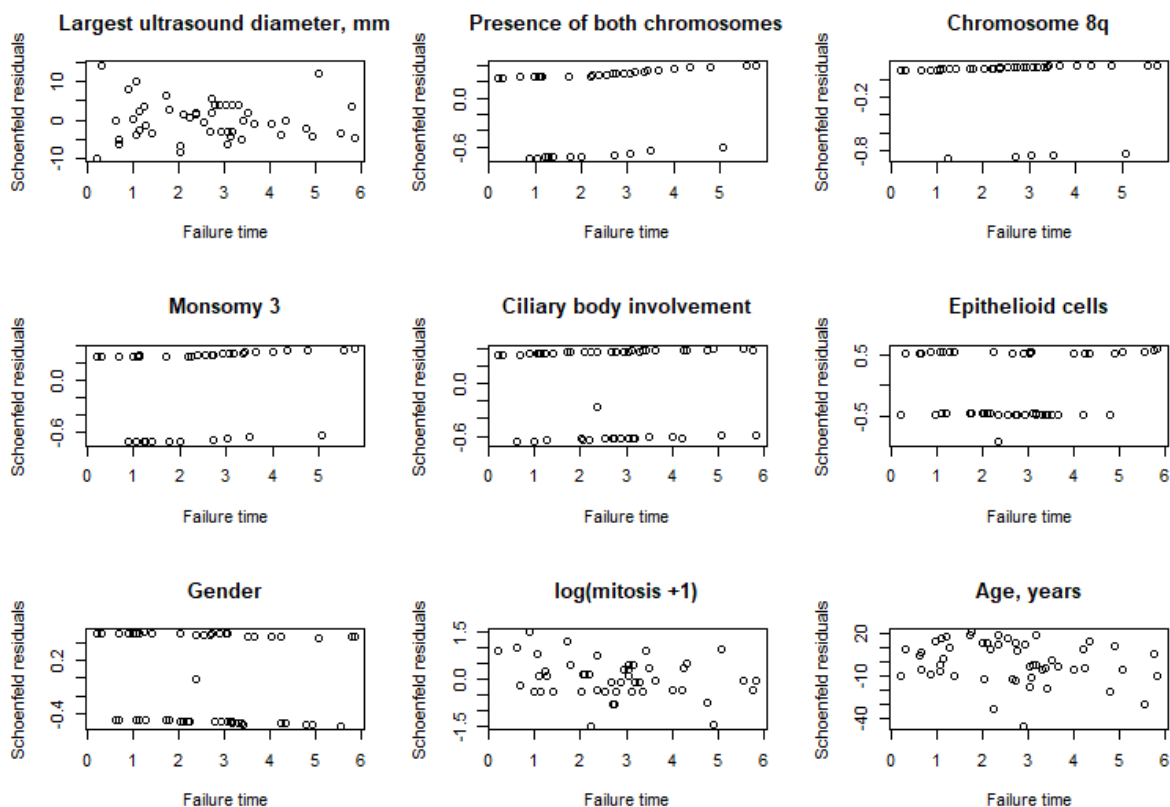
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57   Figure S2. Residual diagnostic plots for Fine-Gray model from multivariable analysis
58   +presence of chromosome 8q
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LBD,

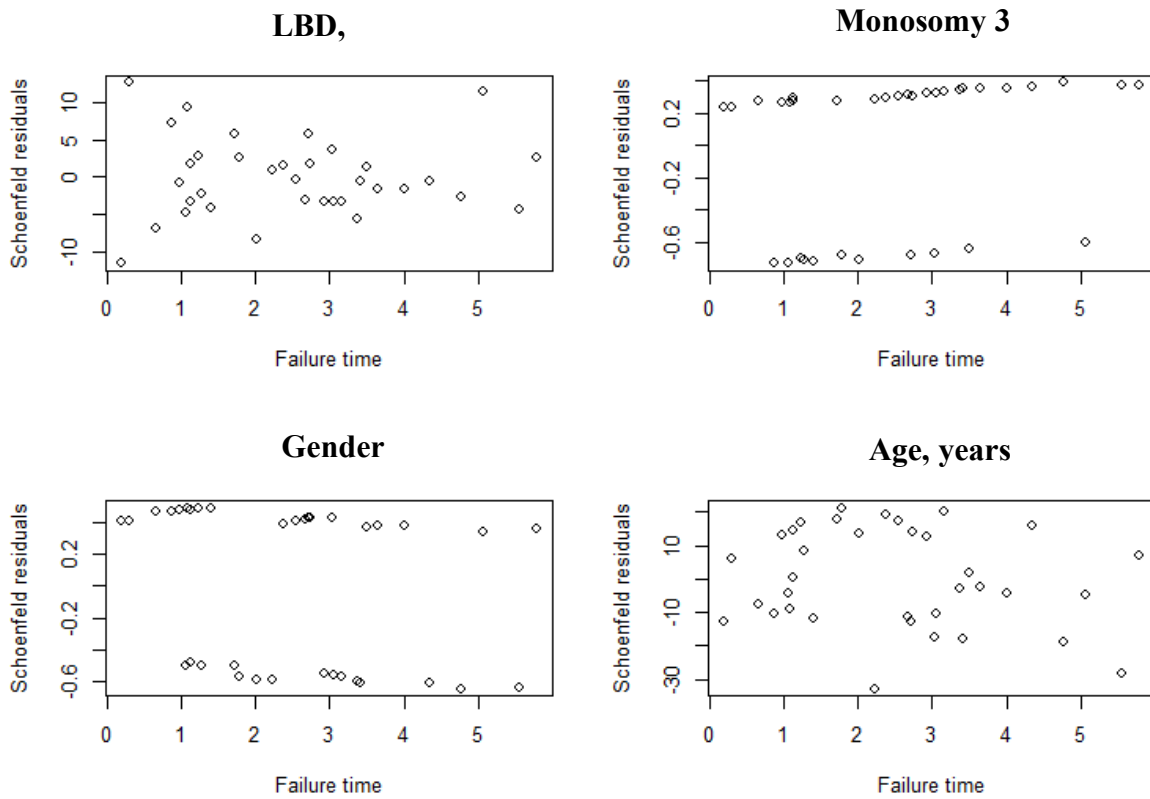

Chromosome 8q

Gender

Age, years

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84 Figure S3. Residual diagnostic plots for Fine-Gray model from multivariable analysis
85 +presence of M3

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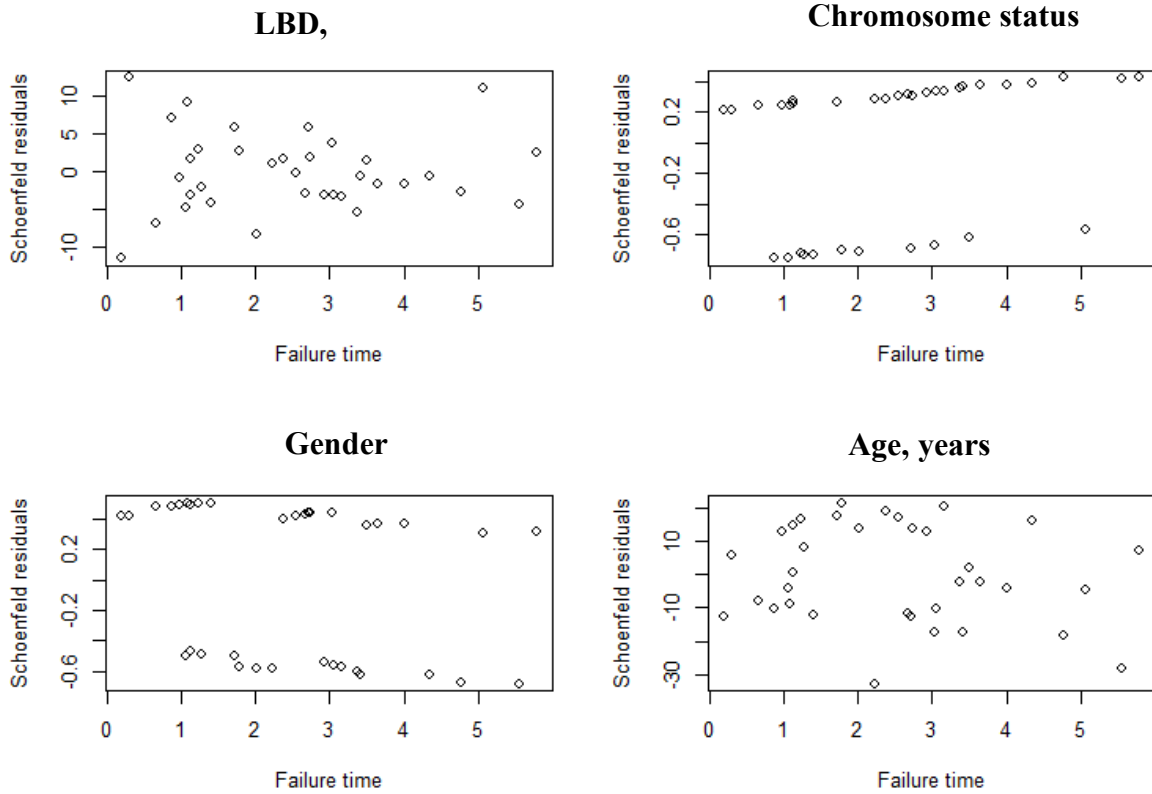91
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Figure S4. Residual diagnostic plots for Fine-Gray model from multivariable analysis
+presence of both chromosomes

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139 Table S4. Univariate analysis using the Fine-Gray model

Characteristic	Melanoma death			Other death		
	HR	95% CI	P value	HR	95% CI	P value
Male	0.66	0.39-1.14	0.14	1.19	0.44-3.24	0.73
Age, years	1.03	1.01-1.06	*0.011*	1.06	1.02-1.1	*0.002*
LBD, mm	1.13	1.06-1.2	*<0.001*	1.06	0.97-1.15	0.21
tumour thickness, mm	1.01	0.91-1.11	0.9	1.05	0.96-1.14	0.27
AJCC stages II III	2.43	1.29-4.58	*0.006*	0.73	0.27-1.97	0.53
Monosomy 3, n(%) Absent Present	Ref 2.92	- 1.43-5.97	- *0.003*	Ref 0.97	- 0.33-2.85	- 0.96
Chromosome 8gain Absent Present	Ref 4.95	- 1.97-12.4	- *<0.001*	Ref 0.83	- 0.28-2.49	- 0.74
Chromosome status **Neither or one** **Both**	Ref 4.73	- 2.31-9.71	- *<0.001*	Ref 0.69	- 0.22-2.2	- 0.53
Extraocular extension Absent Present	Ref 1.81	- 0.93-3.55	- 0.082	Ref 0.29	- 0.04-2.24	- 0.23
Ciliary body involvement Absent Present	Ref 2.01	- 1.15-3.51	- *0.014*	Ref 0.59	- 0.22-1.63	- 0.31
Epithelioid cells Absent Present	Ref 1.48	- 0.86-2.55	- 0.15	Ref 0.75	- 0.26-2.18	- 0.6
Loops Absent Present	Ref 1.53	- 0.88-2.66	- 0.14	Ref 0.84	- 0.28-2.47	- 0.75
Mitosis (log)	2.22	1.29-3.79	*0.004*	1.14	0.51-2.55	0.751

140 Univariate analysis on available cases as opposed to complete-case analysis to preserve sample size
141 and enhance statistical power. Statistically significant p-values(p<0.05) have been *italicized*
142 Mitosis was $\log_e$-transformed, adding 1 prior to transformation, as $\log_e(0)$ is undefined
143 Abbreviations; LBD, largest basal diameter; Loops, closed vascular loops;
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151 Table S5. Univariate analysis using the Cause-specific hazards model

Characteristic	Melanoma death			Other death		
	HR	95% CI	P value	HR	95% CI	P value
Male	0.67	0.39-1.16	0.156	1.04	0.38-2.87	0.939
Age, years	1.04	1.02-1.06	*<0.001*	1.10	1.04-1.16	*<0.001*
LBD, mm	1.14	1.07-1.21	*<0.001*	1.14	1.01-1.29	0.0296
tumour thickness, mm	1.01	0.93-1.10	0.82	1.06	0.91-1.23	0.475
AJCC stages II III	2.31	1.23-4.33	*0.009*	0.93	0.34-2.53	0.892
Monosomy 3, n(%) Absent Present	Ref 3.04	- 1.49-6.18	- *0.002*	Ref 1.45	- 0.48-4.38	- 0.508
Chromosome 8 gain Absent Present	Ref 4.97	- 1.94-12.71	- *<0.001*	Ref 1.28	- 0.42-3.95	- 0.665
Chromosome status **Neither or one** **Both**	Ref 4.85	- 2.38-9.90	- *<0.001*	Ref 1.24	- 0.37-4.09	- .728
Extraocular extension Absent Present	Ref 1.83	- 0.97-3.42	- 0.0605	Ref 0.38	- 0.05-2.89	- 0.35
Ciliary body involvement Absent Present	Ref 1.88	- 1.07-3.3	- *0.0289*	Ref 0.77	- 0.28-2.13	- 0.614
Epithelioid cells Absent Present	Ref 1.55	- 0.90-2.68	- 0.113	Ref 0.88	- 0.30-2.54	- 0.81
Loops Absent Present	Ref 1.62	- 0.92-2.85	- 0.0917	Ref 1.09	- 0.35-3.38	- 0.886
Mitosis (log)	2.22	1.35-3.64	*0.002*	1.14	0.51-2.55	0.751

152 Univariate analysis on available cases as opposed to complete-case analysis to preserve sample size
153 and enhance statistical power. Statistically significant p-values(p<0.05) have been *italicized*
154 Mitosis was $\log_e$-transformed, adding 1 prior to transformation, as $\log_e(0)$ is undefined
155 Chromosome status defined as presence of both chromosomes in any one individual vs absence of
156 both.
157 Abbreviations; LBD, largest basal diameter;;

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161   Table S6. Multivariable analysis (melanoma-related death)

Characteristic (n=99; events=35)	Fine-Gray			Cause-specific model		
	SHR	95% CI	P value	HR	95% CI	P value
Male	0.53	0.27-1.01	0.055	0.57	0.28-1.15	0.12
Age, years	1.02	1.001-1.05	*0.043*	1.02	0.997-1.05	0.10
LBD, mm	1.14	1.05-1.24	*0.003*	1.15	1.06-1.25	*<0.001*
Chromosome status Neither/One Both	 Ref 3.90	 - 1.86-8.18	 - *<0.001*	 Ref 4.17	 - 2.00-8.70	 - *<0.001*

162   Abbreviations: LBD, Largest basal diameter;
163   Statistically significant p-values have been *italicised*
164
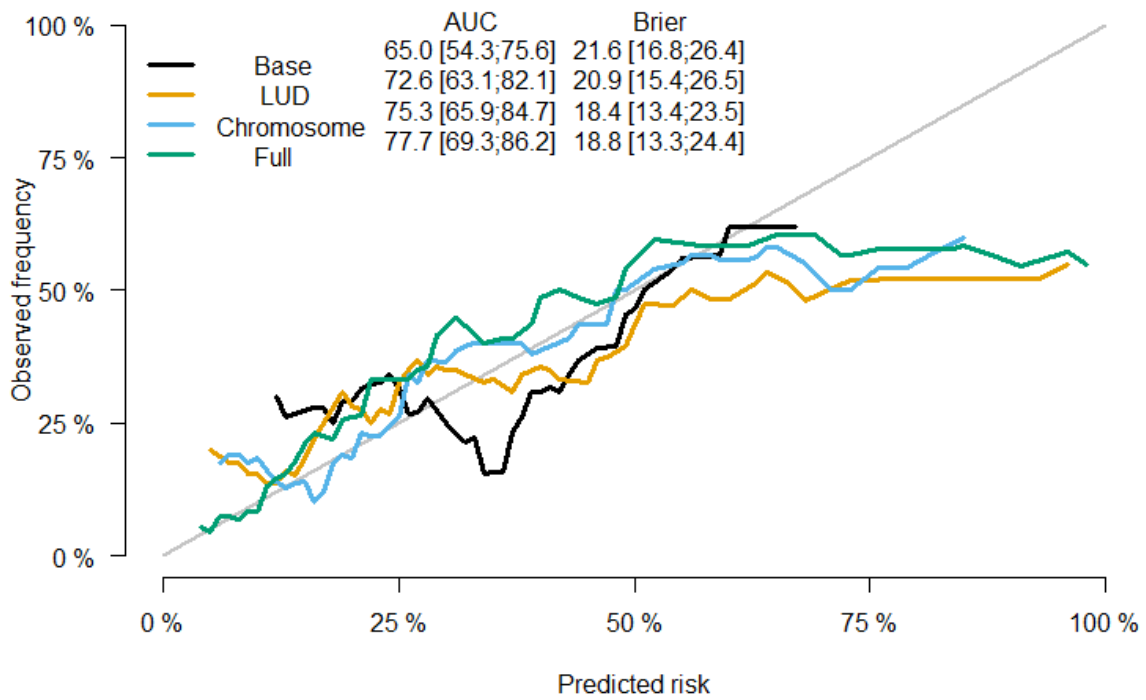
Calibration curves for the Fine-Gray models as per table 4 for Base, +LBD, +Chromosome status and full models.

The Base model includes; age and gender, +LBD model includes; age, gender and LBD, +Chromosome status model includes; age, gender and chromosome status and full model includes; age, gender, chromosome status and largest basal diameter.

Model validation was performed via leave-one-out bootstrap, as per R package *riskRegression*.

AUC and Brier scores point estimates provided as time-dependent measures with their 95% confidence intervals.

176 Table S7. Effect of adding LBD after adjusting for chromosomes and vice versa- competing
177 risk regression analysis based on Fine and Gray model (5-year follow-up)
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Characteristic (n=99; event=35)	Melanoma death		Brier score(95% CI); Bootstrap adjusted (95% CI)	AIC
	C-statistic			
	Apparent; Bootstrap adjusted (95% CI)	AUC $_t$ ROC (95% CI); Bootstrap adjusted (95% CI)		
Base model	63.3; 60.6(56.0,75.3)	65.2(52.9,77.5); 65.0(54.3,75.6)	19.9(15.3,24.4); 21.6(16.8,26.4)	306.9
+LBD	70.1; 66.9(62.4,80.8)	72.5(62.0,83.1); 72.6(63.1,82.1)	18.8(13.7,24.0); 20.9(15.4,26.5)	292.97
+Chromosome status	72.4; 71.2(66.7,84.5)	78.1(67.9,88.3); 75.3(65.9,84.7)	16.3(11.6,21.0); 18.4(13.4,23.5)	289.45
+Chromosome status +LBD	75.8; 72.6(68.6,84.8)	81.9(73.4,90.3); 77.7(69.3,86.2)	16.3(11.3,21.3); 18.8(13.4,24.4)	281.37

180 Base model includes Age and gender. Number of bootstrap samples: 1000, bootstrap sample size: 99
181 for C-statistic
182 Chromosome status defined as; neither chromosome/one chromosome vs two chromosomes
183 Time dependent-ROC curves (95% CI) and leave-one-out bootstrap ROCs based on Blanche et al[17],
184 correlates predictions with binary status at time t, whereas non time-dependent c-statistics presented
185 correlate predictions with actual event times
186 Brier score combines discrimination and calibration - it is defined as the expected square prediction
187 error or distance between observed and predicted probabilities. Smaller values indicate higher
188 predictive accuracy.
189 For AIC, smaller values indicate better model fit
190 Abbreviations: AUC_t, time-dependent ROC; LBD, largest basal diameter; AIC- Akaike Information
191 Criterion, BIC- Bayesian information Criterion, C-Statistic; Concordance-Statistic
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