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# 1 **Molecular characterization of fast-growing melanomas**

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60 **Mendeley Link:** <https://data.mendeley.com/datasets/ysd3vr9yr8/1>

61

62 **IRB statement:** This non-interventional study followed French regulations. All  
63 patients gave written informed consent.

64

65 **Keywords:** Melanoma, fast growth melanoma, mutations of poor prognosis, FGFR2  
66 mutations

67

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69 October 2020, Vilnius, Lithuania

70

71 **Abstract**

72 Background: The rate of growth of primary melanoma is a robust predictor of  
73 aggressiveness, but the mutational profile of fast-growing melanomas (FGMM), and  
74 its potential to stratify patients at high risk of death, has not been comprehensively  
75 studied.

76 Objective: To investigate the epidemiological, clinical and mutational profile of primary  
77 cutaneous melanomas with a thickness  $\geq 1$  mm, stratified by rate of growth (ROG).

78 Methods: Observational prospective study. Deep-targeted sequencing of 40  
79 melanoma driver genes on formalin fixed, paraffin embedded primary melanoma  
80 samples. Comparison of FGMM (ROG  $>0.5$  mm/month) and non-FGMM (ROG  $\leq 0.5$   
81 mm/month).

82 Results: Two hundred patients were enrolled among which 70 were FGMM. The  
83 relapse free survival was lower in the FGMM group ( $p=0.014$ ). FGMM had a higher  
84 number of predicted deleterious mutations within the 40 genes than non-FGMM  
85 ( $p=0.033$ ). Ulceration ( $p=0.032$ ), thickness ( $p=0.006$ ), lower sun exposure ( $p=0.049$ ),  
86 and FGFR2 mutations ( $p=0.037$ ) were significantly associated with fast growth.

87 Limitations: Single-center study, cohort size, potential memory bias, number of  
88 investigated genes.

89 Conclusion: Fast growth is linked to specific tumor biology and environmental factors.  
90 Ulceration, thickness and FGFR2 mutations associate with fast growth. Screening for  
91 FGFR2 mutations might provide an additional tool to better identify FGMM which are  
92 probably good candidates for adjuvant therapies.

93

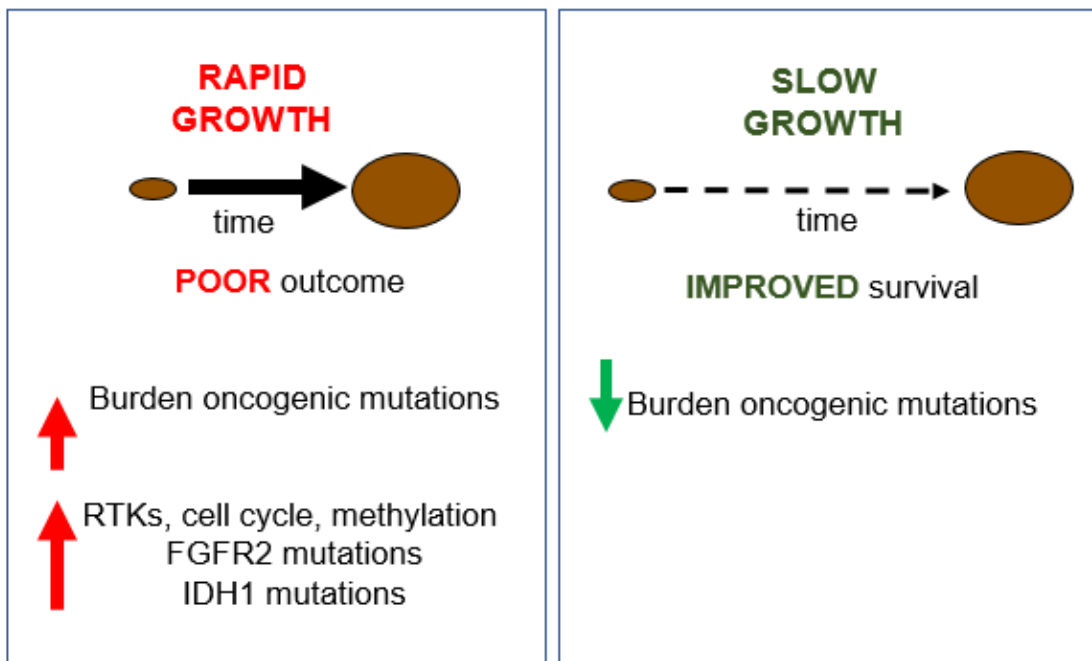
94 **Capsule summary**

- 95
- Fast growing melanomas are aggressive and linked to early death. Rapid
- 96 growth is associated to thicker, ulcerated tumors with FGFR2 mutations; and
- 97 is more frequent in patients with less accumulated sun exposure.
- Ulceration, thickness and FGFR2 mutations are biomarkers for aggressive
- 98 disease and can stratify patients for adjuvant therapy.
- 99

100

101 **Graphical abstract**

102



103

104

## 105 **Introduction**

106       The incidence of cutaneous melanoma continues to rise worldwide<sup>1-4</sup>.  
107       Prevention campaigns promoting early diagnosis underpin an epidemiological shift  
108       towards earlier stage disease<sup>5-10</sup> and the majority of new melanoma cases are  
109       diagnosed at localized stages<sup>8,11,12</sup>. Stage II tumors are considered low risk<sup>13</sup>,  
110       however, the prediction is that, due to the large prevalence of stage II, they will  
111       account for most deaths in the future<sup>8,11,12</sup>.

112       Targeted and immunotherapies have transformed the care for metastatic  
113       melanomas, and adjuvant trials have demonstrated a high efficacy. New trials are  
114       testing anti-PD1 in AJCC IIB/IIC melanomas, expanding the pool of potential  
115       candidates for adjuvant immunotherapy. Identification of new biomarkers of  
116       aggressiveness is paramount to optimize risk-ratio toxicity and ensure optimal  
117       resource allocation.

118       Primary melanoma growth, defined as the ratio of tumor thickness to patient-  
119       reported time of melanoma growth<sup>14</sup>, is a validated robust, reproducible and  
120       independent prognostic factor of outcome<sup>14-17</sup>. Patients with fast-growing melanoma  
121       (FGMM) have aggressive disease that spreads early, leading to shorter survival<sup>14-17</sup>.  
122       Fast growth is associated to nodular subtype, trunk location, male sex, previous non-  
123       melanoma skin cancer, and few childhood sunburns<sup>15-17</sup>. Furthermore, FGMM display  
124       high mitotic rates<sup>15-17</sup>, and frequent NRAS<sup>18</sup> and TERT promoter mutations<sup>19</sup>,  
125       highlighting growth kinetics is a relevant feature that accurately represents melanoma  
126       aggressiveness. The mutational profile of FGMM has not been comprehensively  
127       studied, so we compared the epidemiological, clinical and mutational profile of a  
128       cohort of FGMM and non-FGMM primary cutaneous melanomas.

129

**130 Methods****131 Clinical data**

132 We enrolled prospectively consecutive primary melanoma patients with a  
133 thickness  $\geq 1$ mm referred to the dermatology unit of La Timone Hospital,  
134 Marseilles, France, between February 2012 and October 2014. We collected  
135 epidemiological and clinical characteristics (Mendeley supplemental methods). We  
136 calculated rate of growth (ROG) as the ratio between thickness and time taken to  
137 melanoma development<sup>14</sup>. FGMM were defined by  $ROG > 0.5$ mm/month and non-  
138 FGMM by  $ROG \leq 0.5$  mm/month<sup>15-17</sup>. Patients filled out a standardized questionnaire  
139 to estimate sun exposure (Mendeley supplementary methods). We derived two sun  
140 exposure scores to estimate lifelong sun exposure and lifelong sunburn.

141

**142 Molecular analyses**

143 Tumor DNA was extracted from hand-macrodissected FFPE melanoma  
144 specimens, and normal DNA from patient-matched peritumoural normal skin or wide  
145 local excision. DNA was extracted using the GeneRead DNA FFPE kits (Qiagen,  
146 Hilden, Germany) and DNA integrity assessed using the NGS FFPE QC Kit (Agilent  
147 Technologies). Histopathological variables were extracted from routine reports.

148 We performed deep-targeted sequencing of 40 melanoma genes, selected by  
149 frequency of mutation in the TCGA database ( $>7\%$ ) and/or specific cancer genes of  
150 interest (Mendeley supplementary Table 1). TERT promoter was not analyzed in our  
151 panel. Three different pipelines were used for variant filtering validation and manual  
152 inspection of the “non-consensual” variants was performed on IGV to avoid false  
153 positive calls (Mendeley supplementary methods). Only SNVs predicted as  
154 pathogenic or likely pathogenic by the COSMIC<sup>22</sup> or Varsome<sup>23</sup> database were

155 considered in the final analysis.

156

### 157 **Gene expression analysis**

158 Cutaneous primary and metastatic melanomas (SKCM) from The Cancer Genome  
159 Atlas<sup>24</sup> were divided into *FGFR2* wildtype (WT) and *FGFR2* mutants based on the  
160 presence of a deleterious mutation (missense, frame shift insertion/deletion,  
161 nonsense mutation) in *FGFR2*. Thirty five cases with a missense mutation were  
162 present with matched clinical and gene expression data, and 437 WT cases. For  
163 each sample, the G1/S and G2/M gene expression signature scores were determined  
164 by calculating the geometric mean of all genes in each signature. G1/S and G2/M  
165 specific genes were those used in Tirosh *et al.*<sup>25</sup> to measure proliferating cells.

166 Somatic mutation and RNA-seq (log<sub>2</sub> transformed RSEM) data were downloaded  
167 from UCSC Xena TCGA database (<https://xenabrowser.net/datapages/>) and  
168 statistical analysis was performed in GraphPad Prism v8.2.0. Mann-Whitney test was  
169 used to compare expression and scores between *FGFR2* mutants and WT samples.

### 170 **Statistical analysis**

171 Continuous variables were expressed as means +/- SD or as median with  
172 range and categorical variables as count and percentages. Means were compared by  
173 student t-test, and percentages by Chi-Square test (or Fisher's exact test, as  
174 appropriate). Univariate and stepwise forward multivariate logistic regression models  
175 were used to identify factors associated with FGMM. Variables with a p<.05 in  
176 univariate analysis were included in multivariate analyses. Recurrence free survival  
177 (RFS), distant metastasis free survival (DMFS) and melanoma specific survival  
178 (MSS) were calculated from the melanoma diagnosis. RFS, DMFS and MSS curves  
179 were estimated by the Kaplan–Meier method and the log-rank test. All tests were



180 two-sided, and statistical significance was defined as  $p < .05$ . The false discovery rate  
181 was controlled with a Benjamini-Hochberg procedure<sup>26</sup>. Statistical analyses were  
182 performed using IBM SPSS Statistics version 20 (IBM SPSS Inc., Chicago, IL, USA).  
183 Random forest algorithm, bootstrap and multilayer neural perceptron analyses were  
184 used to estimate the robustness of the results by using the randomForest and caret  
185 packages of R.

186

187 **Results**

188

189 **Clinical and epidemiological variables associated with fast growth**

190 Three hundred and fifty-three patients were referred to our institution during the study  
191 period. ROG calculation, DNA extraction and molecular analyses were successfully  
192 performed for 200 patients (Mendeley Supplementary Figure 1). We compared the  
193 clinical, epidemiological and genetic mutations of FGMM (n=70, ROG >0.5  
194 mm/month) to the features of non-FGMM (n=130, ROG ≤ 0.5/month). The cohort  
195 included 112 men, and the median age at diagnosis was 62 years. Most melanomas  
196 were located on the trunk (84%) and lower limbs (30.5%), and the most common  
197 histological subtype was superficial spreading melanoma (SSM) (63.5%) followed by  
198 nodular melanoma (NM) (29.5%). The median Breslow thickness was 2.25 mm.  
199 Approximately one-third of melanomas were ulcerated and 54% had a mitotic  
200 rate ≥1/mm<sup>2</sup>. Regression was present in 18 samples (9%). A sentinel node biopsy  
201 was performed in 170 patients (75%). Twelve patients had clinical node involvement  
202 at diagnosis (n=12). The AJCC<sup>27</sup> distribution was 35.5% stage I, 39% stage II, 23.5%  
203 stage III and 2% stage IV. The median ROG was 0.26 (IQR 0.09-0.77; Mendeley  
204 Supplementary Table 2).

205 Univariate analysis showed that FGMM were more frequently thick (p<0.001),  
206 ulcerated (p<0.001), nodular (p=0.009), with positive sentinel node (p=0.01). In  
207 addition, they were less frequently located on the upper limb (p=0.047), and more  
208 frequent in patients medicated with betablockers (p=0.02). Furthermore, we found an  
209 association of less lifetime sun exposure (p=0.04) and sunburns (p=0.034) with  
210 FGMM, (Table 1 and Mendeley supplementary Table 3). After multivariate analysis,

211 only ulceration ( $p=0.016$ ), thickness ( $p=0.007$ ) and less lifelong sun exposure  
212 ( $p=0.043$ ) were significantly associated to FGMM (Table 2).

213 We studied the association between survival and melanoma ROG, and found  
214 61 patients relapsed after a median follow-up period of 59.67 months (28 in the  
215 FGMM and 33 in the non-FGMM group). Although the median RFS and median  
216 DMFS were not reached, the RFS was significantly lower in the FGMM group (5-year  
217 FGMM RFS 58.4%, non-FGMM 73.7%,  $p=0.014$  HR 1.9 (1.1-3.1), Figure 1. We  
218 assessed distant recurrences, and documented 56 patients (FGMM=27, non-FGMM=  
219 29). The DMFS was significantly lower in the FGMM cohort (5-year DMFS FGMM  
220 61%, non-FGMM 77.2%,  $p=0.010$  HR 1.9 (1.2-3.3), Mendelely Supplementary Figure  
221 2). Finally, 39 patients died during the follow-up period (FGMM=18, non-FGMM=21),  
222 and we found a trend for lower MSS in the FGMM cohort (5-year FGMM MSS 74.1%,  
223 non-FGMM 83.5%,  $p=0.092$ ).

224

### 225 **Pathogenic mutations associated with FGMM**

226

227 We compared the proportion of samples driven by the common oncogenic  
228 melanoma mutations BRAF, RAS or NF1, or WT for BRAF, NRAS, NF1 (triple WT),  
229 and confirmed a similar distribution to previous cohorts<sup>24,28</sup>. The most commonly  
230 mutated genes were BRAF (49%), NRAS (23.5%) and NF1 (17.5%), together with  
231 TP53 (12%), which appeared in similar proportions in FGMM and non-FGMM  
232 samples. We explored the association between FGMM and mutation burden in the 40  
233 gene panel. FGMM had a higher number of pathogenic mutations than non-FGMM  
234 (mean SNVs FGMM=3.17  $\pm$  3.58, vs. non-FGMM=2.13  $\pm$  1.931;  $p=0.033$ ). We next  
235 investigated the association between pathogenic mutations in each gene and FGMM

236 and found at least one mutation in one of the 40 genes in 179 patients. We  
237 performed univariate analyses and found a higher proportion of pathogenic mutations  
238 in FGFR2, ALK, ERBB4, IDH1, PDGFRA, PREX2 and RB1 in FGMM. We corrected  
239 for multiple comparison, confirming FGFR2 and IDH1 mutations were associated with  
240 fast growth, and noted 15.7% of FGMM presented FGFR2 mutations, in contrast to  
241 2.3% in the non-FGMM group ( $p=0.0049$  HR 7.81 (1.96-45.25; Table 3, Mendeley  
242 Supplementary Tables 4, 5). IDH1 mutations were exclusively found in FGMM at a  
243 rate of 5.7% ( $p=0.049$ ).

244 We reasoned that if FGFR2 mutations are associated to fast growth,  
245 melanomas with FGFR2 mutations should present a transcriptional profile of  
246 increased cell proliferation<sup>25</sup>. We studied the transcriptional profile of melanomas with  
247 FGFR2 mutations using data from TCGA. As the majority of point mutations in  
248 melanoma are acquired early and preserved at the metastatic stage<sup>29</sup>, we opted to  
249 include both primary and metastatic samples of the SKM cohort in our analysis. We  
250 compared the differentially enriched pathways in FGFR2-mutated and FGFR2-wild  
251 type samples and performed unbiased gene set enrichment analysis<sup>30</sup>.  
252 Remarkably, the expression of cell cycle genes indicating increased melanoma cell  
253 proliferation was significantly higher in FGFR2-mutated melanomas (Mendeley  
254 Supplementary Figure 3).

255

### 256 **Alterations in genetic pathways associated with fast growth**

257

258 We analysed the percentage of samples with protein-affecting aberrations in  
259 candidate driver genes, grouped by pathway in our 200 sample cohort (Mendeley  
260 Supplementary Figure 4). FGMM more frequently presented mutations in RTKs

261 (ERBB4/PDGFR/ROS/RET/ALK/KIT/FGFR2; FGMM=32.9%/non-FGMM=18.5%,  
262  $p=0.033$ ), cell cycle pathway (CDKNA/CDK4/BCLAF1; FGMM=10%/non-  
263 FGMM=3.1%,  $p=0.053$ ) and the methylation pathway gene IDH1 (FGMM=5.7%/non-  
264 FGMM=0,  $p=0.042$ ) after Benjamini-Hochberg adjustment (Mendeley Supplementary  
265 Figure 4).

266

## 267 **Integrating clinical and molecular biomarkers of FGMM**

268 To validate the robustness of our biomarkers, we performed stepwise forward  
269 logistic regression analysis including variables that were statistically significantly  
270 associated with FGMM (tumor location, histological subtype, ulceration, mitotic rate,  
271 thickness, sentinel node biopsy, betablocker consumption, lifelong sun exposure,  
272 lifelong sunburn and mutations). The final model, which included 114 patients,  
273 confirmed ulceration ( $p=0.032$ ), thickness ( $p=0.006$ ), less sun exposure ( $p=0.049$ ),  
274 and FGFR2 mutations ( $p=0.037$ ) are independent features associated with FGMM  
275 (Table 4). To further assess how much FGFR2 mutations contributed to fast growth  
276 relative to ulceration and thickness we performed a recursive partitioning analysis  
277 (Mendeley Supplementary Figure 5) as well as bootstrapping and a multilayer  
278 perception network analysis (Mendeley supplementary Figures 6 and 7). All these  
279 analyses demonstrated that FGFR2 mutations allowed additional detection of FGMM  
280 beyond the other factors.

281

## 282 **Discussion**

283 The rapid growth of primary melanoma is recognized as a marker of poor  
284 prognosis, frequently described in tumors with additional hallmarks of aggressive  
285 disease<sup>14-17</sup>. We studied the clinical and genetic characteristics of FGMM, and we  
286 confirm that FGMM metastasize more rapidly, are frequently ulcerated and thicker

287 and inversely associated to lifetime sun exposure<sup>14-17</sup>. Additionally, we observed that  
288 FGMM carry an increased number of pathogenic mutations in melanoma driver  
289 genes and in a novel finding, we show a strong association with FGFR2 mutations.  
290 These results suggest specific molecular changes and environmental factors affect  
291 primary ROG and consequently the disease outcome.

292 Our study confirms previous data<sup>15-17</sup> and sheds light on possible mechanistic  
293 drivers of rapid growth. Extensive studies in cancer research show additive  
294 oncogenic mutations increase the severity of cancer<sup>31,32</sup> and we show that a higher  
295 number of pathogenic mutations in 40 melanoma driver genes is linked to fast  
296 growth. This suggests that additive genetic damage to key genes will move  
297 melanoma forward at a faster pace. Additionally, we show environmental factors  
298 influence the ROG, as patient-reported high levels of sun exposure protects from  
299 rapid growth. In keeping with this finding, previous work revealed a higher burden of  
300 sun-induced mutations across the genome, affecting primarily non-driver genes, is  
301 coupled to better outcome<sup>33</sup>. Taken together, these studies validate that additive  
302 oncogenic drivers accelerate melanoma, but high levels of sun damage protect from  
303 aggressive disease.

304 Genomic aberrations in melanoma frequently affect key signaling pathways to  
305 tumorigenesis. The most affected pathways are the MAP kinase, PI3 kinase and  
306 upstream RTKs. In keeping with a faster proliferation, our study revealed FGMM  
307 accumulated more mutations in RTKs and showed a trend for more mutations in  
308 genes controlling cell cycle. Significantly, we found a robust association between  
309 rapid growth and FGFR2 mutations. FGFR2 is involved in tumor cell proliferation,  
310 angiogenesis, migration and survival in multiple tissues<sup>34-36</sup>, and selective FGFR2  
311 inhibitors show a decrease in tumour cell proliferation and promising results in early

312 phase trials for multiple cancer types with activating FGFR2 mutations<sup>36-39</sup>.  
313 Cutaneous melanoma, however, can present both gain-of-function, oncogenic  
314 mutations<sup>34</sup> as well as loss-of-receptor function mutations<sup>40</sup> through multiple  
315 mechanisms including lower ligand binding affinity, impaired dimerization and  
316 reduced kinase activity<sup>34,36</sup>. These studies highlight FGFR2 signalling can exert  
317 opposing functions, either promoting growth or driving senescence, so it is likely the  
318 contribution of FGFR2 varies depending on cellular context and tumour type. The  
319 signalling consequences of the majority of FGFR2 mutations documented in  
320 melanoma are unknown<sup>41,42</sup>, and further work should address if rapidly growing  
321 melanomas with FGFR2 mutations are candidates for targeted inhibitor FGFR2  
322 therapies. Although the number of samples in our cohort is small, we found IDH1  
323 mutations might associate with fast growth. We identified hotspot oncogenic  
324 IDH1R132C mutations in 4 patients, exclusively in the FGMM group. IDH1 mutations  
325 drive a variety of human cancers in addition to melanoma<sup>43,44</sup>. In vitro studies show  
326 that mutant IDH1 confers growth and metabolic advantage to melanoma and cancer  
327 cells<sup>43,45,46</sup>, and in glioma models, IDH1/2 mutations may shape the immunological  
328 landscape of the tumor microenvironment<sup>47,48</sup>. These findings support that IDH1  
329 mutations might drive more aggressive melanomas.

330 We acknowledge our study has limitations. Twenty percent of patients were  
331 not able to provide information required for ROG calculation and were excluded from  
332 the study. The size of the population was relatively small and missing data reduced  
333 the number of cases included in the multivariate models. Since ROG is calculated  
334 prospectively, and FGFR2 is not routinely analysed, we could not corroborate our  
335 data in a validation cohort. Finally, TERT promoter sequencing was not included in  
336 our panel and is linked to rapid growth<sup>19</sup>. We additionally focused on targeted

337 mutational analysis, omitting overall tumour mutation burden, mutational signatures,  
338 gene fusions and expression.

339 The multivariate analysis reveals FGFR2 mutations, thickness and ulceration remain  
340 robust independent predictor of rapid melanoma growth, a strong indicator of poor  
341 outcome. Only patients with stage III or IV resected melanomas are currently eligible  
342 for adjuvant therapies. Given the ongoing trials in stage II melanomas, one of the  
343 current challenges is to find biomarkers to identify individuals at highest risk of death,  
344 who are most likely to benefit from therapies, and to avoid overtreatment and drug  
345 toxicity. Screening for FGFR2 mutations might provide an additional tool to better  
346 identify the fast-growing tumors which, given their aggressiveness, should be  
347 undoubtedly regarded as strong candidates for adjuvant therapies.

348



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354

355 **Author contributions**

356 CG and JJG conceived the study. CG and AV carried out experiments and managed  
357 the project. CG, AV, NM, AL, EP, LO, TB, PM, GG, LL, MC, RM, RK, RM, EN  
358 contributed to method development and analyses. VA contributed resources and RM  
359 funding. CG, AV, JJG and EN interpreted the data. CG and AV wrote the manuscript,  
360 EN critically reviewed and edited the manuscript.

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362

363

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504 **Figure legends**

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506 **Figure 1.** Relapse-free survival by rate of growth in the 200-patient cohort.

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513 **Tables**

Variable	Non-FGMM (n=130)	FGMM (n=70)	OR 95%	p
<b>Melanoma location</b>				
Head and Neck	11 (8.5%)	9 (12.9%)	1.26 (0.47-3.38)	0.640
Trunk	51 (39.2%)	33 (47.1%)	1	
Upper Limb	20 (15.4%)	4 (5.7%)	0.31 (0.10-0.99)	0.047
Lower Limb	41 (31.5%)	20 (28.6%)	0.75 (0.38-1.51)	0.423
Hands/Feet/Palm/Nail	7 (5.4%)	4 (5.7%)	0.88 (0.24-3.25)	0.852
<b>Histological subtype</b>				
SSM	90 (69.2%)	37 (52.9%)	1	
NM	30 (23.1%)	29 (41.4%)	2.35 (1.24-4.45)	0.009
Other *	10 (7.7%)	4 (5.7%)	1.08 (0.31-3.73)	0.902
<b>Ulceration</b>				
No	92 (73%)	32 (45.7%)	1	
Yes	34 (27%)	38 (54.3%)	3.21 (1.74-5.93)	<0.001
Missing	4	0		
<b>Thickness (mm)</b>				
Median	1.8 (1.4-3)	4 (2.5-6)	1.61 (1.34-1.93)	<0.001
Mean	2.4 ±1.58	5.70 ±6.68		
1.00-2.00	80 (61.5%)	10 (14.3%)	1	
2.01-4.00	33 (25.4%)	30 (42.9%)	7.27 (3.20-16.56)	<0.001
>4.00	17 (13.1%)	30 (42.9%)	14.12 (5.82-34.26)	<0.001
<b>Sentinel node biopsy</b>				
Positive	19 (16.5%)	19 (34.5%)	2.67 (1.27-5.60)	0.010
Negative	96 (83.5%)	36 (65.5%)	1	
Missing	16	14		
<b>AJCC (7<sup>th</sup> classification)</b>				
I	63 (48.5%)	8 (11.4%)	1	
II	43 (33.1%)	35 (50%)	6.41 (2.71-15.11)	<0.001
III	22 (16.9%)	25 (35.7%)	8.95 (3.52-22.74)	<0.001
IV	2 (1.5%)	2 (2.9%)	7.88 (0.97-63.89)	0.053
<b>Beta blockers</b>				
No	123 (94.6%)	59 (84.3%)	1	
Yes	7 (5.4%)	11 (15.7%)	3.28 (1.21-8.88)	0.020
<b>Mean sun exposure score</b>				
during childhood	5.41 ±1.66	4.71 ±1.34	0.75 (0.61-0.92)	0.005
during adulthood	6.88 ±2.11	6.34 ±1.84	0.87 (0.75-1.02)	0.008
all life long	12.3 ±3.42	10.88 ±2.5	0.86 (0.78-0.96)	0.004
<b>Mean sunburn score</b>				
during childhood	1.55 ±0.9	1.25 ±0.8	0.68 (0.47-0.97)	0.033
during adulthood	1.55 ±0.9	1.33 ±0.9	0.77 (0.55-1.08)	0.124
lifelong	3.10 ±1.6	2.57 ±1.5	0.80 (0.65-0.98)	0.034

514 **Table 1. Clinical and epidemiological variables associated with fast growth**

515 Univariate analysis, p value <0.05). \* ALM (acral lentiginous melanoma n=6), LM (lentigo  
516 maligna n=2), Desmoplastic (n=1), spitzoid (n=1), malignant blue (n=1), non-assessable  
517 (n=3), SSM: superficial spreading melanoma, NM: nodular melanoma.

Variables*	non-FGMM (n=130)	FGMM (n=70)	Univariate OR 95%	p	Multivariate OR 95%	p
<b>Melanoma location</b>						
Head and Neck	11 (8.5%)	9 (12.9%)	1.26 (0.47-3.38)	0.64		
Trunk	51 (39.2%)	33 (47.1%)	1		1	
Upper Limb	20 (15.4%)	4 (5.7%)	0.31 (0.10-0.99)	0.047		
Lower Limb	41 (31.5%)	20 (28.6%)	0.75 (0.38-1.51)	0.423		
Hands/Feet/palm/nail	7 (5.4%)	4 (5.7%)	0.88 (0.24-3.25)	0.852		
<b>Histological subtype</b>						
SSM	90 (69.2%)	37 (52.9%)	1		1	
NM	30 (23.1%)	29 (41.4%)	2.35 (1.24-4.45)	0.009		
Other**	10 (7.7%)	4 (5.7%)	1.08 (0.31-3.73)	0.902		
<b>Ulceration</b>						
No	92 (73%)	32 (45.7%)	1		1	
Yes	34 (27%)	38 (54.3%)	3.21 (1.74-5.93)	<0.001	3.18 (1.2-8.2)	0.016
Missing	4	0				
<b>Mitotic rate</b>						
0/mm <sup>2</sup>	37 (37.4%)	13 (22.4%)	0.48 (0.23-1.01)	0.054		
≥1/mm <sup>2</sup>	62 (62.6%)	45 (77.6%)	1		1	
Missing	31	12				
<b>Thickness (mm)</b>						
1.00-2.00	80 (61.5%)	10 (14.3%)	1		1	
2.01-4.00	33 (25.4%)	30 (42.9%)	7.27 (3.20-16.56)	<0.001	4.73 (1.55-14.7)	0.007
>4.00	17 (13.1%)	30 (42.9%)	14.12 (5.82-34.26)	<0.001	7.64 (2.2-27.0)	0.002
<b>Sentinel node biopsy</b>						
Positive	19 (16.1%)	19 (33.3%)	2.67 (1.27-5.60)	0.01		
Negative	96 (81.4%)	36 (63.2%)	1		1	
Missing	16	14				
<b>Beta blockers</b>						
No	123 (94.6%)	59 (84.3%)	1		1	
Yes	7 (5.4%)	11 (15.7%)	3.28 (1.21-8.88)	0.02		
<b>Mean sun exposure score (lifelong)</b>	12.3 (±3.42)	10.88 (±2.5)	0.86 (0.78-0.96)	0.004	0.84 (0.7-0.99)	0.043
<b>Mean sunburn score (lifelong)</b>	3.10 (±1.6)	2.57 (±1.5)	0.80 (0.65-0.98)	0.034		

519

520 **Table 2. Clinical and epidemiological variables associated with fast growth**

521 Multivariate stepwise forward model. \*only variables with a p value <0.05 after univariate  
522 analysis were included in the model\*\*ALM, LM, Desmoplastic, spitzoid, malignant blue, non-  
523 assessable

524



525 **Table 3**

	<b>Non-FGMM (n=130)</b>	<b>FGMM (n=70)</b>	<b>OR 95%</b>	<b>Raw p</b>	<b>Adjusted p Benjamini- Hochberg</b>
<b>ALK mutation</b>					
No	124 (95.4%)	62 (88.6%)	1	0.085	0.085
Yes	6 (4.6%)	8 (11.4%)	2.67 (0.89-8.02)		
<b>ERBB4 mutation</b>					
No	121 (93.1%)	59 (84.3%)	1	0.048	0.112
Yes	9 (6.9%)	11 (15.7%)	2.49 (0.88-7.22)		
<b>FGFR2 mutation</b>					
No	127 (97.7%)	59 (84.3%)	1	0.0007	0.0049
Yes	3 (2.3%)	11 (15.7%)	7.81 (1.96-45.25)		
<b>IDH1 mutation</b>					
No	130 (100%)	66 (94.3%)	1	0.014	0.049
Yes	0	4 (5.7%)	NE		
<b>PDGFRA mutation</b>					
No	128 (98.5%)	65 (92.9%)	1	0.052	0.073
Yes	2 (1.5%)	5 (7.1%)	4.92 (0.93-26.07)		
<b>PREX2 mutation</b>					
No	121 (93.1%)	59 (84.3%)	1	0.048	0.084
Yes	9 (6.9%)	11 (15.7%)	2.51 (0.99-6.38)		
<b>RB1 mutation</b>					
No	127 (97.7%)	64 (91.4%)	1	0.068	0.079
Yes	3 (2.3%)	6 (8.6%)	3.97 (0.96-16.39)		

526 NE: non estimable

527

528 **Table 3. Association of mutations in genes with fast growing and non-fast growing**  
529 **melanoma**

530 Univariate analysis.

531

532 **Table 4**

Variables*	Non-FGMM (n=130)	FGMM (n=70)	Univariate		Multivariate (Stepwise)	
			OR 95%	p	OR 95%	p
<b>Melanoma location</b>						
Head and Neck	11 (8.5%)	9 (12.9%)	1.26 (0.47-3.38)	0.64		
Trunk	51 (39.2%)	33 (47.1%)	1			
Upper Limb	20 (15.4%)	4 (5.7%)	0.31 (0.10-0.99)	0.047		
Lower Limb	41 (31.5%)	20 (28.6%)	0.75 (0.38-1.51)	0.423		
Hands/Feet/palm/nail	7 (5.4%)	4 (5.7%)	0.88 (0.24-3.25)	0.852		
<b>Histological subtype</b>						
SSM	90 (69.2%)	37 (52.9%)	1			
NM	30 (23.1%)	29 (41.4%)	2.35 (1.24-4.45)	0.009		
Other **	10 (7.7%)	4 (5.7%)	1.08 (0.31-3.73)	0.902		
<b>Ulceration</b>						
No	92 (73%)	32 (45.7%)	1		1	
Yes	34 (27%)	38 (54.3%)	3.21 (1.74-5.93)	<0.001	2.90 (1.10-7.63)	0.032
Missing	4	0				
<b>Mitotic rate</b>						
0/mm <sup>2</sup>	37(37.4%)	13(22.4%)	0.48 (0.23-1.01)	0.054		
≥1/mm <sup>2</sup>	62(62.6%)	45(77.6%)	1			
Missing	31	12				
<b>Thickness (mm)</b>						
1.00-2.00	80 (61.5%)	10 (14.3%)	1		1	
2.01-4.00	33 (25.4%)	30 (42.9%)	7.27 (3.20-16.56)	<0.001	5.41 (1.64-17.82)	0.006
>4.00	17 (13.1%)	30 (42.9%)	14.12 (5.82-34.26)	<0.001	8.88 (2.37-33.26)	0.001
<b>Sentinel node biopsy</b>						
Positive	19 (16.1%)	19 (33.3%)	2.67 (1.27-5.60)	0.01		
Negative	96 (81.4%)	36 (63.2%)	1			
Missing	16	14				
<b>Beta blockers</b>						
No	123 (94.6%)	59 (84.3%)	1			
Yes	7 (5.4%)	11 (15.7%)	3.28 (1.21-8.88)	0.02		
<b>Mean sun exposure score (lifelong)</b>	12.3 (±3,42)	10.88 (±2,5)	0.86 (0.78-0.96)	0.004	0.85 (0.71-0.99)	0.049
<b>Mean sunburn score (lifelong)</b>	3.10 (±1.6)	2.57 (±1.5)	0.80 (0.65-0.98)	0.034		
<b>FGFR2 mutation</b>						
No	127 (97.7%)	59 (84.3%)	1		1	
Yes	3 (2.3%)	11 (15.7%)	7.81 (1.96-45.25)	0.005	8.64 (1.14-65.43)	0.037

533 **Table 4. Variables associated with fast growth**

534 Multivariate stepwise forward model. \*only variables with a p value <0.05 after univariate  
535 analysis were included in the model. \*\*ALM, LM, Desmoplastic, spitzoid, malignant blue,  
536 non-assessable.

537 Data missing for the following variables: histological subtype (n=1); ulceration (n=4); SN  
538 biopsy results (n=30); mitotic rate (n=43); sun exposure score (lifelong) (n=12); sunburn  
539 score (lifelong) (n=18).

- FGMM (ROG > 0.5 mm/month)
- Non-FGMM (ROG ≤ 0.5 mm/month)

