



HAL
open science

Molecular characterization of fast-growing melanomas

Caroline Gaudy-Marqueste, Nicolas Macagno, Anderson Loundou, Eric Pellegrino, L'Houcine Ouafik, Timothy Budden, Piyushkumar Mundra, Gabriela Gremel, Victoria Akhras, Lijing Lin, et al.

► **To cite this version:**

Caroline Gaudy-Marqueste, Nicolas Macagno, Anderson Loundou, Eric Pellegrino, L'Houcine Ouafik, et al.. Molecular characterization of fast-growing melanomas. *Journal of The American Academy of Dermatology*, 2021, 10.1016/j.jaad.2021.07.011 . hal-03401110

HAL Id: hal-03401110

<https://amu.hal.science/hal-03401110v1>

Submitted on 22 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

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

2

3 **Authors**

4 Caroline Gaudy-Marqueste MD PhD^{1*}, Nicolas Macagno MD PhD PhD², Anderson Loundou
5 PhD³, Eric Pellegrino MSc⁴ L'houcine Ouafik PhD⁴, Timothy Budden PhD⁵, Piyushkumar
6 Mundra PhD⁶, Gabriela Gremel PhD⁶, Victoria Akhras MD⁷, Lijing Lin PhD⁸, Martin Cook
7 MD⁶, Rajiv Kumar PhD⁹, Jean-Jacques Grob MD¹, Eduardo Nagore MD PhD¹⁰, Richard
8 Marais PhD⁶, Amaya Virós MD PhD^{5*}

9

10 **Affiliations**

11 ¹ Aix Marseille Univ, APHM, CRCM Inserm U1068, CNRS U7258, CHU Timone,
12 Dermatology and Skin Cancer Department, Marseille, France

13 ² Aix Marseille Univ, APHM, INSERM, MMG, CHU Timone, Department of
14 Pathology, Marseille, France

15 ³ Aix-Marseille Univ, SPMC EA3279, Clinical Research Unit, Department of Public Health,
16 Marseille, France.

17 ⁴ Aix Marseille Univ, APHM, CNRS, INP, Inst Neurophysiopathol, Faculté de Médecine
18 Secteur Nord, Service de Transfert d'Oncologie Biologique, Marseille, France.

19 ⁵ Skin Cancer and Ageing Lab, Cancer Research UK Manchester Institute, The University of
20 Manchester, Manchester, UK

21 ⁶ Molecular Oncology, CRUK Manchester Institute, University of Manchester, Manchester,
22 UK

23 ⁷ Department of Dermatology, St. George's NHS Foundation Trust, London, UK

24 ⁸ Division of Informatics, Imaging and Data Sciences, Faculty of Biology, Medicine and
25 Health, University of Manchester, M13 9PL, UK.

26 ⁹ Division of Functional Genome Analysis, German Cancer Research Center, Heidelberg,
27 Germany

28 ¹⁰ Department of Dermatology, Instituto Valenciano Oncología, València, Spain

29

30 **Corresponding authors**

31 Caroline Gaudy-Marqueste: caroline.gaudy@ap-hm.fr

32 Amaya Virós: amaya.viros@cruk.manchester.ac.uk

33

34 **Lead contact:**

35 Amaya Virós, MD, PhD, Wellcome Trust Clinician Scientist

36 Skin Cancer and Ageing Lab, Cancer Research UK Manchester Institute

37 Alderley Park, Alderley SK10 4TG, United Kingdom.

38 Telephone: +44 (0) 7878794211, +44 (0) 161 306 6038

39 Email: Amaya.viros@cruk.manchester.ac.uk

40 Twitter: @DrAmayaViros ORCID: <https://orcid.org/0000-0001-5177-5015>

41

42 **Funding**

43 CG: Société Française de Dermatologie (SFD), Collège des Enseignants en Dermatologie
44 de France (CEDEF) and Fondation de France. EN: Fondo de Investigación en Salud (FIS)
45 PI15/01860, Instituto Carlos III, Spain. RK: TRANSCAN (01KT15511) through the German
46 Ministry of Education and Research (BMBF) and DKFZ. RM: Cancer Research UK (A27412)
47 and Wellcome Trust (100282/Z/12/Z). AV: Wellcome Beit Fellow, personally funded by a
48 Wellcome Intermediate Fellowship (110078/Z/15/Z), additional work funded by Cancer
49 Research UK (A27412) Leo Pharma Foundation and Royal Society RGS\R1\201222. We
50 gratefully acknowledge EORTC support and funding to study primary melanoma tumor
51 markers.

52 **Conflicts of Interest:** No competing interests

53 **Word count:** abstract: 200 text: 2504 capsule summary: 49

54 **Figures:** 1

55 **Tables:** 4

56 **References:** 48

57

58 **Mendeley Supplementary Figures:** 7

59 **Mendeley Supplementary Tables:** 5

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

68 This work was presented during the 16th EADO congress (virtual meeting). 12-14
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 ≥ 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 ≤ 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

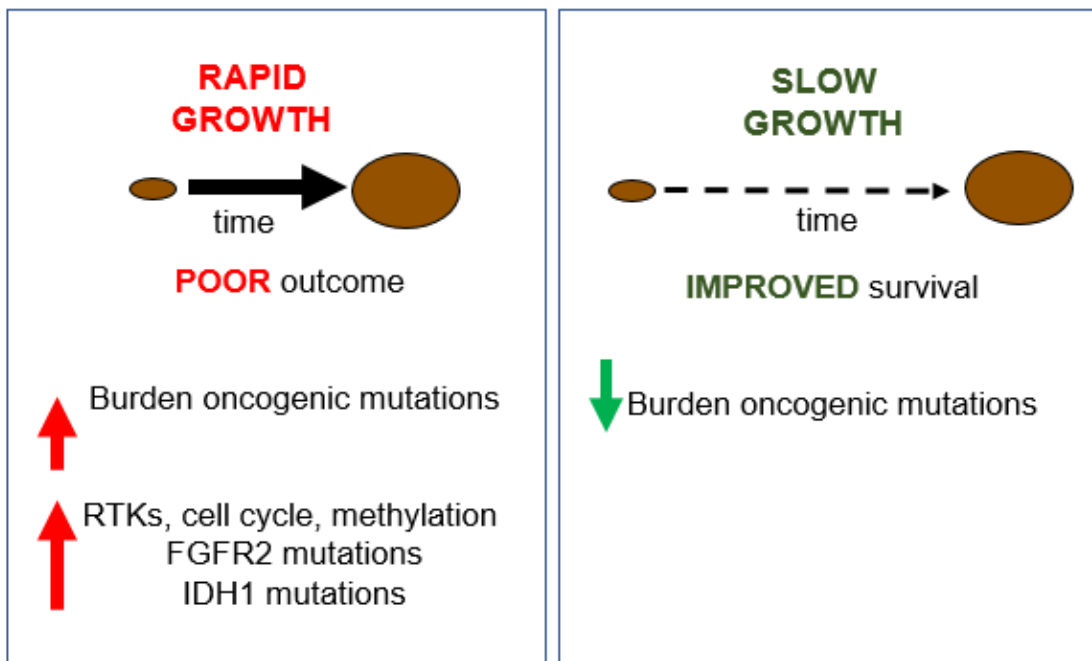
94 **Capsule summary**

- 95
- Fast growing melanomas are aggressive and linked to early death. Rapid growth is associated to thicker, ulcerated tumors with FGFR2 mutations; and
- 96
- is more frequent in patients with less accumulated sun exposure.
- 97
- 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 worldwide¹⁻⁴.
107 Prevention campaigns promoting early diagnosis underpin an epidemiological shift
108 towards earlier stage disease⁵⁻¹⁰ and the majority of new melanoma cases are
109 diagnosed at localized stages^{8,11,12}. Stage II tumors are considered low risk¹³,
110 however, the prediction is that, due to the large prevalence of stage II, they will
111 account for most deaths in the future^{8,11,12}.

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 growth¹⁴, is a validated robust, reproducible and
120 independent prognostic factor of outcome¹⁴⁻¹⁷. Patients with fast-growing melanoma
121 (FGMM) have aggressive disease that spreads early, leading to shorter survival¹⁴⁻¹⁷.
122 Fast growth is associated to nodular subtype, trunk location, male sex, previous non-
123 melanoma skin cancer, and few childhood sunburns¹⁵⁻¹⁷. Furthermore, FGMM display
124 high mitotic rates¹⁵⁻¹⁷, and frequent NRAS¹⁸ and TERT promoter mutations¹⁹,
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 ≥ 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 development¹⁴. FGMM were defined by $ROG > 0.5$ mm/month and non-
138 FGMM by $ROG \leq 0.5$ mm/month¹⁵⁻¹⁷. 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 COSMIC²² or Varsome²³ 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 Atlas²⁴ 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.*²⁵ to measure proliferating cells.

166 Somatic mutation and RNA-seq (log₂ 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 procedure²⁶. 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/mm². 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 AJCC²⁷ 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 We compared the proportion of samples driven by the common oncogenic
227 melanoma mutations BRAF, RAS or NF1, or WT for BRAF, NRAS, NF1 (triple WT),
228 and confirmed a similar distribution to previous cohorts^{24,28}. The most commonly
229 mutated genes were BRAF (49%), NRAS (23.5%) and NF1 (17.5%), together with
230 TP53 (12%), which appeared in similar proportions in FGMM and non-FGMM
231 samples. We explored the association between FGMM and mutation burden in the 40
232 gene panel. FGMM had a higher number of pathogenic mutations than non-FGMM
233 (mean SNVs FGMM=3.17 \pm 3.58, vs. non-FGMM=2.13 \pm 1.931; $p=0.033$). We next
234 investigated the association between pathogenic mutations in each gene and FGMM
235

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 proliferation²⁵. 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 stage²⁹, 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 analysis³⁰.
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 α /ROS/RET/ALK/KIT/FGFR2; FGMM=32.9%/non-FGMM=18.5%,
262 $p=0.033$), cell cycle pathway (CDKN1A/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 disease¹⁴⁻¹⁷. 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 exposure¹⁴⁻¹⁷. 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 data¹⁵⁻¹⁷ 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^{31,32} 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 outcome³³. 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 tissues³⁴⁻³⁶, 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 mutations³⁶⁻³⁹.
313 Cutaneous melanoma, however, can present both gain-of-function, oncogenic
314 mutations³⁴ as well as loss-of-receptor function mutations⁴⁰ through multiple
315 mechanisms including lower ligand binding affinity, impaired dimerization and
316 reduced kinase activity^{34,36}. 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^{41,42}, 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^{43,44}. In vitro studies show
326 that mutant IDH1 confers growth and metabolic advantage to melanoma and cancer
327 cells^{43,45,46}, and in glioma models, IDH1/2 mutations may shape the immunological
328 landscape of the tumor microenvironment^{47,48}. 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 growth¹⁹. 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

349 **Acknowledgments**

350 We are grateful for advice and support from Pr Magnus Rattray-University of
351 Manchester. Bioresources were provided by the Biological Resources Centre of the
352 Assistance Publique des Hôpitaux de Marseille, France (certified NF S96-900 & ISO
353 9001 v2015).

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.

361

362

363

364 **References**

- 365 1. Whiteman DC, Green AC, Olsen CM. The Growing Burden of Invasive Melanoma:
366 Projections of Incidence Rates and Numbers of New Cases in Six Susceptible
367 Populations through 2031. *Journal of Investigative Dermatology*. 2016;136(6):1161-1171.
368 doi:10.1016/j.jid.2016.01.035
- 369 2. Olsen CM, Green AC, Pandeya N, Whiteman DC. Trends in Melanoma Incidence Rates
370 in Eight Susceptible Populations through 2015. *J Invest Dermatol*. 2019;139(6):1392-
371 1395. doi:10.1016/j.jid.2018.12.006
- 372 3. Sacchetto L, Zanetti R, Comber H, et al. Trends in incidence of thick, thin and in situ
373 melanoma in Europe. *European Journal of Cancer*. 2018;92:108-118.
374 doi:10.1016/j.ejca.2017.12.024
- 375 4. Garbe C, Keim U, Eigentler TK, et al. Time trends in incidence and mortality of
376 cutaneous melanoma in Germany. *J Eur Acad Dermatol Venereol*. 2019;33(7):1272-
377 1280. doi:10.1111/jdv.15322
- 378 5. Bordoni A, Leoni-Parvex S, Peverelli S, Mazzola P, Mazzucchelli L, Spitale A.
379 Opportunistic screening strategy for cutaneous melanoma does not change the incidence
380 of nodular and thick lesions nor reduce mortality: a population-based descriptive study in
381 the European region with the highest incidence. *Melanoma Res*. 2013;23(5):402-407.
382 doi:10.1097/CMR.0b013e328363b015
- 383 6. Baade P, Meng X, Youlden D, Aitken J, Youl P. Time trends and latitudinal differences in
384 melanoma thickness distribution in Australia, 1990-2006. *Int J Cancer*. 2012;130(1):170-
385 178. doi:10.1002/ijc.25996
- 386 7. Minini R, Rohrmann S, Braun R, Korol D, Dehler S. Incidence trends and clinical-
387 pathological characteristics of invasive cutaneous melanoma from 1980 to 2010 in the
388 Canton of Zurich, Switzerland. *Melanoma Res*. 2017;27(2):145-151.
389 doi:10.1097/CMR.0000000000000312
- 390 8. Whiteman DC, Baade PD, Olsen CM. More people die from thin melanomas (≤ 1 mm)
391 than from thick melanomas (> 4 mm) in Queensland, Australia. *J Invest Dermatol*.
392 2015;135(4):1190-1193. doi:10.1038/jid.2014.452
- 393 9. Coory M, Baade P, Aitken J, Smithers M, McLeod GRC, Ring I. Trends for in situ and
394 invasive melanoma in Queensland, Australia, 1982-2002. *Cancer Causes Control*.
395 2006;17(1):21-27. doi:10.1007/s10552-005-3637-4
- 396 10. Geller AC, Clapp RW, Sober AJ, et al. Melanoma epidemic: an analysis of six decades of
397 data from the Connecticut Tumor Registry. *J Clin Oncol*. 2013;31(33):4172-4178.
398 doi:10.1200/JCO.2012.47.3728
- 399 11. Criscione VD, Weinstock MA. Melanoma Thickness Trends in the United States, 1988-
400 2006. *Journal of Investigative Dermatology*. 2010;130(3):793-797.
401 doi:10.1038/jid.2009.328
- 402 12. Landow SM, Gjelsvik A, Weinstock MA. Mortality burden and prognosis of thin
403 melanomas overall and by subcategory of thickness, SEER registry data, 1992-2013. *J*
404 *Am Acad Dermatol*. 2017;76(2):258-263. doi:10.1016/j.jaad.2016.10.018

- 405 13. Gershenwald JE, Scolyer RA, Hess KR, et al. Melanoma staging: Evidence-based
406 changes in the American Joint Committee on Cancer eighth edition cancer staging
407 manual. *CA Cancer J Clin.* 2017;67(6):472-492. doi:10.3322/caac.21409
- 408 14. Grob JJ, Richard MA, Gouvernet J, et al. The kinetics of the visible growth of a primary
409 melanoma reflects the tumor aggressiveness and is an independent prognostic marker: a
410 prospective study. *Int J Cancer.* 2002;102(1):34-38. doi:10.1002/ijc.10660
- 411 15. Liu W, Dowling JP, Murray WK, et al. Rate of growth in melanomas: characteristics and
412 associations of rapidly growing melanomas. *Arch Dermatol.* 2006;142(12):1551-1558.
413 doi:10.1001/archderm.142.12.1551
- 414 16. Tejera-Vaquerizo A, Barrera-Vigo MV, López-Navarro N, Herrera-Ceballos E. Growth
415 rate as a prognostic factor in localized invasive cutaneous melanoma. *J Eur Acad*
416 *Dermatol Venereol.* 2010;24(2):147-154. doi:10.1111/j.1468-3083.2009.03367.x
- 417 17. Martorell-Calatayud A, Nagore E, Botella-Estrada R, et al. Defining fast-growing
418 melanomas: reappraisal of epidemiological, clinical, and histological features. *Melanoma*
419 *Res.* 2011;21(2):131-138. doi:10.1097/CMR.0b013e328342f312
- 420 18. Nagore E, Hacker E, Martorell-Calatayud A, et al. Prevalence of BRAF and NRAS
421 mutations in fast-growing melanomas. *Pigment Cell Melanoma Res.* 2013;26(3):429-431.
422 doi:10.1111/pcmr.12082
- 423 19. Nagore E, Heidenreich B, Requena C, et al. TERT promoter mutations associate with
424 fast-growing melanoma. *Pigment Cell Melanoma Res.* 2016;29(2):236-238.
425 doi:10.1111/pcmr.12441
- 426 20. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. *Arch*
427 *Dermatol.* 1988;124(6):869-871. doi:10.1001/archderm.124.6.869
- 428 21. Larnier C, Ortonne JP, Venot A, et al. Evaluation of cutaneous photodamage using a
429 photographic scale. *Br J Dermatol.* 1994;130(2):167-173. doi:10.1111/j.1365-
430 2133.1994.tb02895.x
- 431 22. Tate JG, Bamford S, Jubb HC, et al. COSMIC: the Catalogue Of Somatic Mutations In
432 Cancer. *Nucleic Acids Research.* 2019;47(D1):D941-D947. doi:10.1093/nar/gky1015
- 433 23. Kopanos C, Tsiolkas V, Kouris A, et al. VarSome: the human genomic variant search
434 engine. *Bioinformatics.* 2019;35(11):1978-1980. doi:10.1093/bioinformatics/bty897
- 435 24. Cancer Genome Atlas Network. Genomic Classification of Cutaneous Melanoma. *Cell.*
436 2015;161(7):1681-1696. doi:10.1016/j.cell.2015.05.044
- 437 25. Tirosh I, Izar B, Prakadan SM, et al. Dissecting the multicellular ecosystem of metastatic
438 melanoma by single-cell RNA-seq. *Science.* 2016;352(6282):189-196.
439 doi:10.1126/science.aad0501
- 440 26. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful
441 Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B*
442 *(Methodological).* 1995;57(1):289-300.
- 443 27. Balch CM, Gershenwald JE, Soong S-J, et al. Final version of 2009 AJCC melanoma
444 staging and classification. *J Clin Oncol.* 2009;27(36):6199-6206.
445 doi:10.1200/JCO.2009.23.4799

- 446 28. Tsao H, Chin L, Garraway LA, Fisher DE. Melanoma: from mutations to medicine. *Genes*
447 *Dev.* 2012;26(11):1131-1155. doi:10.1101/gad.191999.112
- 448 29 Vergara IA, Mintoff CP, Sandhu S, et al. Evolution of late-stage metastatic melanoma is
449 dominated by aneuploidy and whole genome doubling. *Nat Commun.* 2021 4;12(1):1434.
- 450 30. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: A
451 knowledge-based approach for interpreting genome-wide expression profiles.
452 *Proceedings of the National Academy of Sciences of the United States of America.*
453 2005;102(43):15545-15550. doi:10.1073/pnas.0506580102
- 454 31. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell.*
455 1990;61(5):759-767. doi:10.1016/0092-8674(90)90186-i
- 456 32. Shain AH, Yeh I, Kovalyshyn I, et al. The Genetic Evolution of Melanoma from Precursor
457 Lesions. *N Engl J Med.* 2015;373(20):1926-1936. doi:10.1056/NEJMoa1502583
- 458 33. Trucco LD, Mundra PA, Hogan K, et al. Ultraviolet radiation-induced DNA damage is
459 prognostic for outcome in melanoma. *Nat Med.* 2019;25(2):221-224.
460 doi:10.1038/s41591-018-0265-6
- 461 34. Katoh M. FGFR2 abnormalities underlie a spectrum of bone, skin, and cancer
462 pathologies. *J Invest Dermatol.* 2009;129(8):1861-1867. doi:10.1038/jid.2009.97
- 463 35. Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. *Nat*
464 *Rev Cancer.* 2010;10(2):116-129. doi:10.1038/nrc2780
- 465 36. Dienstmann R, Rodon J, Prat A, et al. Genomic aberrations in the FGFR pathway:
466 opportunities for targeted therapies in solid tumors. *Ann Oncol.* 2014;25(3):552-563.
467 doi:10.1093/annonc/mdt419
- 468 37. Ivan M, Matei D. Blockade of FGF signaling: therapeutic promise for ovarian cancer.
469 *Cancer Biol Ther.* 2010;10(5):505-508. doi:10.4161/cbt.10.5.13023
- 470 38. Greulich H, Pollock PM. Targeting mutant fibroblast growth factor receptors in cancer.
471 *Trends Mol Med.* 2011;17(5):283-292. doi:10.1016/j.molmed.2011.01.012
- 472 39. Daniele G, Corral J, Molife LR, de Bono JS. FGF receptor inhibitors: role in cancer
473 therapy. *Curr Oncol Rep.* 2012;14(2):111-119. doi:10.1007/s11912-012-0225-0
- 474 40. Gartside MG, Chen H, Ibrahimi OA, et al. Loss-of-function fibroblast growth factor
475 receptor-2 mutations in melanoma. *Mol Cancer Res.* 2009;7(1):41-54. doi:10.1158/1541-
476 7786.MCR-08-0021
- 477 41. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform
478 for exploring multidimensional cancer genomics data. *Cancer Discov.* 2012;2(5):401-404.
479 doi:10.1158/2159-8290.CD-12-0095
- 480 42. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics
481 and clinical profiles using the cBioPortal. *Sci Signal.* 2013;6(269):pl1.
482 doi:10.1126/scisignal.2004088
- 483 43 Shibata T, Kokubu A, Miyamoto M, Sasajima Y, Yamazaki N. Mutant IDH1 confers an in
484 vivo growth in a melanoma cell line with BRAF mutation. *Am J Pathol.* 2011
485 Mar;178(3):1395-402. doi: 10.1016/j.ajpath.2010.12.011. PMID: 21356389; PMCID:
486 PMC3069821

- 487 44 Cancer Genome Atlas Network. Genomic Classification of Cutaneous Melanoma. *Cell*.
488 2015 Jun 18;161(7):1681-96. doi: 10.1016/j.cell.2015.05.044. PMID: 26091043; PMCID:
489 PMC4580370.
- 490 45 Lian CG, Xu Y, Ceol C, et al . Loss of 5-hydroxymethylcytosine is an epigenetic hallmark
491 of melanoma. *Cell*. 2012 14;150(6):1135-46. doi: 10.1016/j.cell.2012.07.033. PMID:
492 22980977; PMCID: PMC3770275.
- 493 46 Dang L, White DW, Gross S, et al. Cancer-associated IDH1 mutations produce 2-
494 hydroxyglutarate. *Nature*. 2009 10;462(7274):739-44. doi: 10.1038/nature08617. PMID:
495 19935646
- 496 47 Amankulor NM, Kim Y, Arora S, et al . Mutant IDH1 regulates the tumor-associated
497 immune system in gliomas. *Genes Dev*. 2017 15;31(8):774-786. doi:
498 10.1101/gad.294991.116. PMID: 28465358; PMCID: PMC5435890.
- 499 48 Tyrakis PA, Palazon A, Macias D, et al. S-2-hydroxyglutarate regulates CD8⁺ T-
500 lymphocyte fate. *Nature*. 2016 8;540(7632):236-241. doi: 10.1038/nature20165. Epub
501 2016 Oct 26. PMID: 27798602; PMCID: PMC5149074.

502

503

504 **Figure legends**

505

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

507

508

509

510

511

512

513 **Tables**

Variable	Non-FGMM (n=130)	FGMM (n=70)	OR 95%	p
Melanoma location				
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
Histological subtype				
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
Ulceration				
No	92 (73%)	32 (45.7%)	1	
Yes	34 (27%)	38 (54.3%)	3.21 (1.74-5.93)	<0.001
Missing	4	0		
Thickness (mm)				
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
Sentinel node biopsy				
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		
AJCC (7th classification)				
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
Beta blockers				
No	123 (94.6%)	59 (84.3%)	1	
Yes	7 (5.4%)	11 (15.7%)	3.28 (1.21-8.88)	0.020
Mean sun exposure score				
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
Mean sunburn score				
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
Melanoma location						
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		
Histological subtype						
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		
Ulceration						
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				
Mitotic rate						
0/mm ²	37 (37.4%)	13 (22.4%)	0.48 (0.23-1.01)	0.054		
≥1/mm ²	62 (62.6%)	45 (77.6%)	1		1	
Missing	31	12				
Thickness (mm)						
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
Sentinel node biopsy						
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				
Beta blockers						
No	123 (94.6%)	59 (84.3%)	1		1	
Yes	7 (5.4%)	11 (15.7%)	3.28 (1.21-8.88)	0.02		
Mean sun exposure score (lifelong)	12.3 (±3.42)	10.88 (±2.5)	0.86 (0.78-0.96)	0.004	0.84 (0.7-0.99)	0.043
Mean sunburn score (lifelong)	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**

	Non-FGMM (n=130)	FGMM (n=70)	OR 95%	Raw p	Adjusted p Benjamini- Hochberg
ALK mutation					
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)		
ERBB4 mutation					
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)		
FGFR2 mutation					
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)		
IDH1 mutation					
No	130 (100%)	66 (94.3%)	1	0.014	0.049
Yes	0	4 (5.7%)	NE		
PDGFRA mutation					
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)		
PREX2 mutation					
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)		
RB1 mutation					
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
Melanoma location						
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		
Histological subtype						
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		
Ulceration						
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				
Mitotic rate						
0/mm ²	37(37.4%)	13(22.4%)	0.48 (0.23-1.01)	0.054		
≥1/mm ²	62(62.6%)	45(77.6%)	1			
Missing	31	12				
Thickness (mm)						
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
Sentinel node biopsy						
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				
Beta blockers						
No	123 (94.6%)	59 (84.3%)	1			
Yes	7 (5.4%)	11 (15.7%)	3.28 (1.21-8.88)	0.02		
Mean sun exposure score (lifelong)	12.3 (±3,42)	10.88 (±2,5)	0.86 (0.78-0.96)	0.004	0.85 (0.71-0.99)	0.049
Mean sunburn score (lifelong)	3.10 (±1.6)	2.57 (±1.5)	0.80 (0.65-0.98)	0.034		
FGFR2 mutation						
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)

