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Vismodegib resistant mutations are not selected in multifocal relapses of locally advanced basal cell carcinoma after vismodegib discontinuation

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Nicole Basset Seguin is an investigator and a consultant for Roche laboratories

Ariel Savina and Fanny Bouquet are employees of Roche Laboratories

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To the editor,

Hedgehog pathway inhibitors (HPI) inactivating SMO [1], have become first line treatment for patients with locally advanced BCC (laBCC). HPI safety and efficacy have been shown in clinical trials[2,3]. Nevertheless, common adverse events lead to treatment discontinuation.

Some laBCC develop acquired resistance (AR) to HPI, illustrated by tumor regrowth under treatment after an initial response. AR is explained by the presence of SMO mutations affecting the binding of the drug or conferring constitutive activation of SMO , (acquired de novo or present before treatment at low frequency and selected during its course) [4,5]. LaBCC patients who discontinued vismodegib after achieving complete remission (CR) frequently develop multifocal relapses, which could harbor vismodegib resistant mutations[6]. We hypothesized that vismodegib resistant clones could lie dormant and regrow after drug withdrawal.

To this end, we studied three laBCC patients who achieved clinically and histologically CR with vismodegib and relapsed after treatment discontinuation. All patients gave their written informed consent for the study, including a non-opposition note and signed agreement for genetic analysis. Frozen or formalin-fixed paraffin-embedded (FFPE) tumor tissue was obtained before vismodegib treatment and after relapse for DNA extraction. DNA sequencing of 21 cancer genes (CDKN2A, CTNNB1, DHH, FBXW11, GLI1, GLI2, GLI3, GSK3B, HHIP, HRAS, IHH, NRAS, PIK3CA, PTCH1, PTCH2, SHH, SMO, STAT5B, STK36, SUFU, TP53) (in-house microarray) was performed using Next Generation Sequencing

(NGS) on PGM sequencer and ThermoFisher technology (Ion PGM™ Hi-Q™ View Chef Kit, ThermoFisher). The preparation of amplicon libraries was made by AmpliSeq. Average sequencing depth was 1125X, and 95% of the target regions were covered. Detection of variants was performed with integrated software dedicated to Ion Torrent technology (Torrent browser and Ion Reporter). Only variants with a high-quality score (p value <0.001) and allelic frequency of a least 0.05 of variant reads were retained.

Multifocal relapses from three laBCC patients who achieved CR with vismodegib and discontinued treatment (Fig.1) were studied. Driver mutations in HP genes were identified: Loss-of-Function PTCH1 mutations in patients 1 and 2 and Gain-of-Function SMO W535L mutation in patient 3 (Table1). That latter was shown to confer partial drug resistance to vismodegib [5]. However CR observed in this patient 3 as well as in 2 other patients with SMO W535L tumors treated in our clinic (data shown) suggest another yet unidentified genomic variants could be implicated in the resistance. TP53 mutations were also observed in patients 2 and 3 (Table 1). All identified mutations are most likely somatic, as they are present in only a fraction of cells (Variant Allele Frequency < 35%), and they inactivate the tumor suppressor gene *PTCH1* or are reported as cancer mutations in the COSMIC database. Another variant not described in BCC was detected in patient 2 in gene hFU (STK36), a positive regulator of the GLI zinc-finger transcription factors [7]. We found no significant differences in the coding regions of sequenced genes in relapsed tumors compared to pre-treatment tumors, especially, no additional SMO mutations (Table1).

Our results suggest that in laBCC, multifocal relapses after vismodegib discontinuation harbor the same mutational pattern than the baseline tumor. These results are interesting as BCCs are amongst the most highly mutated human cancers and could be expected to select drug resistant clones. This suggests that residual disease, after treatment cessation, regrows without the need to acquire further genetic alterations and could be eligible for treatment rechallenge. Accordingly, two of our patients who presented multifocal relapses after treatment discontinuation including the one bearing a SMO mutation were again subjected to vismodegib and achieved apparent clinical CR.

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Tables and figures legends:

Patients	**Patient 1+**			**Patient 1 ++**			**Patient 2++**		**Patient 3+**	
Mutations	Baseline	R 1	R 2	Baseline	R 3	R 4	Baseline	R	Baseline	R
SMO (NM_005631.4)									c.1604G>T (p.Trp535Leu) **33%**	c.1604G>T (p.Trp535Leu) **13%**
PTCH1 (NM_000264.4)	c.1189G>T (p.Glu397*) **12%** c.3153G>A (p.Trp1051*) **11%**	c.1189G>T (p.Glu397*) **20%** c.3153G>A (p.Trp1051*) **22%**	c.1189G>T (p.Glu397*) **10%** c.3153G>A (p.Trp1051*) **10%**	c.1189G>T (p.Glu397*) **14%** c.3153G>A (p.Trp1051*) **15%**	c.1189G>T (p.Glu397*) **7%** c.3153G>A (p.Trp1051*) **4%**	c.1189G>T (p.Glu397*) **4%** c.3153G>A (p.Trp1051*) **4%**	c.3306+1G>T **11%**	c.3306+1G>T **16%**		
STK36 (NM_015690.5)							c.1915-1G>A **12%**	c.1915-1G>A **7%**		
P53 (NM_000546.5)							c.853G>A (p.Glu285Lys) **12%**	c.853G>A (p.Glu285Lys) **15%**	c.855_856 delinsAA (p.Glu286Lys) **34%**	c.855_856 delinsAA (p.Glu286Lys) **15%**

Table 1 : NGS analysis of patient's tumor at baseline and at relapse after drug discountinuation.

* = Stop codon, +Frozen biopsy, ++ FFPE biopsy, R=Relapse. The percentages (%) correspond to the presence of mutation in the tumor

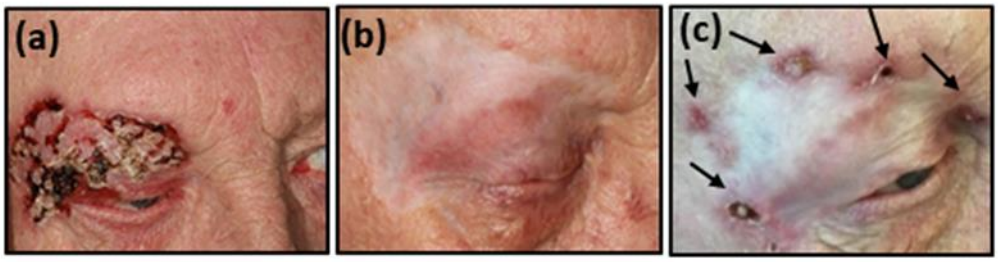

Figure 1:

Illustration of a laBCC and its multifocal relapses in one of the studied patients, a)- Baseline invasive basal cell carcinoma of the upper right eyelid, b)- Complete tumoral remission after 12 months of Vismodegib treatment, c)- Multifocal relapses (indicated by arrows) 1 year after treatment discontinuation..