

1 **JEADV-2019-0507**

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

4 Key words:

5 Basal cell carcinoma, vismodegib, resistance, multifocal relapses, SMO mutations

6 Manuscript word count : 585words

7 Table and figure count : 1 figure and 1 table

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Conflict of interest:

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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Battistella and Sergey Nikolaev have no conflict of interest to declare.

Funding/Support:

Nicole Basset Seguin and Meriem Ighilahriz have received research funding from Roche

laboratories

1 **To the editor,**

2 Hedgehog pathway inhibitors (HPI) inactivating SMO¹, have become first line treatment for
3 patients with locally advanced BCC (laBCC). HPI safety and efficacy have been shown in
4 clinical trials^{2,3}. Nevertheless, common adverse events lead to treatment discontinuation.

5 Some laBCC develop acquired resistance (AR) to HPI, illustrated by tumor regrowth under
6 treatment after an initial response. AR is explained by the presence of SMO mutations
7 affecting the binding of the drug or conferring constitutive activation of SMO, (acquired de
8 novo or present before treatment at low frequency and selected during its course)^{4,5}. LaBCC
9 patients who discontinued vismodegib after achieving complete remission (CR) frequently
10 develop multifocal relapses, which could harbor vismodegib resistant mutations⁶. We
11 hypothesized that vismodegib resistant clones could lie dormant and regrow after drug
12 withdrawal.

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

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

7 Multifocal relapses from three laBCC patients who achieved CR with vismodegib and
8 discontinued treatment (Fig.1) were studied. Driver mutations in HP genes were identified:
9 Loss-of-Function *PTCH1* mutations in patients 1 and 2 and Gain-of-Function *SMO* W535L
10 mutation in patient 3 (Table1). That latter was shown to confer partial drug resistance to
11 vismodegib ⁵. However CR observed in this patient 3 as well as in 2 other patients with *SMO*
12 W535L tumors treated in our clinic (data shown) suggest another yet unidentified genomic
13 variants could be implicated in the resistance. *TP53* mutations were also observed in patients
14 2 and 3 (Table 1). All identified mutations are most likely somatic, as they are present in only
15 a fraction of cells (Variant Allele Frequency < 35%), and they inactivate the tumor suppressor
16 gene *PTCH1* or are reported as cancer mutations in the COSMIC database. Another variant
17 not described in BCC was detected in patient 2 in gene *hFU* (*STK36*), a positive regulator of
18 the *GLI* zinc-finger transcription factors ⁷. We found no significant differences in the coding
19 regions of sequenced genes in relapsed tumors compared to pre-treatment tumors, especially,
20 no additional *SMO* mutations (Table1).

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

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10 **Tables and figures legends:**

Patients	Patient 1+			Patient 1 ++			Patient 2++		Patient 3+	
	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%

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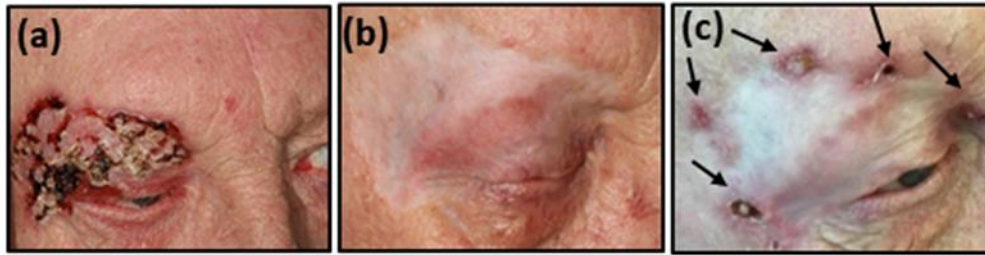
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14 **Table 1** : NGS analysis of patient's tumor at baseline and at relapse after drug
15 discontinuation.

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

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2 **Figure 1:**

3 Illustration of a laBCC and its multifocal relapses in one of the studied patients, a)- Baseline
4 invasive basal cell carcinoma of the upper right eyelid, b)- Complete tumoral remission after
5 12 months of Vismodegib treatment, c)- Multifocal relapses (indicated by arrows) 1 year after
6 treatment discontinuation..

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