Author’s response to reviews

Title: Variation of mutant allele frequency in NRAS Q61 mutated melanomas

Authors:
Zofia Hélias-Rodzewicz (zofia.helias-ext@aphp.fr)
Elisa Funck-Brentano (elisa.funckbrentano@gmail.com)
Nathalie Terrones (nathalie.terrones@yahoo.fr)
Alain Beauchet (alain.beauchet@aphp.fr)
Ute Zimmermann (ute.zimmermann@aphp.fr)
Cristi Marin (cristi.marin@aphp.fr)
Philippe Saiag Saiag (philippe.saiag@uvsq.fr)
Jean-François Emile Emile (jean-francois.emile@uvsq.fr)

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Prof. Ioannis D. Bassukas
University of Ioannina, Greece
Prof. Feroze Kaliyadan
King Faisal University, Saudi Arabia
Xianyong Yin
University of North Carolina at Chapel Hill, USA
Section Editors, BMC Dermatology
Dear Prof Bassukas, Kaliyadan and Yin,

It is our pleasure to resubmit our revised manuscript (BDER-D-17-00003) entitled “Variation of mutant allele frequency in NRAS Q61 mutated melanomas” for your consideration.

We thank you and 2 reviewers for your careful consideration of our initial manuscript submission. We have addressed all the points in our response to reviewers.

In addition to the above, we believe that these data are important to publish because they highlight in a large series of human skin melanomas that the quantity (in percentage) of mutated NRAS are highly heterogeneous and the NRAS Q61 /WT amount are not heterozygous in about 39% of them.

Further studies are needed to evaluate the value of M%NRAS for prediction of response to targeted therapy.

We hope that you find our manuscript suitable for acceptance and thank you for the opportunity to have our work evaluated. Please contact us if you have additional questions or need additional information.

Sincerely yours,

Zofia Hélias-Rodzewicz & Jean-Francois Emile

On behalf of all coauthors

Reviewer reports:

(Reviewer 1): This paper focuses on the NRAS Q61 mutant allele frequency in melanoma. This paper is extremely well written and will add to the general knowledge of somatic mutations in melanoma. The conclusions are drawn appropriately. I see no areas for revision.

We thank the Reviewer for the positive evaluation of our manuscript.
(Reviewer 2): The main conclusion of this study is that NRAS mutant allele frequency is heterogenous in melanoma and only 30% of cases had significantly increased NRAS mutant allele frequency. The most important concern with the data is that there is no validation that the heterogenous NRAS allele frequency is simply not a result of variable tumour cell content. Although the tumour content is noted as being >80%, there is no independent validation of this.

The reviewer is right: the % of tumour cells is a major issue, and we apologize not to have addressed this point in the previous version. In a previous paper on BRAF mutant allele frequencies (MAF) in melanomas (Hélias-Rodzewicz et al. BMC Cancer 2015 15:497), we showed in figure 1a that MAF was closely related to the % of melanoma cells. We also showed that the MAF was no more dependent on % of tumor cells above 80%, and that the inter-observer reproducibility for the evaluation of tumor cell content was substantial for the 80 % cut-off (κ = 0.79), and was only moderate for the 70 % cut-off (κ = 0.49). Thus, we decided to use the cut-off of 80 % of tumor cells in the present paper.

We provide these important arguments within the M&M of the revised version.

Considering the fact that mutation specific NRAS antibodies are now available, I think it is essential that a subset of the tumour samples are rescreened to validate the genotyping data with IHC data of mutant NRAS and melanoma-markers. This will ensure that the genotyping data matches the IHC data, and not simply a result of low tumour content.

We thank the Reviewer for this important point. IHC with an antibody against NRAS Q61R were performed in 40 melanomas of this series and the results were published in 2015 by Ilie and colleagues in J Am Acad Dermatol.

We have added this information in the following sections:

Discussion: "Additionally, the genotyping accuracy of 40 NRAS mutated melanomas, of which 27 had a p.Q61R, were confirmed by immunohistochemistry with an antibody against Q61R [27].

The allele frequencies derived from the TCGA dataset should also be presented as in Figure 2 (potentially as supplementary) for direct comparison with data generated in this work.

We thank the Reviewer for this point.

We have added this information in the following sections:
Results: Among the 85 NRAS mutated cases that were available only 50.6% (43/85) had a heterozygous status of NRAS mutation, while 34.1% (29/85) was High non-HET and 15.3% (13/85) was Low non-HET (additional file 3: Figure S3).

Additional files: Additional file 3: Figure S3. NRAS Q61 mutant allele burden in ATGC melanomas

Histogram representation of NRAS Q61 mutant allele quantity (in percentage) in 85 ATGC NRAS mutated melanomas. The X and Y axis correspond to the percentage of NRAS mutant and to the number of cases, respectively.

It is not clear where the 104 NRAS Q61 mutated melanomas come from here (page 9, line 7)?

We apologize to the Reviewer for the lack of clarity on this point.

We have added this information in the following sections:

Discussion: The frequencies of BRAF V600 and NRAS Q61 mutations were evaluated in 267 FFPE melanoma patients of the series (flow chart in additional file 2: Figure S2) diagnosed between March 2013 and May 2015. NRAS mutation was detected in 48 melanomas, corresponding to 18% (48/267) of all melanomas and 33.8% (48/142) of BRAF V600 wild type cases. Additionally, 63 NRAS Q61 mutated melanomas, diagnosed before this period, were included into the molecular analyses. In total, 111 NRAS Q61 mutated tumors were collected with 60-95% tumor cells.

Quality of Figure 1 is not high enough for easy review

We apologize to the Reviewer for the lack of quality in this figure. The quality was modified and we have added the following modifications to the description of this figure:

FISH: 1 - disomy, 2 - disomy with rare polysomic cells, 3 polysomy (A: 3-4 copy, B: >4 copy), 4 - amplification, 5 - monosomy, 6 - high intra-tumor copy number variations of NRAS/chromosome 1.