Author’s response to reviews

Title: Targeted capture-based NGS is superior to multiplex PCR-based NGS for hereditary BRCA1 and BRCA2 gene analysis in FFPE tumor samples

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Author’s response to reviews:

Reviewer 1 - Gulsah Cecener:
Aim of the study is comparing an adapted targeted capture-based protocol and a multiplex PCR-based approach.

In the manuscript, aim of the study was well described. The method is appropriate for the content and following figures and tables are clear enough to define the way of the study.

Answer to the Reviewer

Thank you for your positive statements.

Reviewer 2:

OBJECTIVE - Full research articles: is there a clear objective that addresses a testable research question(s) (brief or other article types: is there a clear objective)?

Yes - there is a clear objective

DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?

Yes - the approach is appropriate

EXECUTION - Are the experiments and analyses performed with technical rigor to allow confidence in the results?

Yes - experiments and analyses were performed appropriately

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?

No - there are minor issues

OVERALL MANUSCRIPT POTENTIAL - Could an appropriately REVISED version of this work represent a technically sound contribution?

Probably - with minor revisions
GENERAL COMMENTS:

My overall impression is that this is a well-designed study. The authors have shown that their capture-based NGS protocol can reliably identify SNVs, small InDels, as well as CNVs using DNA from FFPE samples. The finding with deceased index patients are especially exciting.

Answer to the Reviewer

Thank you for your positive statements.

ADDITIONAL REQUESTS/SUGGESTIONS:

1. Authors should explain for readers why different minimum read counts are considered for multiplex-PCR based vs. the targeted capture-based approach.

Answer to 1

These thresholds were similarly applied in other studies which are cited in our manuscript. E.g.


To make clearer to the audience why we used these thresholds we added a statement in the result chapter of the manuscript: “We used criteria for the multiplex PCR-based technique routinely applied in daily diagnostic of somatic cancer tissue. Therefore, higher read depths used in the multiplex PCR-based protocol compared to the targeted enrichment-based protocol (500 vs. 30)
is due to necessity for confident identification of somatic variants in tumor specimens because of cancer tissue heterogeneity [26].”


2. There are many typos and some grammatical errors and therefore the manuscript should be gone through to correct these issues.

Answer to 2

The manuscript has undergone a language optimization process. See comprehensive track changes along the entire manuscript.

Reviewer 3 - Matthias Nees:

1. The language used on this manuscript is good but not flawless; especially in the beginning of the text, there are a few grammar issues including e.g. the endings of words (singular/plural). Otherwise, grammar and sentences are okay and acceptable. However, at least one round of additional proofreading is necessary/recommended.

Answer to 2

The manuscript has undergone a language optimization process. See comprehensive track changes along the entire manuscript.

The abstract in particularly is well written and concise, the main message - despite complex patient cohorts analyzed - comes easily across and also appeals to readers not specifically or directly involved in this research.

The same applies to introduction, which summarizes the field of BRCA1/2 biology, genetic research and treatment options in a few short paragraphs; very expertly written in my opinion.

The technical issues introduced in this manuscript are of very real and practical nature, due to the poor and highly variable quality of DNA in paraffin-embedded tissues. The manuscript as such is therefore relevant for the field and should attain significant attention. Moreover, it is written in
a very Hands-on, practical and clear fashion that should be particularly useful for those who adapt, optimize and utilize such laboratory methods in daily clinical practice.

2. Concerning the methods themselves, one may wonder if the originator of the PCR panels (Illumina) has actually compared these or related methods carefully themselves, and if any relevant publications (maybe in the form of posters, short communications, white papers etc) from Illumina exist that would corroborate the findings described here? (This would be interesting for practical reasons again).

Answer to 2

Unfortunately we did not find any information on whether Illumina Inc. performed these or related methods carefully themselves. However, Illumina Inc. recently offered the TruSight Tumor 170 kit which also relies on a targeted capture-based protocol on DNA originating from FFPE tissue and quality checks have been performed demonstrating the robustness of the protocol. We added one sentence in the discussion chapter describing this protocol and its applicability on FFPE tumor material with regards on BRCA1/2 genetic testing in FFPE tissues:

“Recently, Illumina Inc. offered the TruSight Tumor 170 panel targeting 170 genes including BRCA1 and BRCA2. The panel also relies on a targeted capture-based protocol that is applicable on DNA originating from FFPE tissue and might therefore be suitable for our modification steps.”

3. The figures are somewhat difficult to interpret for non-experts in the field, in particular the combined data from 5 or 13 patients in the PCR Panel (Figure 1B, and Suppl. Figure 1B). It is not entirely clear what the red arrows indicate (or description in figure legend is insufficient to clarify). Would the example of just one or 2 exemplary patient data give a more clear representation of the typical differences between the 2 approaches? And illustrate the strength and Advantage of the targeted capture-based NGS?

Answer to 3

We modified the figure 1 by adding the panel 1C showing the normalized coverage differences in low-quality DNAs from FFPE tissue from patients 5 and 10 which better demonstrates the advantages of our modified protocol.

4. It might be more conclusive if 1 or 2 concrete examples that typically affect the quality of genetic testing (and everyone fears) were shown as examples - similar to figure 2, but indicating broader genetic areas. In particular, when based on highly fragmented DNA, from
1 or 2 selected patient samples as discussed in the text. It could be considered if such selected examples might illustrate the power and advantages of the 2 methods more impressively than current figure 1, and suppl. figure 1.

Answer to 4

We generated a novel figure (Figure 2 in the revised manuscript) and incorporated a novel section into the result chapter describing the discussed advantages of our protocol. The figure focusses, as indicated by the novel Figure 1C, on low-quality DNA from FFPE tissues of patient 5 and 10. We focus on the most practical and relevant aspects such as number of zero- and low-covered nucleotides, number of artifacts, polymorphisms and false-positive variants, quality values of the DNA and if the underlying pathogenic BRCA variant was detected or not.

Small issues:

5. There are several small typos or inconsistencies in lines 90 - 92:

90 ….for example in cases where the index patient IS deceased or with suspicion of mosaicism. In addition, testing of tumor tissueS aids interpretation of variants of unknown significance (VUS) with regard to the identification of a possible second hit, represented as loss-of-heterozygosity (LOH) or a second pathogenic somatic variant

Answer to 5

Corrected.

6. line 100: reference inserted wrongly: (respectively[10]-13)

Answer to 6

Corrected.

7. line 104: diagnostic algorithms need to be complemented

Answer to 7

Corrected.