Peer Review File

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Reviewer Comments

1. The study has several limitations. The targeted sequencing analysis only encompasses some of the genes involved in the homologous DNA repair pathway? There are many others in addition to BRCA and PALB2 , principally RAD51 and some in the upstream pathway MRN, OTUB1, MMSET and many other.

Reply 1: Thank you for the constructive feedback, and we certainly acknowledge the limitations of the study, inherent to our methods and available resources. Nonetheless, we consider our NGS panel to be relatively well equipped to study the pathway. As noted in the results (1st paragraph), our 152-gene panel included 14 genes with established roles in the pathway, namely *ATM*, *ATRX*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *MRE11A*, *NBN*, *PALB2*, *RAD50*, *RAD51*, *RAD51B*, *RAD51C*, and *RAD51D*. These genes were selected initially based on known mutational landscape of the pathway at the time of the panel design. Since the completion of this study, a larger NGS panel has been instituted, and the results are subject of a future study.

Changes in the text: No changes were made. Please refer to the results (1st paragraph) and the Supplemental Table 1 (for all 152 genes sequenced).

2. What is the definition of pathogenic HR pathway gene variants? How do you know that such SNPs are conferring higher risk of carcinogenesis?. Are there already known? Are there functional studies as transfection of the variant alleles in cell lines?

Reply 2: The clinical practice of tiering, interpretation and annotation of variants is guided by clinically established system such as the AMP/ASCO/CAP system (as described in the methods). "Disease-associated"/"pathogenic" variants are tiered as such based on "strong clinical significance". For HR genes, clinical significance may be due to known association with familial cancer risks, response to PARP inhibitors, among other lines of evidence. Databases such as BRCA databases and ClinVar provide valuable information with respect to familial cancer risks.

Changes in the text: The method section has been expanded to include further information about variant interpretation practice (see Page 5, line 113-118).

3. What about gene fusions, for example RAD51 gene fusions are well established and described for example in EGFR mutant NSCLC. RAD51 form complexes with BRCA1-PALB2-BRCA2.

Reply 3: With our panel being DNA-based, targeting the coding sequences only (with padding for splice variants), the panel is not suitable for fusion detection. However, additional information may be obtained from our newer NGS workflow, and such are again subject of a future study.

Changes in the text: Not applicable.

What is the difference on what you call SNPs and VUS (variant of unknown significance). Is it possible that what is detected by NGS are VUS?

The "pathogenic "SNPs identified should be detailed in which region domain of the gene are involved.

Reply 3: SNV (single nucleotide variants) refer to single nucleotide differences compared to the reference genomic sequence. SNPs generally denote SNVs of germline derivation. However, the distinction between germline and somatic SNVs are generally not made in the context of tumor-only sequencing assays, such as our NGS workflow. SNVs (which include SNPs) encompass the entire benign-disease associated (/pathogenic) spectrum, including VUS. Indeed, as the reviewer suggests, numerous SNVs were interpreted as VUS, as shown in Supplemental Table 3.

Changes in the text: Please refer to supplemental table 3 for more information on the variants. Also, the method section has been expanded to include further information about the tumor-only sequencing nature of the assay (see Page 5, line 118-119).