Peer Review File

Article information: https://dx.doi.org/10.21037/tlcr-22-594

Review Comments

Comment 1: In the methods, the authors state that "Germline BRCA status was not routinely evaluated for all patients in this cohort. However, we assumed that known founder variants can be considered as germline." It is not clear how this impacts the data presented. Were any of the BRCA mutations detected in this study excluded as germline? The authors state that 12 of the 25 tumors with pathogenic/likely pathogenic BRCA mutations were well-established BRCA founder mutations but go on to define these as somatic BRCA mutations in the figures and discussion. Further explanation of how the authors categorized the BRCA variants for this study would be helpful. The limitations do mention the inability to define the rate of germline BRCA mutations in the cohort, due to the use of commercial NGS testing. Therefore, the BRCA mutations categorized as pathogenic should be consistently referred to as pBRCA throughout the manuscript and figure titles/legends, rather than as being of known somatic origin.

Reply 1:

- In the methods, we modified the text to clarify that germline status was not assessed as part of the study, and therefore none of the 25 pathogenic/likely pathogenic *pBRCA* mutations detected by NGS could be reliably categorized as being of either germline or purely somatic origin.
- In the results, we acknowledge that in 48% of patients with *pBRCA* mutations, the variant was a well-established founder mutation, which might reflect a background germline mutation. Nonetheless, we emphasize that the lack of germline testing precludes making a definitive classification of said variants as being of either somatic or germline origin.
- As suggested, all mentions of pathogenic/likely pathogenic *BRCA* mutations throughout the manuscript have been revised, and these are now consistently referred to broadly as *pBRCA*, without further classification as somatic.
- The title of the manuscript has also been updated to reflect that all pathogenic *BRCA* mutations

Changes in the text:

- Row 122 changed to the following: “Germline *BRCA* status was not evaluated in the study. Therefore, we were not able to reliably categorize any of the pBRCA mutations identified by NGS as being either a manifestation of a background germline BRCA mutation, or of purely somatic origin.”
- Row 155 changed to the following: “BRCA mutation status detected by tumour NGS”

- Row 167 changed to the following: “Notably, in 12 (48%) patients, the pathogenic variant was a well-established BRCA founder mutation, which might potentially reflect a germline origin. However, germline status was not routinely performed in the study, therefore none of the cases could be reliably classified as germline.”
- Row 217 changed to the following: “This study extends the limited literature concerning BRCA status in NSCLC”.
- Row 271 changed to the following: “Notwithstanding the above limitations, our findings do suggest that pulmonary tumours harbouring pathogenic *BRCA* mutations can represent a specific subtype of NSCLC, in which platinum-based therapy as a whole, and chemo-immunotherapy combinations in particular, is an effective strategy
- Supplementary figure 2: heading changed to “Patient distribution by tumor *BRCA* status”
- Table 1: heading changed to “Table 1: Demographic and Disease Characteristics According to Tumour BRCA Mutation Status”
- Table 3: heading changed to: Table 3: Disease Control Rates With Platinum-Based Therapy, According to Tumour BRCA Status”
- Title changed to: “BRCA Mutations Detected by Tumour Next-Generation-Sequencing in NSCLC: Impact on Response to Therapy and Disease Course”

Comment 2: The determination of pathogenicity for BRCA variants in accordance with ACMG guidelines would largely be intended for germline variant classification. A classification strategy using the Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer (PMID: 27993330) may be more appropriate for this study. A statement explaining the rationale for the chosen method of determining pathogenicity for the BRCA variants detected in this study is recommended.

Reply 2:

- We added a description of the process by which *BRCA* variants were classified into four categories of pathogenic potential, in adherence with the Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer. Specifically, a consensus-based approach was used wherein only variants classified as having strong clinical significance or potential clinical significance in both the Clinvar and Varsome databases were considered as such for downstream analysis.

Changes in the text:

- Row 113, the following text was added: For the purpose of classifying BRCA variants by their pathogenic potential, we employed the scheme detailed in the Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer, which includes the following categories: “variants of strong clinical significance”, “variants

of potential clinical significance", "variants of unknown clinical significance" and "benign or likely benign variants"**错误!未找到引用源。**. For each BRCA variant, we performed search queries in both the Varsome and Clinvar databases. A consensus-based approach was used wherein only variants classified as having strong clinical significance or potential clinical significance in both databases were considered as such for downstream analysis. We termed these variants *pBRCA* (pathogenic/likely pathogenic).

Comment 3: - Consistency throughout the manuscript in the use of italics for gene names and in the use of superscript for citations is recommended.

Reply 3:

- As suggested, all gene names through the manuscript are now in italics, and superscript is now used uniformly for citations.

Changes in the text:

- Throughout entire manuscript, all gene names changed to italics, superscript used uniformly for citations

Comment 4: - line 245, should germinal be germline?

Reply 4: text modified as advised from germinal to germline

Changes in the text: row 235: "germinal" changed to "germline"

Comment 5: - While not determined to be statistically significant, how the PD-L1 status was determined for this cohort should be expanded on in the methods section.

Reply 5: In the methods, a section was added under the heading "Molecular Characterization of Tumour Samples", in which we elaborate regarding the measurement and reporting of PD-L1 expression status of tumor samples.

Changes in the text: Row 109, the following text added: "PDL-1 expression level in lung tumor samples was measured using the Dako PD-L1 immunohistochemistry (IHC) 22C3 pharmDx assay. Expression levels were reported as tumor proportion score (TPS) and classified as either negative (<1%), low-positive (1-49%) or positive (≥50%)".

Comment 6: - Table 2 is confusing and is not adequately explained in the results section. Additional specifics about the co-occurring NSCLC driver mutations in these cases would be helpful, as these could also influence the patients' responses to therapy. All abbreviations used in the table should be included in the legend.

Reply 6: We have modified Table 2 as advised. It is now easier to follow and is more comprehensively explained in the results sections, under the heading "*Co-occurring driver mutations".* The table now includes specifics regarding each co-occurring mutation identified in *pBRCA* patients, alongside patient sex & smoking history, response to therapy and PDL-1 status. All abbreviations are included in the legend.

Changes in the text: Row 177, the following text has been added: "Of the 25 patients with a *pBRCA* mutation identified by NGS, 15 (60%) had a co-occurring alteration in at least one of the established NSCLC driver genes *(EGFR, KRAS, ALK, BRAF, MET, ERBB2 or ROS1),* including 13 patients with a characteristic driver mutation. Notably, three *pBRCA* patients had no identifiable driver mutation, and no history of smoking, suggesting a possible oncogenic role for *BRCA* in these patients. Patient-level specifics for *pBRCA* patients, including co-occurring alterations, gender, smoking history, response to treatment (to either platinum-based therapy or single-agent immunotherapy) and PD-L1 expression levels, are shown in Table 2."