Peer Review File

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COMMENT 1: It would be nice to add a Table with an overview of the studies whose data/results you have considered to compile this review. Also, it would be better, if all pertinent data from these studies were included, because sometimes utilization is not uniform. For example, you mention the TMB results of CM-159 nd LCMC3 (line 97-102), but not the TMB results of NADIM, despite the fact that NADIM is mentioned further down regarding the NLR (line 183-186). Similarly, the ctDNA (clearance) results of CM-861 are missing, which is important, because CM-861 is a randomized phase 3 trial.

REPLY 1:

- We added some data. (see Page 3, line 56-58)
  'Currently, there are various ongoing and completed clinical trials (phase I, II and III) underway to assess the role of neoadjuvant ICI-based treatments in patients with resectable NSCLC. (Table 1)'
  'Table 1. Clinical trials of neoadjuvant therapy with ICIs for resectable NSCLC' attached to your mail as a separate file.
- We added TMB results of NADIM. (see Page 5, line 107-108)
  'Additionally, TMB was not found to be associated with survival in the NADIM study (46 patients with stage IIIA NSCLC).'
- We added the ctDNA (clearance) results of CM-816. (CM-861 is a trial about renal cell carsinoma, however CM-816 is a randomized phase 3 trial about our topic) (see Page 9-10, line 215-220)

  'For instance, the Checkmate-816 study, which is the first phase III trial to show a benefit for neoadjuvant ICI (nivolumab) plus platinum-doublet chemotherapy for resectable NSCLC, demonstrated that patients treated with nivolumab plus chemotherapy were more likely to have ctDNA clearance and appeared to be more likely to have a pathological complete response at the time of surgery. (37)'

COMMENT 2: TMB section lines 102-106: there are also nice technical overviews about the relative impact of potential confounders for panel-based TMB, which show that indeed the panel size has the greatest impact (e.g. PMID 32143116), and that there is also considerable spatial variability of the TMB (e.g. PMID 31349062).

REPLY 2:

- We added the results of the two studies mentioned above. (see Page 5, line 110-116)

'A previous study, which analyzed the most important confounders of panel sequencing based measurement of TMB (psTMB) (panel size, germline filtering, biological and technical variance), showed that the limited panel size represented the largest contributor to total psTMB variance. (23) It was also shown that in addition to technical aspects such as germline filtering, the tumour content and spatially divergent mutational profiles within a tumour were relevant factors influencing TMB estimation, revealing limitations of single-sample–based TMB estimations.' (24)

COMMENT 3: TIL section, lines 129-138: for neoadjuvant treatment, the results of the NADIM could be mentioned (Provencio et al, Lancet Oncol 2020, the TIL data are presented in the supplements of the paper). Regarding the potential important of immune cell subsets, there is an interesting finding that specifically a higher proportion of B cells is associated with long-term IO benefit (e.g., PMID 33520406 for NSCLC, also for other tumors, e.g., PMID 31942075, 31942071, 31942077). The study PMID 33520406 also demonstrates the feasibility of TIL/TME-profiling in the routine setting using mRNA assays.

REPLY 3:

- We added the results of the NADIM. (see Page 7, line 148-152)
  'In the NADIM study the found an association between TIL levels in post-treatment surgical specimens and progression-free survival (PFS). They reported that patients with low stromal levels of CD3+CD8+ cells, CD3+PD-L1 cells, or with low tumor levels of CD3+CD8+PD-L1+ cells or CD3+PD-L1+ cells showed all a 100% PFS at both 18 and 24 months.' (22)
- We added the results of the PMID 33520406. (see Page 7, line 154-158)
  'Regarding the potential important of immune cell subsets, a previous study suggested that B cells and total TILes could be complementary predictors of ICI benefit in NSCLC. Because they found that a higher proportion of B cells, CD45+ cells and total TILs was associated with prolonged PFS after ICI treatment. The feasibility of TIL/TME-profiling in the routine setting using mRNA assays was also demonstrated in this study.' (31)

COMMENT 4: Line 31: change "…development of biomarkers…" to "…development of accurate biomarkers…"

REPLY 4:

We have modified our text as advised (see Page 2, line 31).

COMMENT 5: Line 36: "Emerging results from the clinical trials…": please delete the word "the".

REPLY 5:

We have modified our text as advised (see Page 2, line 36).

COMMENT 6: Line 60: “Studies about neoadjuvant ICIs are an ideal setting for exploring **such** biomarkers.” Please elaborate why this is the case.

REPLY 6:

We added some data and modified our text as following. (see Page 3, line 63-65)

‘They are ideal settings for exploring biomarkers due to emerge novel pathways and molecules during studies about neoadjuvant ICIs.’

COMMENT 7: Line 84: Change “may be one of the other …” to “may be another …”

REPLY 7:

We have modified our text as advised (see Page 4, line 89).

COMMENT 8: Line 97: “documented in NSCLC” add Nature. 2013 Aug 22;500(7463):415-21. doi: 10.1038/nature12477.

REPLY 8:

We added Nature. 2013 Aug 22;500(7463):415-21. doi: 10.1038/nature12477 as a referance number 20. (see Page 5, line 102).

COMMENT 9: The authors need to add a figure to make the article more attractive to the readers.

REPLY 9:

We added a figure as Figure 1 (see Page 3, line 68) and figure legends and abbreviations (see Page 22, line 499-505).

Figure 1. ‘Biomarkers under investigation for neoadjuvant immune checkpoint inhibitors in nonsmall cell lung cancer’ attached to your mail as a separate file.