Peer Review File

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Reviewer A:

Comments:

1. In the line 165, the authors says "Several studies have evaluated …" Please cite the studies that you are referring to.

Response: We have included additional references supporting the statement "Several studies have evaluated immunohistochemical (IHC) staining of PD-L1 on tumor cells and tumor-infiltrating immune cells as a biomarker of response to ICIs in the metastatic setting."**. These references are now included in the line 183 of the revised manuscript, page 9 and are listed below:**

1. **Reference 28:** Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 2016;387:1540-50.
2. **Reference 29:** Herbst RS, Garon EB, Kim DW, et al. Long-Term Outcomes and Retreatment Among Patients With Previously Treated, Programmed Death-Ligand 1–Positive, Advanced Non–Small-Cell Lung Cancer in the KEYNOTE-010 Study. J Clin Oncol 2020;38:1580-90.
3. **Reference 30:** Fehrenbacher L, Spira A, Ballinger M, et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet 2016;387:1837-46.
4. **Reference 31:** Rittmeyer A, Barlesi F, Waterkamp D, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet 2017;389:255-65.
5. **Reference 32:** Spigel D, de Marinis F, Giaccone G, et al. LBA78 - IMpower110: Interim overall survival (OS) analysis of a phase III study of atezolizumab (atezo) vs platinum-based chemotherapy (chemo) as first-line (1L) treatment (tx) in PD-L1–selected NSCLC. Annals of Oncology 2019;30:v915.
6. **Reference 33:** Reck M, Rodriguez-Abreu D, Robinson AG, et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. N Engl J Med 2016;375:1823-33.
7. **Reference 34:** Mok TSK, Wu YL, Kudaba I, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. Lancet 2019;393:1819-30.
8. **Reference 35:** Aguilar EJ, Ricciuti B, Gainor JF, et al. Outcomes to first-line pembrolizumab in patients with non-small-cell lung cancer and very high PD-L1 expression. Ann Oncol 2019;30:1653-9.
9. **Reference 36:** Hellmann MD, Paz-Ares L, Bernabe Caro R, et al. Nivolumab plus Ipilimumab in Advanced Non-Small-Cell Lung Cancer. N Engl J Med 2019;381:2020-31.
10. **Reference 37:** Jotte R, Cappuzzo F, Vynnychenko I, et al. Atezolizumab in Combination With Carboplatin and Nab-Paclitaxel in Advanced Squamous NSCLC (IMpower131):

Results From a Randomized Phase III Trial. Journal of Thoracic Oncology 2020;15:1351-60.
11. **Reference 38:** Remon J, Passiglia F, Ahn MJ, et al. Immune Checkpoint Inhibitors in Thoracic Malignancies: Review of the Existing Evidence by an IASLC Expert Panel and Recommendations. J Thorac Oncol 2020;15:914-47.

2. In the line 179 and 180, the authors says "The utility of PD-L1 as a biomarker of response to neoadjuvant ICIs in early-stage NSCLC is being investigated…" Please give us the respective references in the end of this sentence.

Response: We have included supporting references at the end of the statement "The utility of PD-L1 as a biomarker of response to neoadjuvant ICIs in early-stage NSCLC is being investigated." **These references are now cited in line 191 of the revised manuscript, page 9 and are listed below:**

1. **Reference 16:** Forde PM, Chaft JE, Smith KN, et al. Neoadjuvant PD-1 Blockade in Resectable Lung Cancer. N Engl J Med 2018;378:1976-86.
2. **Reference 17:** Kwiatkowski DJ, Rusch VW, Chaft JE, et al. Neoadjuvant atezolizumab in resectable non-small cell lung cancer (NSCLC): Interim analysis and biomarker data from a multicenter study (LCMC3). Journal of Clinical Oncology 2019;37:8503-.
3. **Reference 18:** Cascone T, William WN, Weissferdt A, et al. Neoadjuvant nivolumab (N) or nivolumab plus ipilimumab (NI) for resectable non-small cell lung cancer (NSCLC): Clinical and correlative results from the NEOSTAR study. Journal of Clinical Oncology 2019;37:8504-.
4. **Reference 19:** Bar J, Urban D, Ofek E, et al. Neoadjuvant pembrolizumab (Pembro) for early stage non-small cell lung cancer (NSCLC): Updated report of a phase I study, MK3475-223. Journal of Clinical Oncology 2019;37:8534-.
5. **Reference 20:** Altorki N, Borczuk A, Saxena A, et al. P2.04-92 Neoadjuvant Durvalumab With or Without Sub-Ablative Stereotactic Radiotherapy (SBRT) in Patients with Resectable NSCLC (NCT02904954). Journal of Thoracic Oncology 2019;14:S746.
6. **Reference 21:** Gao S, Li N, Gao S, et al. Neoadjuvant PD-1 inhibitor (Sintilimab) in NSCLC. J Thorac Oncol 2020;15:816-26.
7. **Reference 22:** Shu CA, Gainor JF, Awad MM, et al. Neoadjuvant atezolizumab and chemotherapy in patients with resectable non-small-cell lung cancer: an open-label, multicentre, single-arm, phase 2 trial. Lancet Oncol 2020;21:786-95.

3. In the lines, 182, 183 and 184 please explain the sentence "Interestingly, the investigators reported that post treatment surgical tumor samples showed a greater amount of infiltrating CD8+ T cells and immune cells had a significantly higher PD-L1 expression (16)."

Response: To address reviewer's comment, we have added explanation to the abovementioned sentence **in the lines 193-200, pages 9 and 10 of the revised manuscript, which now reads as follows**:

"PD-L1 staining and multispectral immunofluorescence were performed on tumor sections to analyze PD-L1 expression on tumor and immune cells (16). While MPR was observed in both PD-L1 positive and negative tumors, in one case, the pretreatment biopsy specimen showed PD-L1 negative tumor cells but PD-L1 positive tumor infiltrating immune cells (16). Analysis of the matched posttreatment surgical specimen showed high influx of CD8+ T cells and infiltrating immune cells expressing elevated levels of PD-L1 as compared to the pretreatment biopsy specimen, suggesting perhaps an adaptive immune response (16)."

4. In the lines 246 and 247, please explain the reason of this sentence "Smoking-related NSCLCs have one of the highest TMBs among 247 all cancers (38)"

Response: We have modified the sentence "Smoking-related NSCLCs have one of the highest TMBs among all cancers" to clarify the reason for including this statement and added supporting references. **This information is now included in the lines 276-280, pages 12 and 13 of the revised manuscript. This section of the revised manuscript now reads as follows:**

"Lung squamous cell carcinomas and lung adenocarcinomas, which can be caused by chronic mutagenic exposure (tobacco smoking), have been shown to have one of the highest somatic mutation burden (50), and in some studies, elevated TMB has been found to be associated with clinical benefit of ICIs in patients with advanced NSCLC (51,52)."

1. **Reference 50:** Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. Nature 2013;500:415-21.
2. **Reference 51:** Rizvi H, Sanchez-Vega F, La K, et al. Molecular Determinants of Response to Anti-Programmed Cell Death (PD)-1 and Anti-Programmed Death-Ligand 1 (PD-L1) Blockade in Patients With Non-Small-Cell Lung Cancer Profiled With Targeted Next-Generation Sequencing. J Clin Oncol 2018;36:633-41.
3. **Reference 52:** Carbone DP, Reck M, Paz-Ares L, et al. First-Line Nivolumab in Stage IV or Recurrent Non-Small-Cell Lung Cancer. N Engl J Med 2017;376:2415-26.

5. In the lines 286 and 287, please insert references to the sentence "The T cell receptor (TCR) recognizes tumor neoantigens as peptides bound to major 287 histocompatibility (MHC) molecules.."

Response: We have added references supporting the statement "The T cell receptor (TCR) recognizes tumor neoantigens as peptides bound to major histocompatibility (MHC) molecules**" in the line 310, page 14 of the revised manuscript. The references added are listed below:**

1. **Reference 56:** Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science 2015;348:69-74.
2. **Reference 57:** Ward JP, Gubin MM, Schreiber RD. The Role of Neoantigens in Naturally Occurring and Therapeutically Induced Immune Responses to Cancer. Adv Immunol 2016;130:25-74.
3. **Reference 58:** Schumacher TN, Scheper W, Kvistborg P. Cancer Neoantigens. Annu Rev Immunol 2019;37:173-200.

6. In the section Blood-based biomarkers, page 356, please discuss the possible technologies used to assess the mentioned biomarkers in patient´s bloods.

Response: We have detailed the technologies used to analyze the biomarkers in the peripheral blood of patients and is included in different topics within "Peripheral Blood Biomarkers" section. **This information is now included as shown in the lines below, pages 17-21 of the revised manuscript.**

lines 391-394: "Metrics of the TCR repertoire, including density, diversity, and clonality, were analyzed by sequencing of the CDR3 (complementarity determining region 3) regions in the TCR-β chain involved in antigen binding and correlated with response to therapy (16,18,61,75,76)."

lines 420-423: "In these studies, blood samples were subjected to plasma-based cytokine arrays and analysis of PBMCs (peripheral blood mononuclear cells) by flow cytometry to identify the immunophenotypes of peripheral immune cells potentially associated with efficacy of ICIs (48,79)."

lines 447-449: "Absolute values of circulating blood cells collected during routine laboratory blood draws and ratios of complete blood cell counts have been investigated as potential markers of tumor response to ICIs (79-83)."

lines 496-502: "In some of these studies, ctDNA has been quantified using error-suppressed deep sequencing data which were analyzed for allelic fraction of tumor-derived DNA within the total cell free DNA in the plasma (93). bTMB has been quantified from ctDNA sequencing data using FoundationOne (F1) CDx NGS assay that targets 1.1 Mb of genomic coding sequence and all SNVs (Single nucleotide variants) with allele frequencies of ≥0.5%, excluding driver mutations and identifiable SNPs (Single nucleotide polymorphisms) were counted (91)."

1. **Reference 16:** Forde PM, Chaft JE, Smith KN, et al. Neoadjuvant PD-1 Blockade in Resectable Lung Cancer. N Engl J Med 2018;378:1976-86.
2. **Reference 18:** Cascone T, William WN, Weissferdt A, et al. Neoadjuvant nivolumab (N) or nivolumab plus ipilimumab (NI) for resectable non-small cell lung cancer (NSCLC): Clinical and correlative results from the NEOSTAR study. Journal of Clinical Oncology 2019;37:8504-.
3. **Reference 61:** Zhang J, Ji Z, Caushi JX, et al. Compartmental Analysis of T-cell Clonal Dynamics as a Function of Pathologic Response to Neoadjuvant PD-1 Blockade in Resectable Non-Small Cell Lung Cancer. Clin Cancer Res 2020;26:1327-37.

4. **Reference 75:** Reuben A, Zhang J, Lin HY, et al. T cell repertoire analysis of non-small cell lung cancer patients treated with neoadjuvant nivolumab alone or in combination with ipilimumab (NEOSTAR trial). Journal of Clinical Oncology 2019;37:8532-.
5. **Reference 76:** Reuben A, Zhang J, Chiou SH, et al. Comprehensive T cell repertoire characterization of non-small cell lung cancer. Nat Commun 2020;11:603.
6. **Reference 48:** Oezkan F, He K, Owen D, et al. OA13.07 Neoadjuvant Atezolizumab in Resectable NSCLC Patients: Immunophenotyping Results from the Interim Analysis of the Multicenter Trial LCMC3. Journal of Thoracic Oncology 2019;14:S242-S3.
7. **Reference 79:** Laza-Briviesca R, Cruz-Bermudez A, Casarrubios M, et al. P2.04-10 Biomarkers of Pathological Response on Neo-Adjuvant Chemo-Immunotherapy Treatment for Resectable Stage IIIA NSCLC Patients. Journal of Thoracic Oncology 2019;14:S711.
8. **Reference 80:** Jiang T, Bai Y, Zhou F, et al. Clinical value of neutrophil-to-lymphocyte ratio in patients with non-small-cell lung cancer treated with PD-1/PD-L1 inhibitors. Lung Cancer 2019;130:76-83.
9. **Reference 81:** Jiang T, Qiao M, Zhao C, et al. Pretreatment neutrophil-to-lymphocyte ratio is associated with outcome of advanced-stage cancer patients treated with immunotherapy: a meta-analysis. Cancer Immunol Immunother 2018;67:713-27.
10. **Reference 82:** Soyano AE, Dholaria B, Marin-Acevedo JA, et al. Peripheral blood biomarkers correlate with outcomes in advanced non-small cell lung Cancer patients treated with anti-PD-1 antibodies. J Immunother Cancer 2018;6:129.
11. **Reference 83:** Ren F, Zhao T, Liu B, et al. Neutrophil-lymphocyte ratio (NLR) predicted prognosis for advanced non-small-cell lung cancer (NSCLC) patients who received immune checkpoint blockade (ICB). Onco Targets Ther 2019;12:4235-44.
12. **Reference 93:** Goldberg SB, Narayan A, Kole AJ, et al. Early Assessment of Lung Cancer Immunotherapy Response via Circulating Tumor DNA. Clin Cancer Res 2018;24:1872-80.
13. **Reference 91:** Gandara DR, Paul SM, Kowanetz M, et al. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. Nat Med 2018;24:1441-8.

7. Again, page 420, please insert reference to "investigated as potential markers of tumor response to ICIs."

Response: We inserted references at the end of the sentence **"**Absolute values of circulating blood cells collected during routine laboratory blood draws and ratios of complete blood cell counts have been investigated as potential markers of tumor response to ICIs.**" in the line 449, page 19 of the revised manuscript, as listed below:**

1. **Reference 79:** Laza-Briviesca R, Cruz-Bermudez A, Casarrubios M, et al. P2.04-10 Biomarkers of Pathological Response on Neo-Adjuvant Chemo-Immunotherapy Treatment for Resectable Stage IIIA NSCLC Patients. Journal of Thoracic Oncology 2019;14:S711.
2. **Reference 80:** Jiang T, Bai Y, Zhou F, et al. Clinical value of neutrophil-to-lymphocyte ratio in patients with non-small-cell lung cancer treated with PD-1/PD-L1 inhibitors. Lung Cancer 2019;130:76-83.

3. **Reference 81:** Jiang T, Qiao M, Zhao C, et al. Pretreatment neutrophil-to-lymphocyte ratio is associated with outcome of advanced-stage cancer patients treated with immunotherapy: a meta-analysis. Cancer Immunol Immunother 2018;67:713-27.
4. **Reference 82:** Soyano AE, Dholaria B, Marin-Acevedo JA, et al. Peripheral blood biomarkers correlate with outcomes in advanced non-small cell lung Cancer patients treated with anti-PD-1 antibodies. J Immunother Cancer 2018;6:129.
5. **Reference 83:** Ren F, Zhao T, Liu B, et al. Neutrophil-lymphocyte ratio (NLR) predicted prognosis for advanced non-small-cell lung cancer (NSCLC) patients who received immune checkpoint blockade (ICB). Onco Targets Ther 2019;12:4235-44.

8. In pages 496 – 502, please add an explanation to the interaction of microbioma and immune-response.

Response: We have shortened and summarized the microbiome section in the revised manuscript in response to reviewer B, comment 1. We have included information to highlight the interplay between gut microbiome and immune response in the context of ICI therapy, **as shown in the paragraphs below that are included in the "Host-based microbiome" section, page 23 of the revised manuscript:**

Lines 536-538: "Favorable microbiome species may improve the efficacy of ICIs by activating dendritic cells and increasing CD8+ T cell recruitment to the tumor microenvironment (103), inducing IL-12-dependent Th1 immune response (104), and augmenting T cell responses (105)."

Lines 540-545: "FMTs from responding patients to mice or oral administration of microbiome in combination with FMT from nonresponding patients reinstituted response to ICI therapy by inducing tumor infiltration of CR9+CXCR3+CD4+ T cells through an IL-12-dependent signaling pathway (106). PD-1 blockade triggered local and systemic recall of Th1 immune response against specific microbiome that may enhance cancer immunosurveillance (106)."

Lines 546-549: "In a cohort of Chinese patients with advanced NSCLC, responders to PD-1 inhibition showed greater gut microbiome diversity at baseline and a stable microbiome composition during treatment (107). The authors also found that systemic memory CD8+ T cells and NK cells exerted an antitumor treatment effect (107)."

9. Page 511, insert references to "Studies have shown that oral…"

Response: We have shortened the host-based biomarker section in the revised manuscript in response to reviewer B, comment 1. Therefore, the sentence this reviewer is referring to has been removed in the revised manuscript.

<u>Reviewer B:</u>

Comments:

The authors report a short summary of the current evidence of the efficacy of neoadjuvant ICI in early stage NSCLC patients and summarize the current evidence of potential predictive

biomarkers in this setting. The manuscript is well written, sometimes with additional information not necessary and some references are not updated. I have the following questions / comments:

1. The manuscript is too long and sometime the authors report information out of the scope of the current review. Microbiome section is like a review of this topic and it is not put into the context of current early stage NSCLC (no evidence). It is not necessary summarize all current evidence in metastatic melanoma / NSCLC it is out of the scope of this manuscript.

Response: The microbiome section has been significantly summarized to focus on its emerging role as potential biomarker of ICI efficacy in NSCLC. The text in the "Host-based biomarkers" **section has been modified to reflect these changes on page 23 of the revised manuscript as follows:**

"Accumulating evidence suggests that the composition of bacteria residing in the gut may play a key role in determining the efficacy of anticancer therapy, including ICIs (100,101). Gut microbial-derived metabolites have been shown to have a profound effect on systemic immune function (102). Favorable microbiome species may improve the efficacy of ICIs by activating dendritic cells and increasing CD8+ T cell recruitment to the tumor microenvironment (103), inducing IL-12-dependent Th1 immune response (104), and augmenting T cell responses (105). In patients with metastatic NSCLC and RCC, administration of antibiotics along with anti-PD-1/PD-L1 therapy altered gut microbiome and deterred tumor responses to ICIs (106). FMTs from responding patients to mice or oral administration of microbiome in combination with FMT from nonresponding patients reinstituted response to ICI therapy by inducing tumor infiltration of CR9+CXCR3+CD4+ T cells through an IL-12-dependent signaling pathway (106). PD-1 blockade triggered local and systemic recall of Th1 immune response against specific microbiome that may enhance cancer immunosurveillance (106).

In a cohort of Chinese patients with advanced NSCLC, responders to PD-1 inhibition showed greater gut microbiome diversity at baseline and a stable microbiome composition during treatment (107). The authors also found that systemic memory CD8+ T cells and NK cells exerted an antitumor treatment effect (107). While these early correlative studies demonstrate associations between gut microbiome composition and tumor responsiveness to ICIs, investigative efforts are ongoing to evaluate the impact of gut microbiome on efficacy of neoadjuvant ICIs and ICI-related toxicity in patients with early-stage NSCLC (NCT03158129)."

2. Page 5 line 111, neoadjuvant meaning has been already defined in the previous paragraph.

Response: We have deleted the definition of neoadjuvant in the line 123, page 6 of the revised manuscript.

3. Ref 19. The study with neoadjuvant sintilimab has already been published by Gao et al (J Thorac Oncol. 2020 May;15(5):816-826. doi: 10.1016/j.jtho.2020.01.017). Please check reference. The same for reference 22 al ready published in Lancet of Oncology

Response: We have updated references 19 and 22 which are numbered as **references 21 and 22, respectively, in the revised manuscript, as listed below.**

1. **Reference 21:** Gao S, Li N, Gao S, et al. Neoadjuvant PD-1 inhibitor (Sintilimab) in NSCLC. J Thorac Oncol 2020;15:816-26.
2. **Reference 22:**. Shu CA, Gainor JF, Awad MM, et al. Neoadjuvant atezolizumab and chemotherapy in patients with resectable non-small-cell lung cancer: an open-label, multicentre, single-arm, phase 2 trial. Lancet Oncol 2020;21:786-95.

4. In page 6 and 7 authors report the MPR for nivolumab and sintilimab but MPR and pCR for atezolizumab. It would be clearer if authors report both potential predictive markers for all trials.

Response: To address reviewer's comment, we have included both the MPR and pCR rates reported in the trials discussed **on pages 7 and 8 of the introduction section of the revised manuscript, as detailed in the text below:**

Lines 144-148: "In the first feasibility study (NCT02259621), two doses of neoadjuvant nivolumab (anti-PD-1 antibody) induced a 45% MPR rate in 20 resected patients with NSCLC with no major delays in surgery (16). Three patients achieved pathologic complete response (pCR; 0% viable tumor cells), however, in one of them, residual tumor was seen in hilar lymph nodes (16)."
Lines 151-154: "In a neoadjuvant study evaluating the PD-1 inhibitor sintilimab (anti-PD-1 antibody) in 37 Chinese patients with resectable NSCLC, two doses of neoadjuvant therapy induced an MPR rate of 40.5% in patients, including 16.2% with pCR in the primary tumor and 8.1% in the lymph nodes (21)."
Lines 149-159: "Initial results from the first phase II randomized, single-institution study, NEOSTAR (NCT03158129), which tested neoadjuvant nivolumab as single agent or combined with ipilimumab (anti-cytotoxic T-lymphocyte-associated protein 4 [CTLA-4] antibody) in 44 patients with resectable NSCLC, revealed that three doses of nivolumab monotherapy produced a 17% MPR rate, including a 9% pCR rate, whereas combination therapy induced a 33% MPR rate, including a 29% pCR rate, in the intention-to-treat patient population (18)."
Lines 159-163: "ICIs are now being tested in combination with platinum doublet chemotherapy in patients with resectable NSCLC and early results have demonstrated MPR rates ranging between 57% and 83% following chemotherapy plus PD-1/PDL-1 inhibition, and pCR rates reported between 33% and 59% in two studies (22,23)."

5. I would suggest deleting the entire paragraph about KN024 and KN042 and just saying that in metastatic setting PD-L1 correlates with outcome.

Response: The entire paragraph describing the KN024 and 042 studies has been deleted, and we have summarized the results of PD-L1 and outcome from the studies in the metastatic setting **in the lines 187-189, page 8 of the revised manuscript, as reported below:**

"In patients with metastatic NSCLC, higher levels of tumor PD-L1 expression correlated with improved outcomes to pembrolizumab (anti-PD-1 antibody) treatment (33,34)."

6. In the biomarkers section the authors explain again the NEOSTAR trail, and it has been previously explained in the introduction section. Please check it.

Response: We have deleted the repeated description of the NEOSTAR trial in the biomarker section of the revised manuscript.

7. I would suggest deleting or modifying the paragraph regarding LVI. These patients did not receive ICI. Probable the most relevant of this study is that dual high: PDL1 and TMB correlate with higher intratumoral densities of CD3 and it could be a negative prognostic factor for ICI efficacy.

Response: We agree with this comment in that patients in the cohort analyzed by Mitchell et al. were not treated with ICIs, and, therefore, the description of LVI as a potential biomarker for neoadjuvant immunotherapy is not supported by this study. This section has been deleted from **page 13 of the revised manuscript. The revised section now reads as follows:**

"Some small-scale studies of neoadjuvant ICIs have investigated the association between tissue TMB and responses to therapy. In the pilot study of 21 patients with resectable NSCLC treated with neoadjuvant nivolumab, MPR was significantly associated with TMB on pretreatment tumor biopsies (16). Patients with MPR also had significantly higher TMB than those without MPR (16). The candidate mutation-associated neoantigens (MANAs) predicted to be produced as a result of somatic gene alterations correlated with tumor pathologic regression, and a greater number of predicted MANAs in pretreatment tumors was associated with a lower percentage of residual viable tumor after neoadjuvant nivolumab (16). In the LCMC3 study (NCT02927301), TMB at baseline or surgery was not found to correlate with MPR or pathological tumor regression following neoadjuvant atezolizumab, and no significant associations were noted between tumor genomic aberrations and MPR (17). Additional larger studies are needed, and it is critical to consider that several factors may limit the use of TMB as a biomarker of response to neoadjuvant ICIs, including the lack of standard threshold for high TMB, variability in the approaches to genetic sequencing, and the turnaround time required for tumor sequencing results."

8. Please define ITH

Response: We have defined ITH **in the lines 302-304, pages 13 and 14 of the revised manuscript and included a reference, as follows**:

"neoantigen ITH (Intratumoral heterogeneity), which refers to genetic and biological diversity within single tumor specimen as a result of tumor cell evolution (55),…"

Reference 55: Jamal-Hanjani M, Quezada SA, Larkin J, et al. Translational implications of tumor heterogeneity. Clin Cancer Res 2015;21:1258-66.