Peer Review File

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Reviewer A:

Comment 1: The role of B cells in lung cancer should be compared to the role of B cells in other solid cancer types. In particular, commonalities and differences in B cell biology between lung cancer and other cancer types should be discussed.

Reply 1: Thank you to the reviewer for this comment. We agree that it is important to describe the role of B cells in other solid cancer types and contrast with lung cancer. In particular, it highlights the complexity of B cell biology across all solid tumors, and the need to advance knowledge in this area. We have expanded our discussion to include review of B cell infiltrates in solid cancers including breast cancer and melanoma (revised manuscript page 8).

Comment 2: Lung cancer is a histopathological inhomogeneous disease including adenocarcinoma and squamous cell carcinoma as the most important subtypes of NSCLC. Lung cancer subtypes and possible relations to the immune tumor microenvironment should be discussed more carefully.

Reply 2: We thank the reviewer for this insightful comment and agree that a hallmark of lung cancer is its heterogeneity in histological, molecular and likely immunological characteristics. With regards to the latter, this continues to be a relatively evidence-poor area. We have revised the manuscript to include an expanded description and discussion (revised manuscript pages 10-11).

Comment 3: Recently, B cells have been described as predictive marker for response to immune checkpoint blockade in sarcoma, melanoma and renal cell cancer (Petitprez et al., Nature 2020; Helmink et al., Nature 2020). What are the implications for lung cancer? Any similar results for lung cancer available?

Reply 3: Thank you to the reviewer for this comment and suggested recent references. While B cell density has been reported as a potential predictive marker of response to immune checkpoint blockade in some solid tumors, similar findings are yet to be observed in lung cancer. This is an important point, as it highlights the important role of B cells in other solid cancers and that the specific characterization of B cells in lung

cancer microenvironment is yet to be thoroughly investigated and should be specifically pursued in NSCLC. Untangling the role(s) of B cells in NSCLC will reveal specific pathophysiological mechanisms of disease as well as opportunities to exploit anti-tumorigenic properties of B cells and thus has great potential for targeted therapies. We have edited the manuscript to include a review of these studies and implications for NSCLC (revised manuscript pages 19-20).

Comment 4: Figure 1: An arrow is missing between the pictograms of Th1 and CTL cells. Please explain why these are printed next to each other without an arrow in case that this is intended.

Reply 4: We have revised our figure to incorporate other changes and adjusted the CD8 T cell and Th1 juxtaposition to avoid confusion. Cytotoxic T cells and Th1 T cells can exert their anti-tumor effects via different activation pathways, that both involved B cells (plasma cells via dendritic cell activation for CD8 T cells and B cells acting as antigen presenting cells where they present tumor antigens to CD4+T cells).

Comment 5: The authors focus on reviewing results related to B cell infiltrates in the tumor microenvironment. However, processes in the lymph nodes as well as the migration of immune cells should be also addressed as a part of the immune response to tumors and in particular as important part of B cell functionality.

Reply 5: We agree that secondary lymphoid tissue are dynamic structures and processes in lymph nodes as well as migration and trafficking of immune cells in the tissue microenvironment is a critical part of tumor immunity and have amended our review to include discussion (page 9). Movement of B cells and other immune cells in response to immunological threat is a carefully regulated process, balanced by coordinated expression of chemokine and chemokine receptors expression to ensure appropriate migration and positioning of immune cells in tissues. Expression of chemokines/chemokine receptors mediate anti-tumor immunity by directing the migration of B cells and other leukocytes to the tumor site. The outcome of this migratory response is context-specific, as it may involve trafficking of leukocytes that have tumor-promoting or anti-tumor activities. Chemokine-mediated regulation of immune cell recruitment into tumor sites as well as emerging evidence to support the direct targeting of chemokines/chemokine receptors as a strategy to disrupt tumor promoting microenvironment towards anti-tumor immunity was recently reviewed,

including potentially inhibiting expression of CCR4 to deplete regulatory T cells in lung cancer (Velgilm et al., Front Immunol 2019; Poeta et al., Front Immunol 2019).

Reviewer B:

Comment 1: page 3, line 2: 80% metastatic NSCLC is very high, to my knowledge the percentage is slightly over 50%. The cited reference is also very old (2013), I suuggest correcting the statement based on a more recent source. Also, the life expectancy < 12 months for metastatic disease is too low. Even if we consider chemotherapy only, with modern chemotherapy, median life expectancy is 12-15 months, but nowadays patients generally get chemoimmunotherapy with median OS 20-22 months. For TKI life expectancy is even longer. A possible adaption of the passage could be "where life expectancy under chemotherapy has traditionally not exceeded 1-1.5 years".

Reply 1: Thank you to the reviewer for these comments and the opportunity to amend our statements. Indeed 57% of patients are diagnosed with distant metastases, and 22% are diagnosed with regional metastases (Siegel RL et al., CA Cancer J Clin 2020). The manuscript has been revised accordingly with this updated reference. We thank the reviewer for the suggested re-phrasing and the manuscript has been revised accordingly (revised manuscript page 3).

Comment 2: The abbreviation AID on page 5 is not explained (activation-induced cytidine deaminase).

Reply 2: This oversight has been corrected (revised manuscript page 6).

Comment 3: page 14: rituximab is no standard treatment for solid cancers or T-NHL, the phrase "B cell depletion with rituximab is widely used and ..." should be changed. For example, "B cell depletion with rituximab is widely used for B-cell malignancies, but has also demonstrated some anti-tumor activity is small series of patients with other malignancies, like colon cancer, melanoma, or cutaneous T-cell lymphoma". I would also suggest to to amend the last sentence of the first paragraph on page 16 to "Furthermore, in therapy-resistant metastatic melanoma patients, depletion of B cells by anti-CD20 antibody may result in tumor control (72)."

Reply 3: Thank you to the reviewer for these comments and the suggested re-phrasing. The manuscript has been revised accordingly (revised manuscript page 17):

“B cell depletion via anti-CD20 monoclonal antibody, Rituximab, is widely used for B-cell malignancies, but has also demonstrated anti-tumor activity in small series of patients with other cancers such as melanoma, colorectal carcinoma, and cutaneous T cell lymphoma (68-70)”

Comment 4: page 15, section "B cells can predict response to anti-cancer therapies": an additional aspect is missing here, namely the predictive value of tumor-infiltrating B cells for benefit from immune checkpoint inhibitors. Data about this are accumulating for various cancers (e.g. PMID 31942075, PMID: 29892065).

Reply 4: Thank you to the reviewer for this comment and references. As discussed above characterization and specific investigation of B cells in other solid tumors has revealed avenues for novel application of therapeutics. These finding are not directly translated to lung cancer in the absence of the careful characterization of B cells and subsets proposed in the current review. The manuscript has been revised and relevant references added (revised manuscript pages 19-20).

Comment 5: "The importance of B cells in the anti-cancer immune response is supported by long term follow up of CD20 deletion with the chimeric anti-CD20 monoclonal antibody, Rituximab, in patients with lymphoma, where CD20 depletion was an independent risk factor for the development of secondary solid malignancy (22)." -> I suggest modifying this sentence. The mentioned study looked specifically to NHL patients receiving high-dose chemotherapy with stem-cell support. Several other studies examining conventional chemotherapy together with rituximab did not find an increasing risk of cancer associated with rituximab (e.g. PMID 25750347 and PMID 26681685).

Reply 5: Thank you to the reviewer for this insightful comment. We have chosen to remove the paragraph described by the reviewer as we agree that references to CD20 depletion are more thoroughly addressed in the section “Therapies depleting or inhibiting B cells” where we acknowledge and describe the conflicting responses to Rituximab.