Peer Review File

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Reviewer A:

Comment 1: *Line 45: The authors should consider that the NK cell antitumor activity has become a relevant therapeutic approach primarily in hematological cancers. Please add comments.*

Reply 1: In this review, we have directed the discussion towards solid tumors to be in-line with findings applicable to lung cancer. To make this point clearer, we have included in line 48 "…NK cells are a key cytotoxic immune mediator in controlling the dissemination of solid tumors."

Comment 2: *Lines 56,57: NK cells do not kill through the release of cytokines such as TNFalpha, but they play a regulatory role by releasing this and others (IFNgamma, GM-CSF) soluble factors. Please edit the sentence.*

Reply 2: We thank the reviewer for highlighting this and we have made our points clearer. We introduce TNFalpha as an indirect mode of cell death, which has been clarified on lines 58-59 "…and indirectly induce apoptosis through the release of tumor necrosis factor…".

Comment 3: *Lines 59,60: The authors should rephrase the sentence: ".. such as interleukins and interferons, recruiting an antigen-specific response to the site of infection or disease" because it is incorrect. ... Please add comments about this point and references.*

Reply 3: We have re-worded the section on lines 60-62 to clarify the sentiment: "Moreover, NK cells play a broad immunomodulatory role through the secretion of cytokines, interleukins and interferons to mobilize the innate and adaptive immune response to the site of infection or disease."

Comment 4: *Lines 64,65: Only CD56bright NK cells (expressing CCR7 can directly migrate from blood to lymph nodes. Please modify this sentence.*

Reply 4: We have updated the manuscript two-fold to clarify this point. We have removed the sentence related to migration "Upon maturation in the bone marrow NK cells are circulated around the body and depending on chemokine receptor expression trafficked to specific destinations such as secondary lymphoid organs." and included an explanation of CD56 NK cells on lines 55-57 "Functional human NK cells are identified by flow cytometry as $CD3^{-}CD56^{+}$ and are categorized as cytotoxic ($CD56^{dim}CD16^{+}$) or cytokine producing ($CD56^{bright}CD16^{-}$)"

Comment 5: *Lines 67-70: Please explain better these sentences.*
Reply 5: We thank the reviewer for highlighting these difficult sentences. We have reworded lines 70-76 to provide more clarity: "The large trNK cell population are reliant on cytokines, such as Interleukin 15 (IL-15) for their survival and development, which is secreted by bronchiolar epithelial cells in the lung. Unlike the majority of trNK cells, NK cells that reside in the lung are predominantly in the cytokine producing state, likely due to prolonged IL-15 exposure."

Comment 6: *Lines 71,72: Note that mature NK cells are CD56dim CD16+ NK cells and fully mature NK cells are CD56dim CD16++ KIR+ NKG2A- CD57+ NK cells. CD56bright NK cells are immature NK cells. Please edit the sentences accordingly.*
Reply 6: We have addressed this comment through omission of the word "mature". Use of the terminology "cytokine-producing" or "cytotoxic" NK cells is more consistent and aligns with leaders in the NK field (Guillerey, C., Huntington, N.D. and Smyth, M.J. (2016) *Targeting natural killer cells in cancer immunotherapy.* Nature Immunology 17, 1025–1036).

Comment 7: *Lines 77-96: The authors should differentiate human NK cell receptors (e.g. KIRs, LILRB1, CD94/NKG2A, NCRs, NKG2D, DNAM-1, 2B4, NTBA,..) from mouse NK cell receptors (e.g. Ly49). Furthermore, NKG2D is an activating NK cell receptor and not a ligand for NK cell activating receptors and recognize not only MIC-A/B but also ULPB1-4. The main activating NK receptors are NCRs. The ligands for these receptors have been recently identified. For example, the main NKp30 lIgand is B7/H6 that may be also present as a soluble form in the tumor microenvironment.*
Reply 7: We agree with the comments raised, and have now modified this paragraph to (1) distinguish better between human and mouse proteins and (2) clarified the ligand/receptor interactions. This paragraph is line 79-100 in the revised version of the manuscript.

Comment 8: *Line 170: Note that IL-15 activates NK cells whereas IiFNgamma and TNFalpha are released by NK cells. Please clarify this point in the sentence.*
Reply 8: We have clarified this point by removing IFNg and TNFa from the sentence on line 173-175 "Cigarette smoke reduces the production of IL-15, thereby impairing NK cell cytolytic capabilities".

Comment 9: *Figure 2: Please check the abbreviations (e.g. SASP). Please explain better each symbol in the figure.*

Reply 9: We thank the reviewer for highlighting the figure legend. We have included a detailed figure legend for Figure 2 and have confirmed the correct abbreviations.

Comment 10: *Line 356 and subsequents (NK cell therapy). In this section, the authors include a comprehensive list of clinical trials, including ongoing and completed studies. In order to improve navigation within the data, the authors might add a table with the agent, setting (tumor), trial phase, number of patients and observed outcome (for completed trials) or planned endpoints (for ongoing trials).*
Reply 10: We agree that a table would better summarize this section of the review. Table 2 has now been added to the revised manuscript.

Comment 11: *Line 388: The authors should include more specific clinical data; while no response were observed, we should take into account that cytokine release syndrome was observed, which needed immune-modulatory treatments.*
Reply 11: This clinical observation has been included on lines 391-393 "Patients additionally exhibited cytokine release syndrome, which required additional immune-modulatory treatment. Though no objective response was observed, the trial has established a clinical potential of rhIL-15 in enhancing NK cell activity".

Comment 12: *Line 390: "Nivolumab NCT03388632; Ipilimumab NCT03388632; Avelumab NCT03905135"; please specify the malignancies treated in these ongoing trials*
Reply 12: We have now included the indications these trials refer to on lines 395-396 "Nivolumab and/or Ipilimumab in metastatic solid tumors: NCT03388632; Avelumab in T-cell malignancies: NCT03905135".

Comment 13: *Lines 395-398: with regards to ALT-803, the authors conclude that the trial results are suggestive for efficacy in NSCLC. While encouraging, such data are collected from a phase Ib trial with a limited number of patients and the main findings of increasing doses of ALT-803 involve safety; hence, suggesting efficacy might sound a bit over-optimistic. I suggest to be more cautious and specify that the finding are compatible with a manageable safety profile and suggest that the treatment may be active at the RP2D, although further phase II data are pending. I would also specify that we are considering non-small cell lung cancer, rather than the generic "lung cancer".*
Reply 13: We have toned down the language as suggested and specified NSCLC on lines 401-402 "…3 subjects exhibited a partial response to the combination with ALT-803, suggesting that the IL-15 super-agonist may be efficacious in NSCLC" and line 405 "…highlighting the potential utility".

Comment 14: *Line 410: galunisertib. Actually, as the reported data are from a single-arm phase II trial, we cannot conclude that galunisertib improved OS in HCC patiants; we can, however, conclude that the drug is manageable and active in HCC, and that the magnitude of benefit is associated with TGF-B and AFP levels.*
Reply 14: We have toned down the wording in relation to Galunisertib on line 416 "…galunisertib treatment is manageable and active in…".

Reviewer B:

Comment 1: *There are many kinds of NK cells (haploidentical NK cells, umbilical cord blood NK cells, stem cell-derived NK cells, NK cell lines, adaptive NK cells, cytokine-induced memory-like NK cells and chimeric antigen receptor NK cells). The authors should mention the NK cell categories while discussing the NK cell function and immune therapy.*

Reply 1: We thank the reviewer for their contribution. We have included a new table (**Table 2**) to the revised version of the manuscript, which highlights the clinical trials in which these NK cells have been used.