Reviewer #1 (Remarks to the Author):  
  
In this manuscript, authors analyzed expression profiles of flow sorted lymphoid and myeloid single cells of tumor and peripheral blood from three treatment-naïve ccRCC patients using single cell RNA-seq (scRNA-seq) technique. Based on cell clustering method, cell types were identified and characterized to identify sub-populations of immune cells. Each sub-cluster were described extensively with the gene expression profile. Finally, authors proposed that the immune cells in tumor microenvironment of clear cell renal cell carcinoma (ccRCC) might be important for the response of immunotherapy on ccRCC. Although this paper covers immune cell profiles of ccRCC, supporting evidence on their conclusion was not fully demonstrated.   
  
**1. Three ccRCC patients seems to be not enough to generalize their findings as an immune landscape of ccRCC. At least, authors need to validate the pattern of sub-population of immune cells in another pool of patients with immunohistochemistry or other method.**

We have added additional analysis and data sources. After identifying proliferative CD8+ T cells as a poor prognostic marker, we used mass CyTOF data from Sade-Feldman et alia 2018 to identify the cell population across 68 patients with enrichment in advanced-grade diseases (Figure 6).

**2. Authors need to check their findings described as a specific to tumor in matched normal tissue immune cells. Immune profile in tissue and peripheral blood should be different even in healthy normal people.**

**3. Authors also need to compare healthy normal PBMC for their conclusion on ccRCC-specific feature of peripheral immune of patients.**

We have added scRNA-sequencing immune cell results from a healthy peripheral blood and 3 normal kidney tissues (a total of 11,382 more cells). At a global level, we observed an increase in unique myeloid cells in normal renal tissue (Figure 1B) and tumors were enriched for populations with expression consistent with CD8+ T cells and macrophages (Figure 1B). We did not observe a notable difference in healthy vs ccRCC peripheral blood samples at the global level. The overlap of all the samples can be visualized in Supplemental Figure 1.

**4. What was unique immune feature of ccRCC? Is it possible to compare the distribution of immune sub-population with other cancer types from public dataset?**

This is an excellent question, especially as ccRCC seems to be unique in the immunotherapy-responsive solid tumors. Although beyond the scope of this specific project, we and others are looking into just that. We hope our publicly provided data will allow for additional analysis on this subject.

Reviewer #2 (Remarks to the Author):  
  
Vishwakarma et al use single-cell RNA and TCR sequencing to survey the immune populations present in the tumor and peripheral blood of patients with clear cell renal cell carcinoma (ccRCC). They find that CD4+ and CD8+ T cell subpopulations are more heterogeneous in tumor compared to blood. Furthermore, the exhausted CD8+ T cell population was the most abundant in all three patients and was found exclusively in the tumor but not peripheral blood. TCR analysis revealed a substantial amount of overlap of TCRs between T cell subpopulations and between tumor and blood. The authors also describe the heterogeneity of the myeloid population within the tumor and blood, identifying both anti- and pro-inflammatory macrophage subsets, classical and non-classical monocytes, as well as monocyte-derived DCs. Overall, this is an important paper, which will be of great interest to the field and serve as a useful dataset for many future studies. The analyses were performed well and described  
thoroughly in the methods to enable reproducibility. We have a few suggestions that may improve the manuscript.   
  
Major comments:  
**1. The pseudotime trajectory analysis of CD8+ T cell differentiation (Fig 2E) is interesting. One question that follows is the characterization of the cells around the branch point between exhausted and proliferative cells. Looking at the colors by eye, there appears to be an enrichment of cluster 10 cells at the branch point, although cells from this cluster are also spread along the projection from naïve to the branch point. It would be helpful to include a visualization of each cluster separately to show where cells from each cluster lie along the trajectory. Furthermore, it would be informative to incorporate the finer effector/memory T cell clusters (e1, e2, cm, rm1-3) into this pseudotime analysis. This would allow for more specific interpretation of which factors might be important in specifying T cell fate, which could be a step closer towards a more mechanistic understanding of T cell exhaustion in ccRCC.**

As part of the suggestions by other reviewers, we have added additional samples and re-performed the trajectory analysis (Figure 3E), however, we do find similar divergence between exhausted and proliferative CD8+ T cells. We have changed the visualization to overlay onto the UMAP plot itself, to increase ease for readers.

**2. The authors state that “enriched TCRs were more common in intratumoral CD8+ T cells compared to single TCRs in peripheral blood.” However, in Fig. S5A, the difference between % CD8+ TCRs expanded in tumor and peripheral blood does not appear to be significant. Perhaps the authors could clarify this point.**

We have substantially modified the clonotype analysis for both CD4+ and CD8+ T cells (see figure 2) and the discussion of clonotype analysis in the manuscript. We see a relatively stable clonotype overlap between the tumor and peripheral blood of the 3 patients (overlap coefficient 0.127-0.144) in CD8+ T cells, but not CD4+ T cells (with clonal expansion or overlap).  
  
**3. It would be exciting to do a bit more with the TCR analysis, specifically in the integration with gene expression data and phenotypic clusters. Some questions that could be answered using the data include:**

**a. For the top 5 clonotypes shared in the tumor and peripheral blood (Fig 4C), what are the associated phenotypes of these clones? Is there enrichment of a certain CD4+ or CD8+ subpopulation among these shared clonotypes?**

As mentioned above, we have substantially modified the clonotype analysis as part of the revision process. We found, in general, the top clonotypes in patient 1 and 2 shared between tumor and blood (Figure 2D), while the top clonotypes in patient 3 were seen in tumor-specific manner.

**b. Which CD4+ and CD8+ sub-populations are most clonally expanded? Is this consistent across patients? How might the level of expansion correspond to specific gene signatures?**

We have added analysis of clonotypes to sub-analysis of CD8+ T cells (Figure 3E,F) and CD4+ T cells (Figure 4D) and expanded the analyses to include discussion of gene signatures.

Minor comments:  
**1. Comparing the tSNE colored by patients (Fig 1A, bottom right) and the t-SNE split by tumor and blood (Fig 1E), it seems the pink tumor CD8 T cell (T) cluster 0 is dominated by cells from patient 2. And from Table S1, cells from patient 2 make up >60% of total cells in this cluster. Is there some clinical/pathological indication for this exhaustion cluster would not be seen in patients 1 and 3? Perhaps some discussion on this subject would be interesting to the reader.**

Although we have reclustered and renamed the patient samples (now patient 3), the reviewer makes a great point. As part of the expansion of clonotype and broader analysis, we note that this patient has 2 dominant clones in the tumor that accounts for tumor-exclusive populations. We have added discussion of this to the manuscript.  
  
**2. A slightly more detailed explanation of why lymphoid and myeloid cells were mixed in different proportions (7:3, as stated in Results section; 3:1 in Methods) would be helpful to the reader.**

We have clarified the text to be consistent with the 3:1 ratio. In addition, to the methods, we added the underlying basis for the ratio approach. This ratio was used to ensure similar coverage for lymphoid and myeloid cells across the 3 ccRCC patients, as myeloid cells tend to express a greater number of features.   
  
**3. The examination of the exhausted CD8+ T cell population found in the tumor was informative, revealing sub-populations with distinct profiles of exhaustion markers (Fig 2D). However, the authors’ claim that these subsets have ‘distinct functional properties’ might be overstated, since no functional experiments were performed to validate this statement.**

Thank you for the suggestion and we agree, we have modified the language for better clarity and with limitation to the extent of what the data shows.

**4. Please provide a color legend for 4D.**

Manuscript has substantially been changed with reviewer’s suggestion; this is no longer relevant.

**Reviewer #3 (Remarks to the Author):**  
  
**Vishwakarma et al analyzed 24,904 lymphoid and myeloid cells in matched tumor and blood from 3 patients with clear cell renal cell carcinoma (ccRCC )by single-cell RNA sequencing using 10x genomics platform. both single-cell transcriptome and lymphocyte repertoire were analyzed. This is a descriptive report aimed to characterize ccRCC immune landscape. This is considered as the first report of the immune landscape of ccRCC using scRNA-seq. However, none of the major conclusions were validated by a secondary method.**

Towards validation, we have 1) performed highthroughput immunohistochemistry on the ccRCC samples showing enrichment of CD8+ and PD-1+ cells in tumor compared to adjacent normal tissue, 2) developed a signature to examine survival using bulk sequencing data in the Cancer Genome Atlas, 3) identifying proliferative CD8+ T cells as a poor prognostic marker, we used mass CyTOF data from Sade-Feldman et alia 2018 to identify cell the cell population in ccRCC in advanced-grade diseases.   
  
**Another major flaw of this study is the authors ignored the multiplet issue by drop-let based approach. "Cell suspensions were loaded onto a Chromium Single-Cell Chip along with the reverse transcription (RT) master mix and single cell 5′ gel beads, aiming for 7,500 cells per channel." at this recovery rate, approximately 6% of the cells are multiplets and the authors did not attempt to address how this would impact their analyses.**

We have clarified that cells were removed with UMIs > 5,000 to control for multiplets, please see SCRS integration in the methods section.