**Reviewers' comments:**  
  
Reviewer #1 (Remarks to the Author):  
In the revised manuscript, authors added more scRNA-seq data from one healthy normal PBMC and three normal kidney tissue samples to have scRNA-seq data of 37,055 immune cells in total. Authors also validated the outcome prediction model based on machine-learning algorithm using TCGA ccRCC RNA-seq dataset. Authors conclude we can predict the clinical outcome of ccRCC with the immune signature from CD8+ T cells and TAM. Authors did not fully answer to the question on the immune suppression mechanism directed by those CD8+ T cells and TAM, which are too broad to specify the mechanism. To clarify the activity of CD8+ T cells or TAM in ccRCC, authors were asked to analyze the subtypes of CD8+ T cells and macrophages, which might give us a clue on the immune suppression mechanism such as increased population of exhausted CD8+ T cells. Because the number of cells analyzed in this manuscript is not many, authors can use public datasets to identify those subtypes of CD8+ T cells. In addition, we need to check whether those signatures related to ccRCC of CD8+ T cells and TAM is specific to ccRCC. To this end, authors were asked to test those signatures in other cancer types. Still revised manuscript does not provide scientific advancement in the field of tumor immunology.

Extracted Points:

**Authors did not fully answer to the question on the immune suppression mechanism directed by those CD8+ T cells and TAM, which are too broad to specify the mechanism. To clarify the activity of CD8+ T cells or TAM in ccRCC, authors were asked to analyze the subtypes of CD8+ T cells and macrophages, which might give us a clue on the immune suppression mechanism such as increased population of exhausted CD8+ T cells.**

As part of the resubmission and after incorporating additional single-cell normal kidney and healthy peripheral controls, we performed the requested analyses on both CD8+ T cells (Figure 3) and TAM populations (Figure 4). For the CD8+ T cells, expression trajectory analysis found several distinct branches associated with exhaustion and proliferation (Figure 3E, G - enriched in the tumor CD8+ T cells) and independently associated with clonal expansion (Figure 3E, F). Specifically, CD8\_0 was defined as “exhausted” T cells, with elevated expression of *CTLA4*, TIM-3, PD-1 and *TIGIT* (Figure 3D) and were increased in tumor-infiltrating CD8+ T cells (Figure 3B). Further, we identified 5 distinct macrophage clusters, defining 3 as TAMs (Figure 4E), each of these populations had several common and distinct gene expression patterns (Figure 4F) and gene set enrichment (Figure 4H).  
  
**In addition, we need to check whether those signatures related to ccRCC of CD8+ T cells and TAM is specific to ccRCC. To this end, authors were asked to test those signatures in other cancer types**

Thank you for the suggestions, we have applied the KNN models trained on renal patient samples across all 33 cancer types in PANCAN TCGA below (included as Supplemental Figure 8 and included below). Although the T signature was more broadly applicable, segregated overall survival in 13 of 33 cancers, the TAM signature discriminated overall survival in only 3 cancer types, which may indicate a microenvironment-dependent expression pattern for TAMs. Although the latter signature may seem limiting, however we did not design these signatures to broadly apply to all tumor types. If we were aiming to develop a more broadly applicable signature, we would have sampled all tumor types or immunogenic tumor types in the TCGA during the training of the KNN model.

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**Still revised manuscript does not provide scientific advancement in the field of tumor immunology.**

We respectfully disagree with the statement of this reviewer. As mentioned previously, we are interested in further inquiry into specific populations of immune cells in ccRCC and are planning on additional mechanistic studies (which is stated in the final paragraph of the discussion as well). However, concluding this manuscript does not offer scientific advancement is unfair. First and foremost, the manuscript is the first to combine single-cell gene expression and immune receptor sequencing of immune cells in renal clear cell carcinoma. In addition, the manuscript is the first tumor-based project to utilize the scRepertoire package we developed, allowing for assignment of clonotype by both TCRA and TCRB chains. This facilitates a more in-depth analysis for clonotype dynamics in both CD4+ and CD8+ T cells than other works in the single-cell tumor immunology field. Our work moves beyond just the characterization of a single-cell data set with the inclusion of multimodal points of data quantification – including bulk RNA sequencing and mass cytometry data sets. Both of which point towards more generalizable trends in renal tumor immunology. This is all in addition to providing high-quality single-cell sequencing of paired peripheral blood and tumor-infiltrating immune cells from renal carcinoma patients to the field for further inquiry by tumor immunologists and other researchers. We have added some portions of this response to the discussion to highlight the novelty of the study.

Reviewer #2 (Remarks to the Author):  
Zakharia and colleagues have significantly revised their original manuscript, incorporating additional single cell sequencing data of healthy peripheral blood and normal kidney tissue into their detailed characterization of the CD4+ T cell, CD8+ T cell, and myeloid compartments within clear cell renal cell carcinoma (ccRCC). The authors also identified CD8+ T cell and macrophage gene signatures that correlate with patient survival by training machine learning models on ccRCC data from TCGA. Overall, this revision presents a strengthened story of immune dynamics in ccRCC that sufficiently addresses the comments brought up by us and other reviewers.  
  
Comments:  
**1. In addition to the presence of expanded clonotypes in NK cell clusters (as discussed by the authors), there also appears to be a nontrivial amount of T cell clones found in the monocyte and macrophage clusters (Figure 2A). Some discussion about potential reasons for this observation may be helpful for the reader.**

Excellent point, we have added this discussion to the manuscript:

*Pg 12 “Single clones and clones with 1-5 copy numbers were seen across myeloid clusters (Figure 2A), which may be a result of partial loss of finer gene expression differentiation during the expression integration.”*

As part of another reviewer’s comments, we also performed doublet analysis and found cluster 21 which clustered with B cells and had recovered TCRs consisted of roughly 20% of doublets. We have also noted this discrepancy in the revision and included the doublet analysis as supplemental figure 2. Anecdotally, during the writing and usage of the scRepertoire package, recovered TCR in myeloid and B cells has been an issue we have encountered beyond just renal clear cell carcinoma, but also autoimmune processes of human and mouse skin, peripheral blood in lymphoma, and lung tumors.

**2. The authors show that the CD8+ subcluster 6 and TAM subcluster 3 gene signatures are able to discriminate overall survival (Figure 6B and C). Does a model using a feature set combining these two gene signatures further improve performance? Perhaps this would suggest that the immune response in ccRCC is not driven solely by one cell type, but rather is coordinated across both lymphoid and myeloid responses.**

We want to thank the reviewer for this point, the latter sentiment is what we hypothesize. With our experience in developing signatures from the bulk TCGA data, we have observed significant survival differentiation with 25 to 30 randomly assigned genes using the KNN modeling (as well as SVM), with < 20 randomly selected genes unable to differentiate survival. Thus, combining the two 15-gene signatures would provide disingenuous conclusions. Instead, we have provided a breakdown in the independent association of both signatures across the patients in both the results and discussion.

Pg19 *“However, there was a significant association between the CD8\_6 and TAM\_3 classifications, which shared a high degree of overlap in patients classified into good-prognosis (188 in both signatures) and poor-prognosis (35 in both signatures), Fisher p-value = 9.3e-15.”*

pg23 “*The TAM\_3 classification had an independently high degree with the CD8\_6, suggesting the possible interaction or coordination between lymphoid and myeloid cells in ccRCC*.”  
  
Reviewer #3 (Remarks to the Author):  
**In this revised manuscript, the authors provided plenty new data to validate their scRNAseq findings using several other platforms. However, they did not fully address the multiplet issue. Multiplet identification tools such as Scrublet are recommended.**

We thank the reviewer for the suggestion, we have iperformed doublet/multiplet estimation using the scDblFinder R package, including the analysis as Supplemental Figure 2 (below). Based on the estimation, only a single cluster had doublet estimation > 10% - corresponding to Cluster 21. This was a unique cluster, as it has recovered TCR, but clusters with B cells, possibly indicating interacting cell-cell interaction of B and T cells. The downstream analysis of T cells had already excluded this cluster as well. Other clusters in the integrated object had minimal doublet estimates, which is likely due to the aforementioned filtering by number of unique features. With the further downstream analysis of CD8+, CD4+, and APC cells (Figure B), no predominant doublet clusters were visualized. Percent of doublets in each subcluster were < 2%, with the exception of APC subcluster 12 (arrow) with 4.87% doublets. Overall there is minimal effect of doublets/multiplet on the downstream analysis. We have added these points to the results and expanded the methods to reflect this new analysis.

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