**Methods**

*Subject Details and Tissue Collection*

Fresh blood and primary clear cell renal cell carcinoma (ccRCC) samples were obtained from the University of Iowa Tissue Procurement Core and GUMER repository through the Holden Comprehensive Cancer Center from subjects providing written consent approved by the University of Iowa ethics board committee. The patients ranged from 67 to 74 years old; the tumor samples were of diverse tumor stages and sourced from male subjects. Tumor grades were histologically determined by a pathologist. Three ccRCC tumor specimens paired with individual blood samples were used in the study. Will need IRB FOR publication

*Tumor Dissociation and Isolation of Mononuclear Cells*

Renal tumor samples were dissociated into single cells by a semi-automated combined mechanical/enzymatic process. The tumor tissue was cut into pieces of (2-3mm) in size and transferred to C Tubes (Miltenyi Biotech, Bergisch Gladbach, Germany) containing a mix of Enzymes H, R and A (Tumor Dissociation Kit, human; Miltenyi Biotech). Mechanical dissociation was accomplished by performing three consecutive automated steps on the gentleMACS Dissociator (h\_tumor\_01, h\_tumor\_02 and h\_tumor\_03). To allow for enzymatic digestion, the C tube was rotated continuously for 30 min at 37°C, after the first and second mechanical dissociation step (cite [here](https://pubmed.ncbi.nlm.nih.gov/25867267/)). Cells from fresh tumor specimens were incubated with FcR blocking reagent (StemCell Technologies, Vancouver, Canada) for 10 min at 40C and labelled with 1ug/ml of the FITC anti-human CD45 antibody (BioLegend, San Diego, CA) per 107 cells for 20 min at 40C. CD45+ cells were isolated using the EasySepTM FITC Positive Selection Kit (StemCell Technologies). Alternatively, mononuclear cells (MNCs) from whole peripheral blood of paired subjects were isolated using SepMate Tubes (StemCell Technologies) by density gradient centrifugation. Cells were then viably frozen in 5% DMSO in RPMI complemented with 95% FBS. Cryopreserved cells were resuscitated for flow cytometry analyses by rapid thawing and slow dilution.

*Cell Sorting for Single-Cell RNA sequencing*

Viable immune (CD45+ Hoechst-) single cell suspensions generated from three ccRCC tumor samples and blood were FACS sorted on a FACS ARIA sorter (BD Biosciences) for lymphoid and myeloid cells (Ratio 3:1). The cells were sorted into ice cold Dulbecco’s PBS + 0.04% non-acetylated BSA (New England BioLabs, Ipswitch, MA). Sorted cells were then counted and assessed viability MoxiGoII counter (Orflo Technologies, Ketchum, ID) ensuring that cells were re-suspended at 1000 cells/ul with a viability >90%.

Library Preparation, Single-Cell 5’ and TCR Sequencing

Single-cell library preparation was carried out as per the 10X Genomics Chromium Single Cell 5' Library and Gel Bead Kit v2 #1000014 (10x Genomics, Pleasanton, CA). 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. Following generation of single-cell gel bead-in-emulsions (GEMs), reverse transcription was performed using a C1000 Touch Thermal Cycler (Bio-Rad Laboratories, Hercules, CA); 13 cycles were used for cDNA amplification. Amplified cDNA was purified using SPRIselect beads (Beckman Coulter, Lane Cove, NSW, Australia) as per the manufacturer’s recommended parameters. Post-cDNA amplification reaction QC and quantification was performed on the Agilent 2100 Bioanalyzer using the DNA High Sensitivity chip. For input into the gene expression library construction, 50ng cDNA and 14 cycles was used. To obtain TCR repertoire profile, VDJ enrichment was carried out as per the Chromium Single Cell V(D)J Enrichment Kit, Human T Cell #1000005 (10x Genomics) using the same input sample. Sequencing libraries were generated with unique sample indices (SI) for each sample and quantified. Libraries were sequenced on an Illumina HiSeq 4000 using a 150-pair-end sequencing kit. Gene expression FASTQ files were aligned to the human genome (GRCh38) using the CellRanger v2.2 pipeline, while clonotype sequencing was aligned to the vdj\_GRCh38\_alts\_ensembl genome build provided by the manufacturer.

*Single-cell Data Analysis*

Initial processing of cells isolated form the heart in myocarditis run 1 (n=2,617), myocarditis run 2 (n=10,618), control run1 (n=1,528), and control run 2 (n=8,201) was performed using the Seurat R package (v3.0.2) (cite [here](https://www.nature.com/articles/nbt.4096) and [here](https://www.cell.com/cell/fulltext/S0092-8674(19)30559-8)). Samples were normalized using the *SCTtransform* approach (cite [here](https://genomebiology.biomedcentral.com/articles/10.1186/s13059-019-1874-1)) with default settings. Preperation for integration used 3,000 anchor features and *PrepSCTIntegration*. The integration of sequencing runs occurred with the SCT-transformed data. The dimensional reduction to form the uniform manifold approximation and project (UMAP) utilized the top 30 calculated dimensions and a resolution of 0.7. The schex R package (v1.1.5) was used to visualize mRNA expression of lineage-specific or highly differential markers by converting the UMAP manifold into hexbin quantifications of the proportion of single-cells with the indicated gene expressed. Default binning was set at 80, unless otherwise indicated in the figure legend.

Cell type identification utilized the SingleR (v1.0.1) R package (cite [here](https://www.nature.com/articles/s41590-018-0276-y)) with correlations of the single-cell expression values with transcriptional profiles from pure cell populations in the ENCODE (cite [here](https://www.nature.com/articles/nature11247)). In addition to correlations, canonical markers for cell lineages (Supplemental Table X) and corresponding TCR sequences were used. Differential gene expression utilized the Wilcoxon rank sum test on count-level mRNA data. For differential gene expression across clusters or subclusters, *FindAllMarkers* function in the Seurat package using the log-fold change threshold > 0.25, minimum group percentage = 10%, and the pseudocount = 0.1. Differential comparisons between condition utilized the *FindMarkers* function in Seurat, without filtering and a pseudocount = 0.1. Multiple hypothesis correction was reported using the Bonferroni method. Cell cycle regression was performed in Seurat using the *CellCycleScoring* function and genes derived from Nestorowa et alia (cite [here](https://ashpublications.org/blood/article/128/8/e20/35749/A-single-cell-resolution-map-of-mouse)). Genes were isolated by calling *cc.genes.updated.2019* in R.Gene set enrichment analysis was performed using the escape R package (v0.99.0). Differential enrichment analysis was performed using the *getSignificance* function in escape that is based on the limma R package linear fit model. Cell trajectory The code for all analysis is available at <https://github.com/ncborcherding/ccRCC> (this link is private until publication).

*Statistical Analysis*

Statistical Analyses were performed in R (v3.6.3). Two-sample significance testing utilized Welch’s T test, with significance testing for more than three samples utilizing one-way analysis of variance (ANOVA) with Tukey honest significance determination for correcting multiple comparisons. Two-proportion Z-tests was performed using the total number of cells in each condition as the number of trials and without a prior for proportion.