ONCOIMMUNOLOGY
2025, VOL. 14, NO. 1, 2481109
https://doi.org/10.1080/2162402X.2025.2481109



# Cellular and secretome profiling uncover immunological biomarkers in the prognosis of renal cell carcinoma patients

Le Tong[a,b*], Veronika Kremer[a*], Shi Yong Neo[a,c], Christina Seitz[d], Nicholas P. Tobin[a], Barbara Seliger[e,f], Ulrika Harmenberg[a], Eugenia Colón[g], Ann-Helén Scherman Plogell[h], Lisa L. Liu[a,i,j], and Andreas Lundqvist[a]

[a]Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden; [b]Key Laboratory of Quantitative Synthetic Biology, Shenzhen Institute of Synthetic Biology, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China; [c]Singapore Immunology Network, Agency for Science, Technology and Research, Singapore, Republic of Singapore; [d]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; [e]Institute for Medical Immunology, Martin-Luther University Halle-Wittenberg, Halle, Germany; [f]Institute of Translational Immunology, Medical School "Theodor Fontane", Brandenburg an der Havel, Germany; [g]Department of Women's and Children's Health, Karolinska Institutet and S:t Göran's Hospital-Unilabs, Stockholm, Sweden; [h]Department of Surgery and Oncology, S:t Göran's Hospital, Stockholm, Sweden; [i]Theme Cancer, Karolinska University Hospital, Stockholm, Sweden; [j]Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden

**ABSTRACT**

Renal cell carcinoma (RCC) is recognized as an immunogenic tumor, yet tumor-infiltrating lymphocytes often exhibit diminished effector function. However, the mechanisms underlying reduced T and NK cell activity in RCC remain unclear. Here, we examined the immune contexture in RCC patients undergoing nephrectomy to identify immune-related biomarkers associated with disease progression. Immune cell phenotypes and secretion profiles were assessed using flow cytometry and Luminex multiplex analysis. Supervised multivariate analysis revealed several changes of which frequencies of T and NK cells expressing CCR5, CXCR3, and PD-1 were elevated within tumors compared with peripheral blood. In addition, higher levels of regulatory T cells, PD-1+, and CXCR3+ T and NK cells were observed in patients with relapse following nephrectomy. With regards to soluble factors, tumor-derived CXCL8 was associated with higher Fuhrman grade and increased frequency of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs). These biomarkers demonstrate potential relevance in the progression of RCC and merit further investigation in prospective studies.

**ARTICLE HISTORY**
Received 5 January 2025
Revised 11 March 2025
Accepted 13 March 2025

**KEYWORDS**
Biomarkers; CXCL8; NK cells; PD-1; prognosis; renal cell carcinoma; T cells

## Background

Renal cell carcinoma (RCC) is the most common form of kidney cancer, comprising approximately 90% of all kidney malignancies. Although localized tumors are effectively surgically removed, approximately 20% of patients relapse, thereby emphasizing the need for reliable biomarkers to identify patients with a heightened risk of recurrence.[1]

The tumor microenvironment (TME) of RCC is inherently complex, characterized by hypoxia, activity, an abundance of soluble immunoregulatory factors as well as a dynamic interplay between cancer immune cells and stromal cells. Immune cells play an important role in RCC progression as evidenced by the incidence of spontaneous regression,[2] as well as responsiveness to immune checkpoint inhibition.[3]

RCC tumors are highly infiltrated by immune cells and contain, in contrast to many other solid tumors, high frequencies of natural killer (NK) cells, which have been linked to improved patient outcome.[4–8] However, RCC-infiltrating NK cells have a suppressed phenotype and defects in proliferation, cytokine production, and cytotoxicity.[4,9–11] There are many potential factors that contribute to impaired function of tumor-infiltrating lymphocytes, which are also associated with a poor patient's prognosis. For example, the frequencies of polymorphonuclear (PMN) and monocytic (M) myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Treg), but also the expression of HLA-G on tumor cells are elevated in RCC patients and associated with worse overall survival.[12–15]

The mechanisms governing infiltration and activity of immune cells in RCC are poorly understood. In this study, we investigated immune cell phenotypes and secretion signatures in blood and tumors of RCC patients with localized disease and performed supervised multivariate analysis to correlate such immune signatures with disease parameters. Several cellular and soluble factors, including programmed cell death protein 1 (PD-1) positive T and NK cells are enriched in low-stage RCC tumors, and chemokine (C-X-C motif) ligand (CXCL) 8 levels are higher in high-grade tumors and correlate with a poor prognosis in RCC patients.

## Materials and methods

### *Patients and sample collection*

The study was approved by the Regional Ethical Review Board in Stockholm and adheres to the Declaration of Helsinki

**CONTACT** Andreas Lundqvist andreas.lundqvist@ki.se Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden
*Equal contribution.
Supplemental data for this article can be accessed online at https://doi.org/10.1080/2162402X.2025.2481109

(Ethical approval # 2013-570-31). Following written informed consent for participation in the study, blood samples were collected at the time of surgery and at a median of 46 days (range: 31–71 days) after surgery from 14 patients (Table 1 and Table S1). The median follow-up time after surgery is 9 years and 11 months. Peripheral blood was collected in heparinized tubes, and plasma was recovered by centrifugation. Remaining cellular sediment was used for Ficoll density gradient isolation (Ficoll-Paque plus, GE Healthcare) of peripheral blood mononuclear cells (PBMC). Pieces of surgically resected primary tumors were either embedded in OCT (VWR) or snap-frozen in liquid nitrogen and stored at −80°C. Tissue lysates were prepared from snap-frozen tissue using CelLytic MT extraction buffer according to the manufacturer's procedure (Sigma). Protein concentration was measured using NanoDrop™ (Thermo Fischer Scientific), and protein concentrations were equalized between blood and tumor samples. To obtain single-cell suspensions, freshly isolated tumor samples were cut and passed through a metal sieve with 80 µm mesh size (Bellco Glass), followed by filtration through a 70 µm and 40 µm cell strainer. No enzymes were used for the isolation of single-cell suspensions.

### Flow cytometry

Blood and tumor cell suspensions were stained with antibody mixes (Tables S2 and S3) within 2 hours after collection following incubation with 100 µg human intravenous IgG (Thermo Fisher Scientific) to block unspecific binding. The post-surgery blood sample from patient 8 as well as both blood samples and the tumor cell suspension from patient 9 were cryopreserved in fetal bovine serum (FBS) with 10% DMSO and analyzed by flow cytometry at a later time point. Cells were incubated with fluorescence-conjugated antibody mixes at 4°C for 30 min for surface marker staining. Intracellular staining was performed following the protocol of Fixation/Permeabilization Kit (BD Biosciences) or Transcription Factor Staining Buffer Set (Thermo Fisher Scientific). LIVE/DEAD Fixable Near-IR Dead Cell Stain kit (Thermo Fisher Scientific) was used to exclude dead cells. PBMC was cultured with RCC cell lines A498, 786–0, and Caki-1 (ATCC) at a 2:1 ratio for 2 days and thereafter analyzed for the expression of PD-1 on T and NK cells. Cells were acquired by flow cytometry using the Novocyte (ACEA Biosciences) instrument. Data analysis was performed with FlowJo software (v10.8.1). Gating strategies for individual cell populations are shown in Figure S1.

**Table 1.** Patient characteristics.

| Patients, *n* | 14 |
|---|---|
| **Age**, median (range) | 70 (35–83) |
| **Gender**, *n* (%) | |
| female | 9 (64) |
| male | 5 (36) |
| **Histology**, *n* (%) | |
| clear cell | 11 (79) |
| papillary type 2 | 2 (14) |
| chromophobe (sarcomatoid) | 1 (7) |
| **Fuhrman grade**, *n* (%) | |
| 1 | -7 (50) |
| 2 | 5 (36) |
| 3 | 1 (7) |
| 4 | 1 (7) |
| NA* | |
| **pT stage**, *n* (%) | |
| T1 | 8 (57) |
| T2 | 4 (29) |
| T3 | 2 (14) |
| **Follow up**, *n* (%) | |
| alive, disease free | 11 (79) |
| alive, progressed | 2 (14) |
| dead of disease | 1 (7) |

*Chromophobe histology.

### Multiplex analysis of cytokines and chemokines

Soluble factors in pre- and post-surgery plasma as well as in tumor lysates from RCC patients were quantified using a 40-parameter Bio-Plex Pro Human Chemokine Panel and a 37-parameter Bio-Plex Human Inflammation Panel (Bio-Rad) on a Milliplex Magpix instrument (Merck Millipore) according to the manufacturers' instructions. In total, 67 analytes were used for further analysis after exclusion of those with more than 50% missing values for either sample type.

### Cluster heatmap

Variables with more than 50% of values missing across tumor and pre- and post-surgery blood samples were removed and those remaining were used to cluster samples (row scaled, Pearson correlation, average linkage) in a semi-supervised manner with the *heatmap3* package in R version 3.4.2.

### Multivariate data analysis

The multivariate statistical tools Orthogonal Projections to Latent Structures (OPLS) and OPLS-Effect Projections (OPLS-EP) were applied to find correlations between 177 blood-derived and 154 tumor-derived variables obtained from flow cytometry (cellular) and multiplex analyses (soluble) (X) and clinical parameters or effects (Y). OPLS is a supervised modeling approach that separates the systematic variation in X into a predictive component $t^1$ that is correlated to Y and an orthogonal component $to^1$ that is unrelated to Y.[16] OPLS-EP, a multivariate version of a paired *t*-test[17] was used to investigate differences between tumor and blood parameters as well as between blood parameters before and after surgery. An "effect matrix" that contains the differences between each paired variable for each patient was created by means of subtraction, and OPLS was used to find the relation between this matrix and the target vector Y (here termed "effect"; target values were set to 1). For OPLS, data were scaled to unit variance and mean-centered prior to analysis. For OPLS-EP, data were scaled to unit variance, but not mean-centered. Model quality was assessed by internal cross-validation and described by the parameters $R^2$ (explained variation) and $Q^2$ (predictive ability), with a value of 1% or 100% indicating a perfect model. For biological data, a $Q^2$ value of >0.4 or 40% is generally considered as good predictive capacity.[18] Model loadings represent the influence of the original variables on OPLS components. Variables significantly contributing to the clinical parameters or projected effects were thus reported as OPLS predictive

loadings and their 95% jack-knife confidence intervals. Some OPLS loadings plots show only the most influential model variables selected based on indicated VIP (Variable Importance for Projection) values and absolute values of loading weights scaled as correlation coefficients. All multivariate analyses were performed using SIMCA 14.1 software (Sartorius Stedim Biotech).

### TCGA data analysis

The transcriptome profiling data of ccRCC samples, along with their corresponding clinical annotations, were acquired from The Cancer Genome Atlas (TCGA-KIRC) in September 2023. Patients lacking survival information or follow-up time were excluded from the analyses. The threshold for high and low expression levels of *CXCL8* was set at 50%. The log-rank test was utilized to assess statistical differences in overall survival and disease-specific survival, and survival curves were generated using Kaplan–Meier analysis. In various clinical groups, which include Fuhrman grade, primary tumor stage, and the presence or absence of new tumor events, the expression level of *CXCL8* was compared and analyzed using an unpaired *t*-test.

### Statistics

Paired and unpaired Student's *t*-tests were performed for individual comparisons of two paired and unpaired groups, respectively, that had a normal data distribution. Non-normally distributed paired groups were analyzed with Wilcoxon matched-pairs signed rank tests. For multiple unmatched group comparisons with non-normally distributed data, Kruskal–Wallis tests with the two-stage step-up method of Benjamini, Krieger, and Yekutieli were applied. Correlation analyses were performed using Pearson correlation for normal distributed data and Spearman correlation for non-normal distributed data. Significance was defined by p-values less than 0.05 using a two-tailed test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$, and ns = not significant.

## Results

### Immune cell and secretory phenotypes in blood and tumors of RCC patients

To investigate immune signatures in RCC, cellular and soluble factors of blood and tumors of RCC patients with locally restricted disease were analyzed (Figure 1a and Table 1). Flow cytometry panels were designed with a focus to investigate T and NK cell activation and migration as well as the frequencies and function of Treg and MDSC (Table S2). While no significant differences were observed in lymphocyte frequencies between pre- and post-surgery blood and tumors, the frequency of myeloid cells was significantly lower in post-surgery blood (median 16%) compared with pre-surgery blood (median 30%, $p = 0.034$). The lymphocyte frequencies exhibited greater variability in tumors compared to peripheral blood, indicating higher heterogeneity (Table 2).

A semi-supervised hierarchical clustering algorithm was used to differentiate between tumor and pre-surgery blood samples based on immune cell populations and secretion phenotypes. Tumors displayed overall higher chemokine and cytokine levels and an accumulation of CD8 T cells compared with peripheral blood (Figure 1b, red subgroup, and Table S4a). Additionally, an elevated expression of chemokine receptors, primarily chemokine (C–C motif) receptor (CCR) 5 and chemokine (C-X-C motif) receptor (CXCR) 3, as well as increased levels of PD-1 on T and NK cells was observed in tumors (Figure 1b, blue subgroup, Figure S2a-b, and Table S4b). Since the frequency of PD-1 was higher in tumors compared with blood in both T and NK cells, we investigated if contact with tumor cells increase the expression of PD-1 on T and NK cells. Indeed, the frequency of PD-1 positive T and NK cells was significantly increased upon culture with tumor cells (Figure S2C). The disparity between blood and tumor for these markers was less distinct than that observed for chemokines and cytokines. In contrast, peripheral blood was marked by a heightened expression of DNAX accessory molecule-1 (DNAM-1) on both T and NK cells and of the chemokine receptor CXCR2, specifically on NK cells (Figure 1b, green subgroup, and Table S4c).

Given the observed changes in immune repertoires between tumors and peripheral blood, a paired comparison using the multivariate statistical analysis tool Orthogonal Projections to Latent Structures (OPLS) that has been further developed to OPLS-Effect Projections (EP) to account for paired sample information was used.[17] In the resulting model, 42% of the variability in the data was due to the analyzed tissue (blood vs. tumor), and the model could explain 99% and predict 95% of it (Table S5). The 96 significant predictive loadings were consistent with the heat map patterns from the hierarchical clustering algorithm (Table S6).

Similar to the results from the hierarchical clustering algorithm, multivariate OPLS-EP modeling showed that the expression of the chemokine receptors CCR5 and CXCR3 were higher on intratumoral T and NK cells compared with peripheral blood T and NK cells. In contrast, a reduced expression of CXCR2 was observed in intratumoral NK cells compared with peripheral blood NK cells (Figure 1c and Table S7). The majority (55%) of the assessed parameters that were enriched in patient tumors compared with peripheral blood were cytokines, primarily with an inflammatory function, comprising 75% of all modeled soluble factors. The most influential variable for the distinction was the tumor necrosis factor (TNF) superfamily member APRIL with a 27-fold higher concentration in tumors than in plasma ($p < 0.0001$). Other TNF family ligands, including BAFF, TWEAK, and TNF-α, were also enriched in tumors compared with blood. Chemokines that accumulated in tumors and influenced the distinction from blood included ligands for the chemokine receptor CXCR2 (CXCL1, CXCL2, CXCL6, and CXCL8) and CXCR3 (CXCL9 and CXCL11) (Table S6). Together, these results highlight differences in immune cell parameters in blood and tumors in RCC patients with locally restricted disease.

### Immune secretome is associated with tumor stage in RCC patients

Since the majority of the assessed parameters enriched in tumors compared with peripheral blood were soluble factors, a comprehensive analysis of 52 chemokines and cytokines was

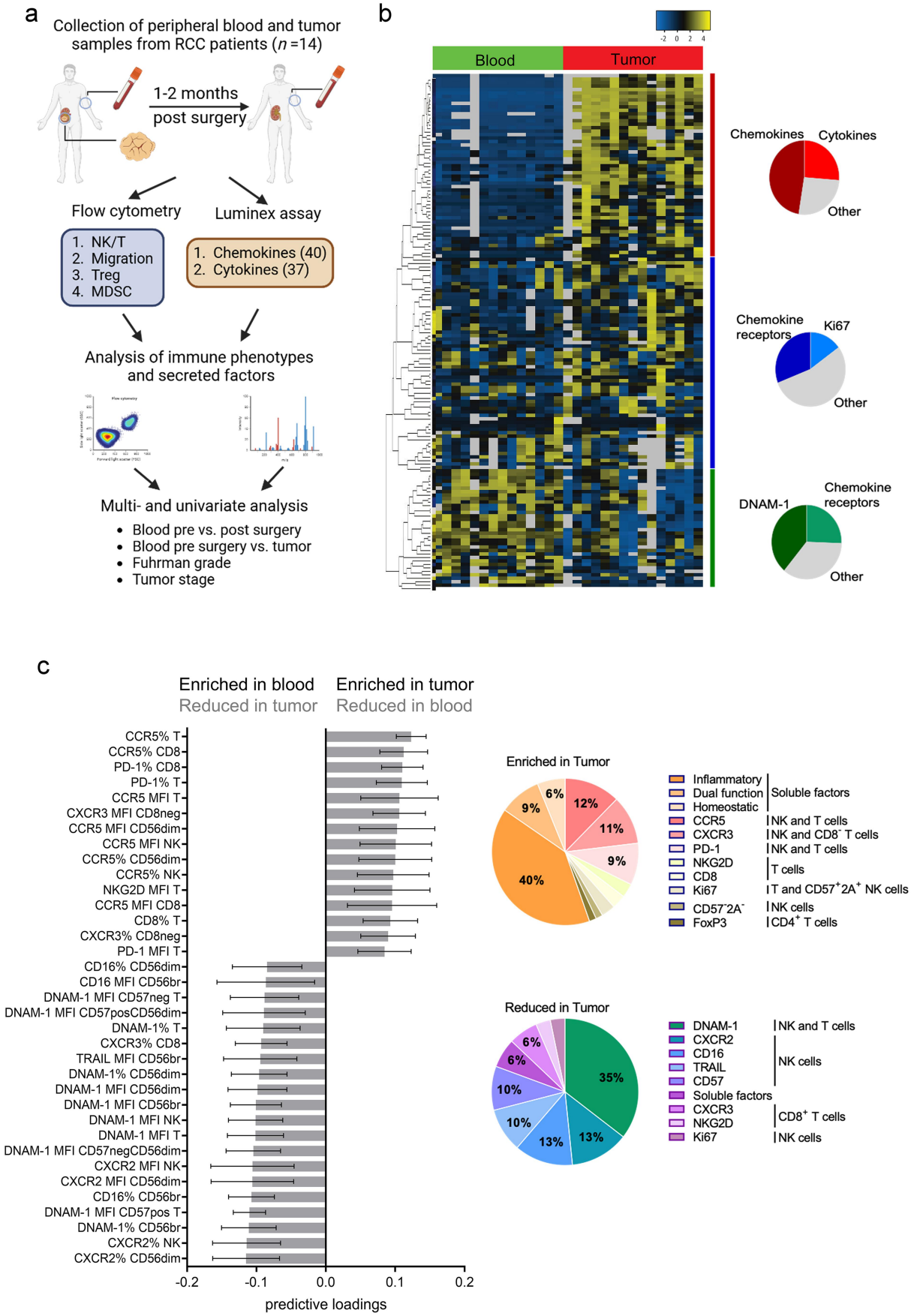


**Figure 1.** Immune cell phenotypes and secretion profiles in peripheral blood and tumors of RCC patients. (a) Experimental and analysis workflow of sample collection and analysis. (b) Semi-supervised hierarchical clustering of immune cell and soluble factor variables in tumor and pre-surgery blood. Compositions of three subgroups are summarized in pie charts. Three distinct subgroups were separated based on differences in the expression of chemokines and cytokines (red), chemokine receptors and Ki67 (blue), and DNAM1 and the chemokine receptors (green). Details of specific cell phenotypes and secretion profiles are specified in Table S4A-C. (c) OPLS-ep analysis of differences between paired tumor and blood variables for each patient at the time of surgery. Immune cell and soluble variables enriched or reduced in tumors compared with blood. Pie charts highlighting factors enriched and reduced in tumors compared with peripheral blood.

**Table 2.** Frequencies of immune cell populations.

| | Blood pre-surgery | | Blood post-surgery | | Tumor | |
|---|---|---|---|---|---|---|
| | Median (range) | | Median (range) | | Median (range) | |
| T cells (% of lymphocytes) | 72 | (56–92) | 74 | (42–84) | 70 | (28–87) |
| NK cells (% of lymphocytes) | 19 | (3–37) | 16 | (6–37) | 20 | (2–65) |
| Treg (% of T cells) | 1.74 | (0.25–4.43) | 1.54 | (0.64–5.59) | 1.71 | (0.06–11.3) |
| Myeloid cells (% of PBMC) | 30 | (10–90) | 16 | (4–61) | | |

performed in tumor lysate, pre-surgery and post-surgery plasma. Although the majority of the detected chemokines were found at higher concentrations in tumor lysates compared to blood, MMP-2, CCL15, and sIL-6 Rα/β were observed at elevated levels in patient blood relative to tumor lysates (Figure 2a). When coupled with analysis of clinical parameters, a general trend toward higher concentrations of chemokines in low-stage tumors (primary tumor (pT) stage 1) compared with advanced tumors (pT stages 2 and 3) was observed (Figure 2b). However, this distinction was not evident based on Fuhrman grades (Figure 2c). Univariate analysis confirmed that secretome profiling delineated low-stage tumors with significantly higher levels of soluble factors including CCL7, CCL15, CCL22, CCL25, IL10, IL16, and sTNF-R1 (Figure S3A). Unlike tumor lysates, no trend of higher concentrations of soluble factors in pre-surgery plasma samples were observed in patients with low-stage tumors (Figure S3B). Similar to tumor lysates, no trend of higher concentrations of soluble factors in pre-surgery plasma samples was found based on Fuhrman grade (Figure S3C). Still, selected soluble factors including CCL27, CXCL10, and CXCL9 were present at higher levels in patients with low-stage tumors (Figure S3D). Together, these results suggest that low-stage RCC tumors are more inflamed.

### *Low-stage RCC tumors are enriched for peripheral blood PD-1$^+$ T and NK cells*

Given differences in selected tumor-derived soluble factors in low vs. high stage RCC tumors, supervised OPLS analysis was applied to correlate the pT stage and variables measured in the tumor. Although a lower pT stage was associated with the presence of inflammatory cytokines and chemokines as well as with high DNAM-1 expression on NK cells, the model could explain 60% and predict 32% of the variation, which should thus be interpreted with caution (Table S5 and Figure S4a).

An analogous OPLS model assessing blood variables separated patients according to their pT stage and explained 94% of the variation, albeit with a predictive ability of 36% (Table S5). The distinction was based on 13 significant predictive loadings. Among cellular components, low-stage tumors had a higher CD8$^+$ to CD4$^+$ T cell ratio (Figure S4b). Furthermore, a high PD-1 expression on peripheral blood NK and CD8$^+$ T cells was associated with a lower pT stage. In contrast, high expression of CXCR2 on T cells was associated with a high pT stage (Figure S4b). In univariate analysis, PD-1 expression was 78% lower on CD56$^{dim}$ NK cells ($p = 0.010$) and 80% lower on CD8$^+$ T cells ($p = 0.0109$) in pT2 when compared with pT1 patients (unpaired t-test, Figure S4c). These results demonstrate that high PD-1 expression on T and NK cells is associated with lower tumor stage.

### *Tumor-derived CXCL8 correlates with high Fuhrman nuclear grade*

Since differences in immune parameters were observed between low and high stage RCC tumor, supervised OPLS analysis was next applied to investigate putative associations between the Fuhrman nuclear grade and immune variables in blood and tumors. The resulting model for blood variables had a predictive ability of only 4% for the Fuhrman grade (Table S5). On the other hand, the model calculated with tumor variables could separate patients according to their Fuhrman grade and explain 96% of the variation with a predictive ability of 56% based on 16 significant predictive loadings (Figure 3a). Tumors with a higher Fuhrman grade showed increased frequencies of regulatory T cells and CXCR3$^+$ CD56$^{bright}$ NK cells and T cells (Figure 3a,b). The most influential soluble factor in the OPLS model was CXCL8, which was significantly increased in tumors with a higher Fuhrman grade (Figure 3a,c).

To investigate if surgical removal of the tumor influences immune parameters, OPLS analysis was applied to pre- and post-surgery blood samples. The generated model identified 32 significantly different variables (Table S5 and Figure S5A). While no differences in CXCL8 levels were observed, the expression of other soluble factors including CCL20, CXCL13, IL-6, and APRIL was higher in pre-surgery vs. post-surgery blood (Figure S5B). Among cellular compartments, proliferation of CD56$^{dim}$ NK cells showed the greatest difference between pre- and post-surgery blood. The frequency of Ki67$^+$ cells CD56$^{dim}$ NK cells proliferated less after surgery regardless of their CD57 or NKG2A status (Figure S5C). Also, among cellular compartments, the frequencies of total myeloid cells and inducible nitric oxide synthase (iNOS) positive myeloid cells were higher in pre-surgery compared with post-surgery blood. Furthermore, the frequency of PMN-MDSCs, but not M-MDSCs, was significantly lower in post-surgery compared with pre-surgery peripheral blood (Figure S5C).

Since CXCL8 can influence the recruitment of MDSCs,[19] we investigated if the frequency of MDSCs correlates with CXCL8 levels in blood. Indeed, CXCL8 levels did correlate with the frequency of PMN-MDSC, but not with that of M-MDSCs (Figure 3d). These results demonstrate that CXCL8 levels are higher in high-grade tumors and surgical removal of primary RCC influences both lymphoid and myeloid cellular compartments as well as specific soluble factors.

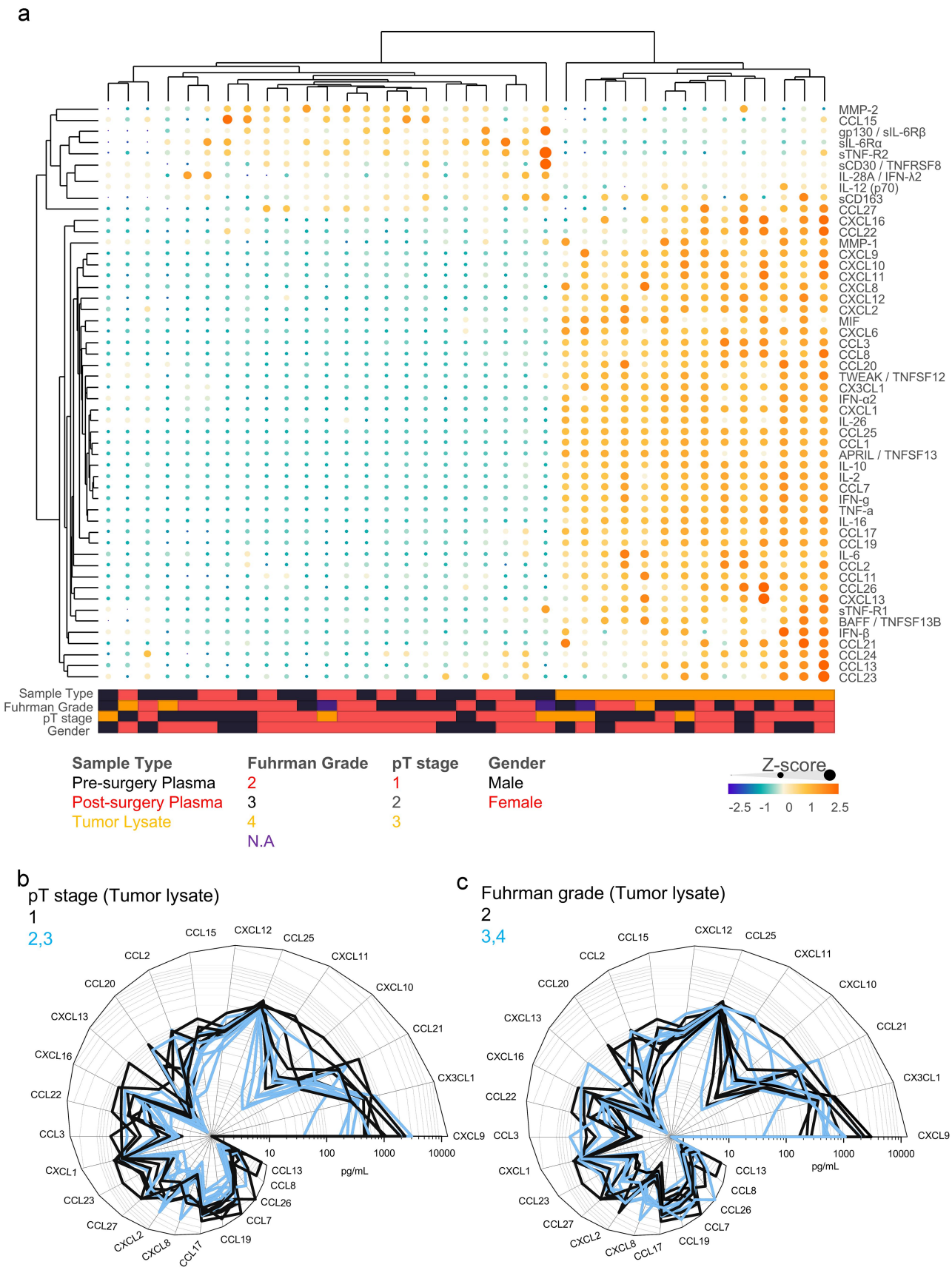


**Figure 2.** Immune secretome is associated with tumor stage and Fuhrman grade in RCC patients. (a) Unsupervised hierarchical heatmap showing normalized z scores of all soluble analytes detected in pre-surgery blood, post-surgery blood, and tumor lysates in relation to clinical and patient parameters. Radar chart (snail plot) showing concentrations of chemokines (pg/mL) detected in tumor lysates and differentiated by tumor stage (b) and tumor grade (c).

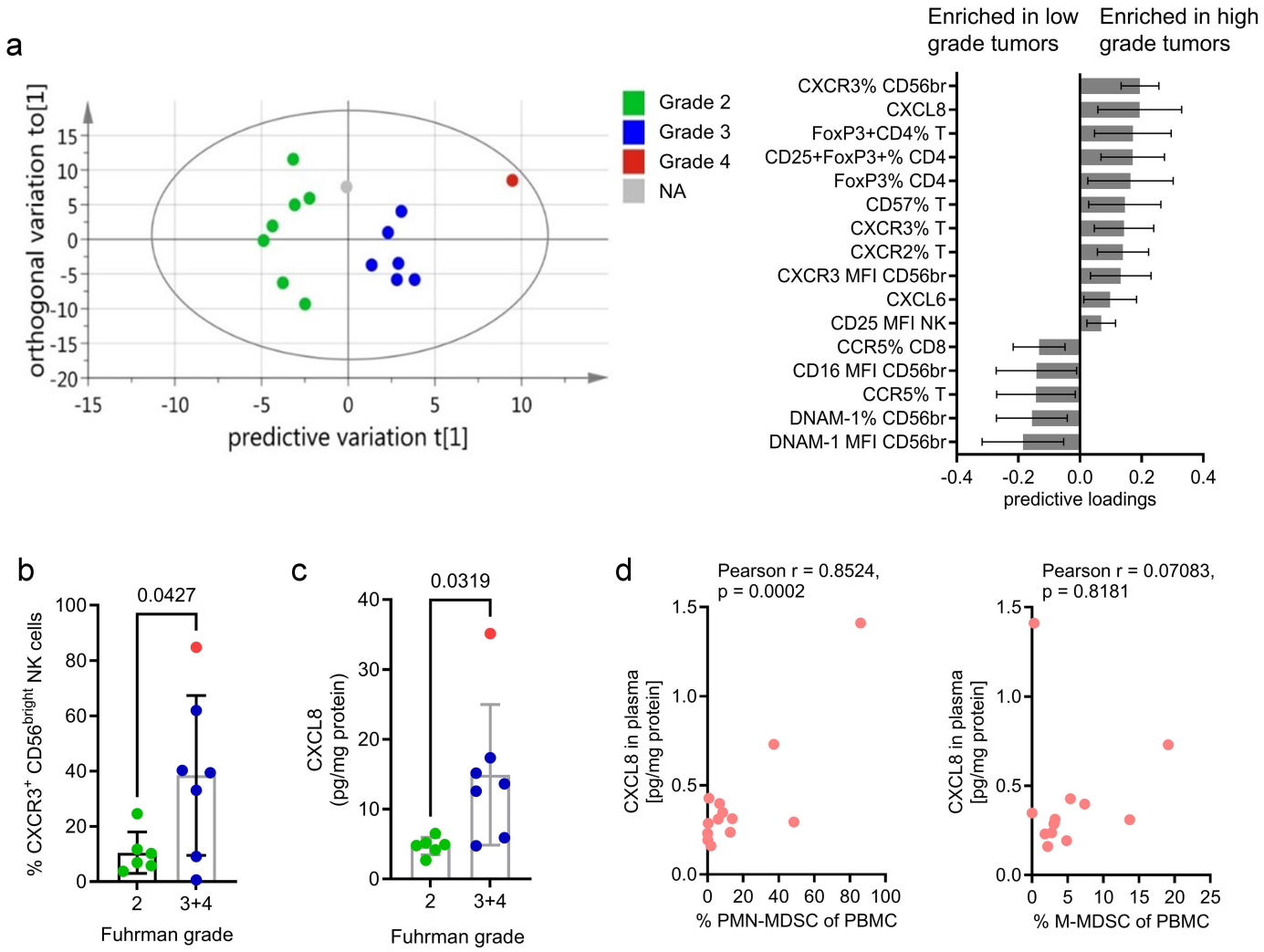


**Figure 3.** CXCL8 correlates with high tumor grade and increased PMN-MDSCs. (a) LEFT: OPSL analysis separating RCC patients according to Fuhrman grade (x-axis, predictive variation; ellipse, Hotelling's T2 95% confidence region). Fuhrman grade for the patient with chromophobe histology is not available (NA). RIGHT: the 16 significant predictive loadings correlating with Fuhrman grade. (b) Frequency of CXCR3+CD56bright NK cells, and (c) CXCL8 concentration in patients with Fuhrman grade 2 vs. 3 + 4. Mean values ± SD are shown. (d) Correlation of CXCL8 concentration and MDSC in peripheral blood.

### *CXCL8 and IFNβ levels correlate with disease relapse in RCC patients undergoing nephrectomy*

Given the risk of relapse following nephrectomy, the identification of reliable biomarkers to predict relapse is mandatory. Within our cohort, three patients relapsed (Table 1 and Table S1). In these patients, several T and NK cell populations were present in blood at different frequencies compared with patients without relapse. For example, the frequency of pre-surgery regulatory T cells, PD-1$^+$ and CXCR3+ T and NK cells was higher in relapse patients compared with non-relapse patients (Figure 4a). With regards to soluble factors, interferon (IFN) β and albeit not significant CXCL8 were lower and higher in relapse and non-relapse patients, respectively (Figure 4b). Notably, CXCL8 was associated with increased frequencies of PD-1 positive T and NK cells in blood (Figure S5d).

Since CXCL8 was observed at higher concentrations in patients with higher Fuhrman grade, the prognostic value of *CXCL8* across TCGA database was investigated. Indeed, *CXCL8* correlates with the Fuhrman grade rather than the tumor stage, which is also consistent within our patient cohort (Figure 4c). Furthermore, high expression of *CXCL8* is associated with poorer overall and disease-specific survival in RCC patients (Figure 4d). In addition, patients, who exhibited a new tumor event following their initial treatment, displayed a higher baseline expression of *CXCL8* in their primary tumors compared to patients, who did not manifest new tumors after treatment (Figure 4e). Since the levels of IFNβ were significantly higher in non-relapse patients, we analyzed the ratio between CXCL8 and IFNβ with regards to relapse in our cohort. Indeed, patients who relapse had a significantly higher CXCL8 to IFNβ ratio in pre-surgery blood (Figure 4f). Taken together, these findings demonstrate that CXCL8 levels in both tumors and blood correlates with a poorer prognosis in patients with localized RCC, and further studies are warranted to investigate CXCL8 as a potential biomarker in patients with RCC.

## Discussion

Using multivariate analysis of cellular compartments and soluble factors in blood and tumors of RCC patients undergoing

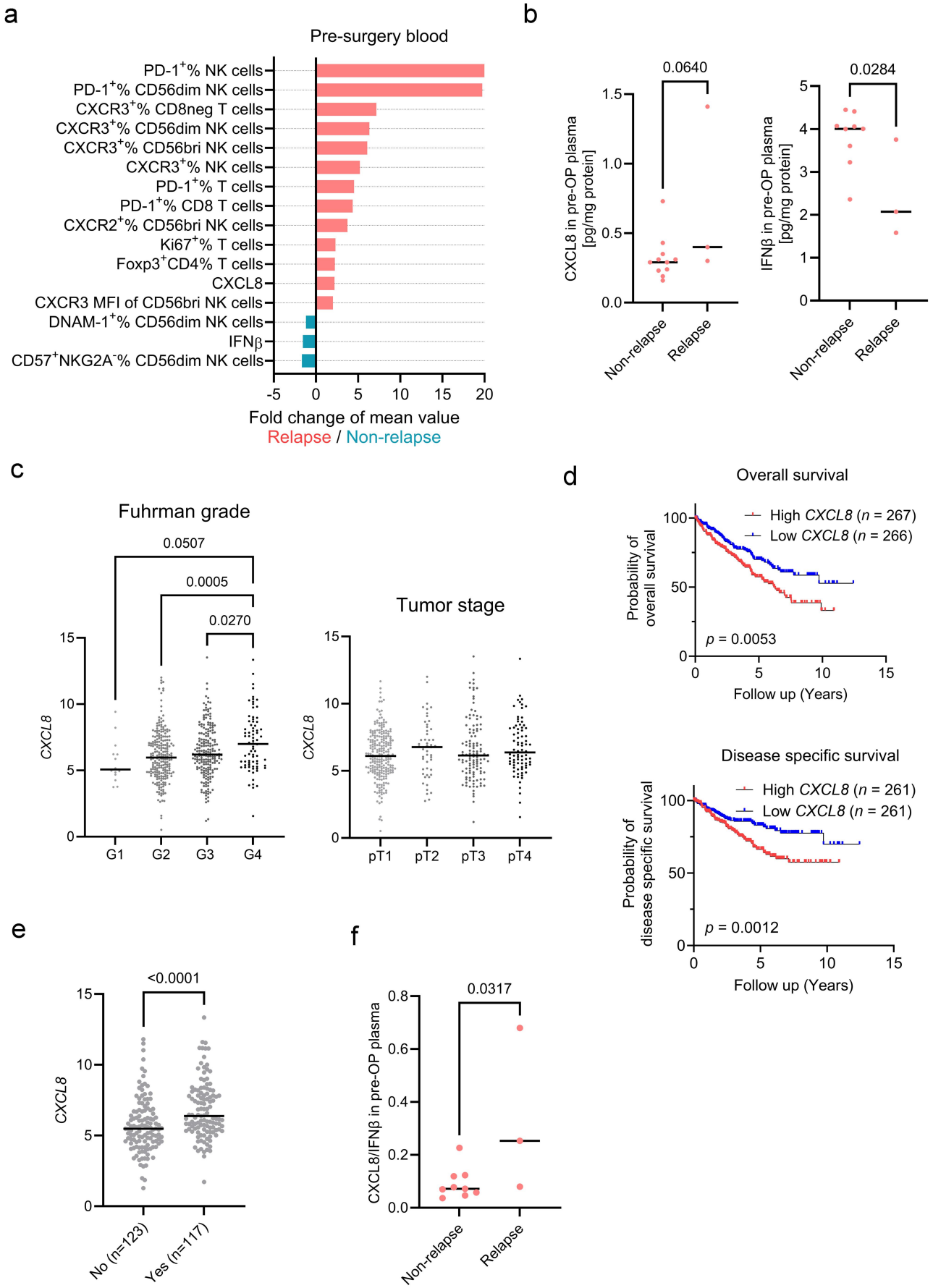


**Figure 4.** CXCL8 and IFNβ levels correlate with disease relapse in RCC patients undergoing nephrectomy. (a) Significant changes ($p < 0.05$) of cellular factors displayed as fold change of mean values in pre-surgery blood samples between relapse and non-relapse patients. (b) CXCL8 and IFNβ levels in relapse and non-relapse patients. (c) *CXCL8* expression across Fuhrman grade and primary tumor stage in TCGA-KIRC cohort. (d) Overall survival and disease specific survival in RCC patients stratified by high and low *CXCL8* expression (set as 50%). *p* values were calculated by log-rank test. (e) *CXCL8* expression in patients without (no) and with (yes) a new tumor event within the TCGA-KIRC cohort. (f) Ratio of CXCL8/IFNβ in relapse vs. non-relapse patients.

surgical removal of their primary tumor, we here describe several putative biomarkers related to disease parameters. Among the most prominent changes in tumors were higher frequencies of T and NK cells expressing the chemokine receptors CCR5 or CXCR3 compared with T and NK cells in peripheral blood. Our data is consistent with previous reports that found that the majority of tumor-infiltrating $CD4^+$ and $CD8^+$ cells express CCR5 in contrast to cells in the peripheral blood in patients with RCC.[5,20,21] The expression of CCR5 on tumor-infiltrating NK cells has not been well studied in the context of human cancers, but CCR5 has been shown to mediate NK cell homing to murine tumors and to sites of inflammation in the context of infection and autoimmunity in both humans and mice.[22,23] The CXCR3/CXCR3 ligand axis is crucial for the recruitment of activated T and NK cells to tumor and inflammation sites.[24,25] This axis has been shown to play a role to mediate tumor regression in a murine model of RCC and associated with favorable outcomes in RCC patients.[8,20,26] Our results that the expression of CXCR3 was higher on $CD8^-$ T cells and NK cells in RCC tumors compared with peripheral blood are in agreement with earlier studies in RCC patients showing that the majority of intratumoral T cells express CXCR3.[20,21]

Both T and NK cells expressed higher levels of PD-1 within the tumor compared with peripheral blood. These results agree with a recent study showing higher frequency of PD-1 positive T and NK cells within the tumor compartment compared with peripheral blood.[27] Our results are furthermore in agreement with those of MacFarlane *et al*. that reported increased levels of PD-1 expression on peripheral blood $CD56^{dim}$ NK cells and on effector and effector memory $CD8^+$ T cells of patients with stage 1 and stage 4 RCC compared with healthy controls.[28] Here, we report that PD-1 expression is higher on peripheral blood NK and $CD8^+$ T cells in RCC patients with lower pT stages. Although induction of PD-1 on NK cells in a tumor setting is not well established, PD-1 has been reported to be elevated on tumor-associated NK cells of patients with ovarian and hepatocellular carcinomas.[29,30] Since PD-1 expression on tumor-infiltrating lymphocytes and programmed death-ligand 1 (PD-L1) expression on tumor cells have been associated with poor clinical outcome in RCC patients,[14,31,32] it may be of value to explore anti-PD-1/L1 immune checkpoint therapy in a neoadjuvant setting. However, a recent Phase II trial of neoadjuvant sitravatinib plus nivolumab in patients undergoing nephrectomy for locally advanced RCC did not substantially increase the overall response rates.[33] We previously reported that RCC-infiltrating NK cells express lower levels of DNAM-1 compared with peripheral blood NK cells.[34] Here, we extend these findings and show that DNAM-1 positive NK cells are enriched in low stage and grade RCC.

The majority of the investigated soluble factors were enriched in RCC tumors compared with plasma, indicating an inflammatory TME. The intratumoral TNF superfamily member APRIL, the factor with the greatest impact on the separation between tumors and blood, as well as TNF-α have been shown to be expressed in more aggressive and advanced RCC tumors and associated with poor survival.[35,36] In general, there was a trend toward higher levels of inflammatory cytokines in low-stage tumors, indicating that these tumors are inflamed. In contrast, no such trend was observed based on tumor grade.

Upon surgical removal of the primary tumor, the concentration of several cytokines decreased. Within cellular compartments, no change in Treg frequencies was observed, as has been reported previously.[37] Yet, while there was no difference between tumors and peripheral blood, high intratumoral Treg frequencies were associated with a higher Fuhrman nuclear grade. These results are in agreement with Liotta *et al*., who reported that intratumoral Treg frequencies correlated with both the primary tumor stage and nuclear grade.[38] MDSCs play an important role in RCC. The frequencies of MDSCs are increased in metastatic RCC patients compared with healthy individuals and is negatively correlated with the survival of patients treated with a peptide vaccine.[12] It has also been described that PMN-MDSCs is the dominant subset of MDSCs to infiltrate RCC tumors to suppress T cell function through the production of reactive oxygen species, nitric oxide, and arginase 1.[39–41] Although no difference in blood MDSCs frequencies between high and low stage or grade patients was observed, decreased frequencies of blood myeloid cells and in particular PMN-MDSC were observed following surgery.

In addition to increased Treg frequency in high-grade tumors, the frequency of $CXCR3^+/CD56^{bright}$ was higher in high-grade tumors. Furthermore, CXCL8 was significantly higher in high-grade tumors compared with low-grade tumors. CXCL8, a chemokine able to attract cells expressing the CXCR2 receptor. CXCR2 ligands including CXCL8 have previously been shown to play important roles in RCC progression. For instance, they are associated with angiogenesis, tumor growth, metastatic potential, and attraction of PMN-MDSCs.[19,39,42,43] Najjar *et al* observed a correlation between the infiltration of the PMN-MDSCs in RCC with the expression of IL-1β, CXCL8, CXCL5, and MIP-1α.[39] Here, we extend these observations demonstrating a positive association between CXCL8 levels and PMN-MDSCs frequencies in peripheral blood. Elevated serum levels of CXCL8 are found in various cancers including RCC and is associated with high tumor burden and an unfavorable prognosis.[37,42,44–47] Also, Corro *et al*. showed that CXCL8 along with CXCR1 expression is associated with cancer stem cell-like properties of RCC.[48]

Changes in serum CXCL8 can serve as a biomarker to monitor immunotherapy outcomes when using checkpoint inhibitors.[44,49] Two clinical studies treating patients with advanced RCC, urothelial carcinoma, melanoma, and non-small-cell lung cancer with immune checkpoint inhibitors showed that a high baseline level of CXCL8 was associated with poor survival.[50,51] With regards to potential biomarkers of relapse following nephrectomy, we found that PD-1 and CXCR3 positive T and NK cells were present at higher frequencies in patients with relapse. Notably, we also found higher and lower levels of CXCL8 and IFNβ in relapse patients, respectively.

In conclusion, we report multiple changes in cellular and soluble factors in patients with RCC undergoing nephrectomy (Figure 5). In addition, we highlight several potential immune-related disease progression biomarkers in peripheral blood and tumors of RCC patients. However, serum levels of CXCL8 represent among the most notable

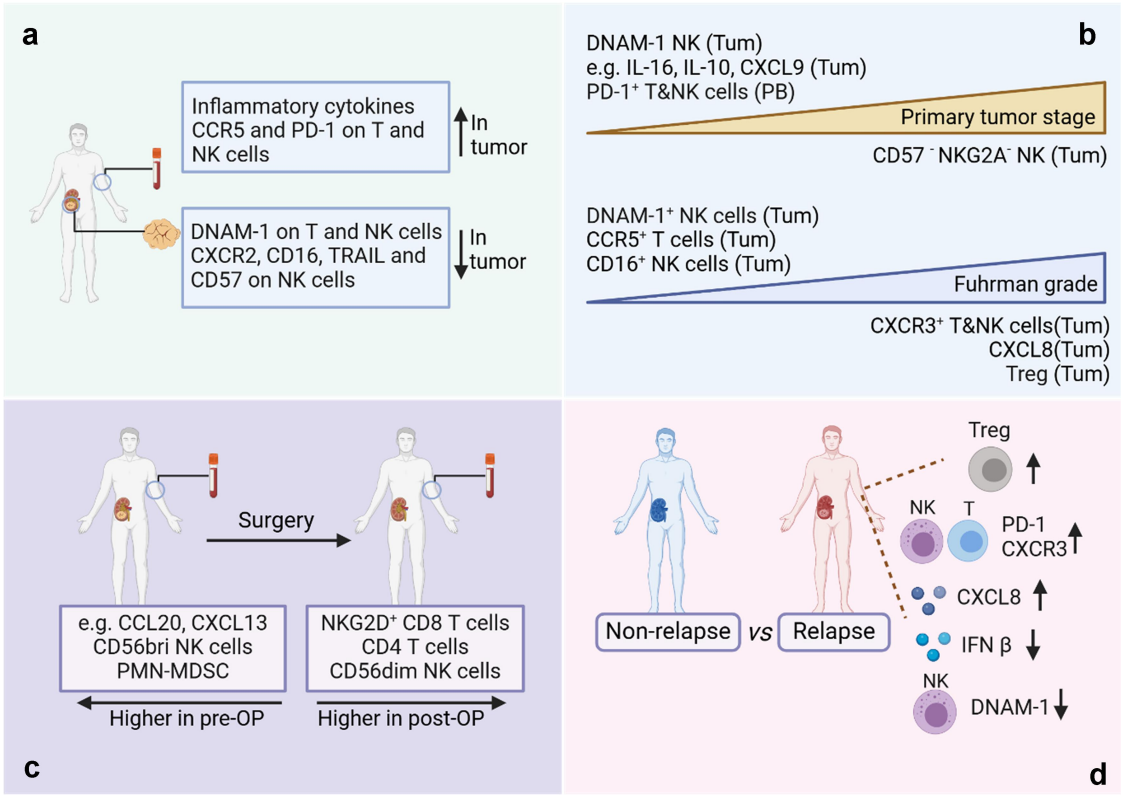


**Figure 5.** Cellular and soluble factors in RCC patients. (a) Difference of immune phenotypes and secretome between blood and tumor. (b) Correlation of tumor and peripheral blood derived variables with tumor stage and Fuhrman grade. (c) Difference in pre- vs. post-surgery blood. (d) Factors correlating with RCC relapse.

findings, where higher levels are present in more aggressive tumors and in patients with relapse. Despite limitations in sample size and diversity of our cohort, our findings warrant for further detailed investigation of CXCL8, potentially coupled with analysis of IFNβ levels, to identify high-risk patients following nephrectomy. Furthermore, since CXCL8 has been shown to upregulate the expression of PD-1 on T cells it may be of value to explore combined PD-1 and CXCL8 blockade.[52] Indeed, Liu and colleagues recently showed that CXCL8 blockade synergizes with PD-1 blockade to delay tumor progression in a model of murine glioma.[53]

## Acknowledgments

We thank Elina Staff for assistance with multivariate analysis.

## Author contributions

CRediT: **Le Tong:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Visualization, Writing – original draft, Writing – review & editing; **Veronika Kremer:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization; **Shi Yong Neo:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation; **Christina Seitz:** Methodology; **Nicholas P. Tobin:** Formal analysis; **Barbara Seliger:** Conceptualization, Resources; **Ulrika Harmenberg:** Resources; **Eugenia Colón:** Resources; **Ann-Helén Scherman Plogell:** Formal analysis, Resources; **Lisa L. Liu:** Conceptualization, Data curation, Funding acquisition, Resources, Supervision, Writing – original draft, Writing – review & editing; **Andreas Lundqvist:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Supervision, Writing – original draft, Writing – review & editing.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

This work was supported by The Swedish Cancer Society [#CAN21 1524 Pj and #CAN24 3655 Pj], The Cancer Research Funds of Radiumhemmet [#211253 and #241293], The Swedish Society for Medical Research [P17-0134], The Swedish Society of Medicine [SLS-960960], Region Stockholm [FoUI-974888 and FoUI-987561], Clas Groschinsky Foundation, Stiftelsen Tornspiran, Karolinska Institutet [#2024-03034], National Medical Research Council [OFIRG24jan-0111], and The Robert Lundberg Memorial Foundation [#2023-01810].

## ORCID

Andreas Lundqvist http://orcid.org/0000-0002-9709-2970

## Author contributions statement

## Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## References


1. Dabestani S, Beisland C, Stewart GD, Bensalah K, Gudmundsson E, Lam TB, Gietzmann W, Zakikhani P, Marconi L, Fernandéz-Pello S, et al. Intensive imaging-based follow-up of surgically treated localised renal cell carcinoma does not improve post-recurrence survival: results from a European multicentre database (RECUR). Eur Urol. 2019;75(2):261–264. doi: 10.1016/j.eururo.2018.10.007.
2. Janiszewska AD, Poletajew S, Wasiutynski A. Spontaneous regression of renal cell carcinoma. Contemp Oncol (Pozn). 2013;2 (2):123–127. doi: 10.5114/wo.2013.34613.
3. Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, Tykodi SS, Sosman JA, Procopio G, Plimack ER, et al. Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. N Engl J Med. 2015;373(19):1803–1813. doi: 10.1056/NEJMoa1510665.
4. Balch CM, Riley LB, Bae YJ, Salmeron MA, Platsoucas CD, Von Eschenbach A, Itoh K. Patterns of human tumor-infiltrating lymphocytes in 120 human cancers. Arch Surg. 1990;125(2):200–205. doi: 10.1001/archsurg.1990.01410140078012.
5. Cozar JM, Canton J, Tallada M, Concha A, Cabrera T, Garrido F, Ruiz-Cabello Osuna F. Analysis of NK cells and chemokine receptors in tumor infiltrating CD4 T lymphocytes in human renal carcinomas. Cancer Immunol Immunother. 2005;54(9):858–866. doi: 10.1007/s00262-004-0646-1.
6. Donskov F, von der Maase H. Impact of immune parameters on long-term survival in metastatic renal cell carcinoma. J Clin Oncol. 2006;24(13):1997–2005. doi: 10.1200/JCO.2005.03.9594.
7. Eckl J, Buchner A, Prinz PU, Riesenberg R, Siegert SI, Kammerer R, Nelson PJ, Noessner E. Transcript signature predicts tissue NK cell content and defines renal cell carcinoma subgroups independent of TNM staging. J Mol Med (Berl). 2012;90(1):55–66. doi: 10.1007/s00109-011-0806-7.
8. Geissler K, Fornara P, Lautenschlager C, Holzhausen H-J, Seliger B, Riemann D. Immune signature of tumor infiltrating immune cells in renal cancer. Oncoimmunology. 2015;4(1): e985082. doi: 10.4161/2162402X.2014.985082.
9. Schleypen JS, Von Geldern M, Weiss EH, Kotzias N, Rohrmann K, Schendel DJ, Falk CS, Pohla H. Renal cell carcinoma-infiltrating natural killer cells express differential repertoires of activating and inhibitory receptors and are inhibited by specific HLA class I allotypes. Int J Cancer. 2003;106(6):905–912. doi: 10.1002/ijc.11321.
10. Krzywinska E, Kantari-Mimoun C, Kerdiles Y, Sobecki M, Isagawa T, Gotthardt D, Castells M, Haubold J, Millien C, Viel T, et al. Loss of HIF-1α in natural killer cells inhibits tumour growth by stimulating non-productive angiogenesis. Nat Commun. 2017;8 (1):1597. doi: 10.1038/s41467-017-01599-w.
11. Chevrier S, Levine JH, Zanotelli VRT, Silina K, Schulz D, Bacac M, Ries CH, Ailles L, Jewett MAS, Moch H, et al. An immune atlas of clear cell renal cell carcinoma. Cell. 2017;169(4):736–749 e18. doi: 10.1016/j.cell.2017.04.016.
12. Walter S, Weinschenk T, Stenzl A, Zdrojowy R, Pluzanska A, Szczylik C, Staehler M, Brugger W, Dietrich P-Y, Mendrzyk R, et al. Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. Nat Med. 2012;18(8):1254–1261. doi: 10.1038/nm.2883.
13. Griffiths RW, Elkord E, Gilham DE, Ramani V, Clarke N, Stern PL, Hawkins RE. Frequency of regulatory T cells in renal cell carcinoma patients and investigation of correlation with survival. Cancer Immunol Immunother. 2007;56(11):1743–1753. doi: 10.1007/s00262-007-0318-z.
14. Kang MJ, Kim KM, Bae JS, Park HS, Lee H, Chung MJ, Moon WS, Lee DG, Jang KY. Tumor-infiltrating PD1-positive lymphocytes and FoxP3-positive regulatory T cells predict distant metastatic relapse and survival of clear cell renal cell carcinoma. Transl Oncol. 2013;6(3):282–289. doi: 10.1593/tlo.13256.
15. Jasinski-Bergner S, Stoehr C, Bukur J, Massa C, Braun J, Hüttelmaier S, Spath V, Wartenberg R, Legal W, Taubert H, et al. Clinical relevance of miR-mediated HLA-G regulation and the associated immune cell infiltration in renal cell carcinoma. Oncoimmunology. 2015;4(6):e1008805. doi: 10.1080/2162402X.2015.1008805.
16. Trygg J, Wold S. Orthogonal projections to latent structures (O-PLS). J Chemometr. 2002;16(3):119–128. doi: 10.1002/cem.695.
17. Jonsson P, Wuolikainen A, Thysell E, Chorell E, Stattin P, Wikström P, Antti H. Constrained randomization and multivariate effect projections improve information extraction and biomarker pattern discovery in metabolomics studies involving dependent samples. Metabolomics. 2015;11(6):1667–1678. doi: 10.1007/s11306-015-0818-3.
18. Eriksson L, Byrne T, Johansson E, Trygg J, Vikström C. Multi- and megavariate data analysis basic principles and applications. Third revised ed. Malmö, Sweden: MKS Umetrics AB; 2013.
19. Alfaro C, Teijeira A, Onate C, Pérez G, Sanmamed MF, Andueza MP, Alignani D, Labiano S, Azpilikueta A, Rodriguez-Paulete A, et al. Tumor-produced interleukin-8 attracts human myeloid-derived suppressor cells and elicits extrusion of neutrophil extracellular traps (NETs). Clin Cancer Res. 2016;22 (15):3924–3936. doi: 10.1158/1078-0432.CCR-15-2463.
20. Kondo T, Nakazawa H, Ito F, Hashimoto Y, Osaka Y, Futatsuyama K, Toma H, Tanabe K. Favorable prognosis of renal cell carcinoma with increased expression of chemokines associated with a Th1-type immune response. Cancer Sci. 2006;97 (8):780–786. doi: 10.1111/j.1349-7006.2006.00231.x.
21. Oldham KA, Parsonage G, Bhatt RI, Wallace DMA, Deshmukh N, Chaudhri S, Adams DH, Lee SP. T lymphocyte recruitment into renal cell carcinoma tissue: a role for chemokine receptors CXCR3, CXCR6, CCR5, and CCR6. Eur Urol. 2012;61(2):385–394. doi: 10.1016/j.eururo.2011.10.035.
22. Liu C, Lou Y, Lizee G, Qin H, Liu S, Rabinovich B, Kim GJ, Wang Y-H, Ye Y, Sikora AG, et al. Plasmacytoid dendritic cells induce NK cell–dependent, tumor antigen–specific T cell cross-priming and tumor regression in mice. J Clin Invest. 2008;118 (3):1165–1175. doi: 10.1172/JCI33583.
23. Gregoire C, Chasson L, Luci C, Tomasello E, Geissmann F, Vivier E, Walzer T. The trafficking of natural killer cells. Immunol Rev. 2007;220(1):169–182. doi: 10.1111/j.1600-065X.2007.00563.x.
24. Qin S, Rottman JB, Myers P, Kassam N, Weinblatt M, Loetscher M, Koch AE, Moser B, Mackay CR. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. J Clin Invest. 1998;101(4):746–754. doi: 10.1172/JCI1422.
25. Wendel M, Galani IE, Suri-Payer E, Cerwenka A. Natural killer cell accumulation in tumors is dependent on ifn-γ and CXCR3 ligands. Cancer Res. 2008;68(20):8437–8445. doi: 10.1158/0008-5472.CAN-08-1440.
26. Tannenbaum CS, Tubbs R, Armstrong D, Finke JH, Bukowski RM, Hamilton TA. The CXC chemokines IP-10 and Mig are necessary for IL-12-mediated regression of the mouse RENCA tumor. J Immunol. 1998;161(2):927–932. doi: 10.4049/jimmunol.161.2.927.
27. Lee MH, Järvinen P, Nísen H, Brück O, Ilander M, Uski I, Theodoropoulos J, Kankainen M, Mirtti T, Mustjoki S, et al. T and NK cell abundance defines two distinct subgroups of renal cell carcinoma. Oncoimmunology. 2022;11(1):1993042. doi: 10.1080/2162402X.2021.1993042.
28. MacFarlane A, Jillab M, Plimack ER, Hudes GR, Uzzo RG, Litwin S, Dulaimi E, Al-Saleem T, Campbell KS. PD-1 expression on peripheral blood cells increases with stage in renal cell carcinoma patients and is rapidly reduced after surgical tumor resection. Cancer Immunol Res. 2014;2(4):320–331. doi: 10.1158/2326-6066.CIR-13-0133.
29. Pesce S, Greppi M, Tabellini G, Rampinelli F, Parolini S, Olive D, Moretta L, Moretta A, Marcenaro E. Identification of a subset of human natural killer cells expressing high levels of programmed

death 1: a phenotypic and functional characterization. J Allergy Clin Immunol. 2017;139(1):335–346 e3. doi: 10.1016/j.jaci.2016.04.025.
30. Liu Y, Cheng Y, Xu Y, Wang Z, Du X, Li C, Peng J, Gao L, Liang X, Ma C. Increased expression of programmed cell death protein 1 on NK cells inhibits nk-cell-mediated anti-tumor function and indicates poor prognosis in digestive cancers. Oncogene. 2017;36 (44):6143–6153. doi: 10.1038/onc.2017.209.
31. Thompson RH, Dong H, Lohse CM, Leibovich BC, Blute ML, Cheville JC, Kwon ED. PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. Clin Cancer Res. 2007;13(6):1757–1761. doi: 10.1158/1078-0432.CCR-06-2599.
32. Thompson RH, Dong H, Kwon ED. Implications of B7-H1 expression in clear cell carcinoma of the kidney for prognostication and therapy. Clin Cancer Res. 2007;13(2):709s–715s. doi: 10.1158/1078-0432.CCR-06-1868.
33. Karam JA, Msaouel P, Haymaker CL, Matin SF, Campbell MT, Zurita AJ, Shah AY, Wistuba II, Marmonti E, Duose DY, et al. Phase II trial of neoadjuvant sitravatinib plus nivolumab in patients undergoing nephrectomy for locally advanced clear cell renal cell carcinoma. Nat Commun. 2023;14(1):2684. doi: 10.1038/s41467-023-38342-7.
34. Tong L, Kremer V, Neo SY, Liu Y, Chen Y, Wagner AK, Yang Y, Chen Z, Seitz C, Tobin NP, et al. Renal cell carcinoma escapes NK cell-mediated immune surveillance through the downregulation of DNAM-1. Cancer Commun (Lond). 2023;43(7):855–859. doi: 10.1002/cac2.12446.
35. Pelekanou V, Notas G, Theodoropoulou K, Kampa M, Takos D, Alexaki V-I, Radojicic J, Sofras F, Tsapis A, Stathopoulos EN, et al. Detection of the TNFSF members BAFF, APRIL, TWEAK and their receptors in normal kidney and renal cell carcinomas. Anal Cell Pathol (Amst). 2011;34(1–2):49–60. doi: 10.1155/2011/108631.
36. Mikami S, Mizuno R, Kosaka T, Saya H, Oya M, Okada Y. Expression of TNF -α and CD 44 is implicated in poor prognosis, cancer cell invasion, metastasis and resistance to the sunitinib treatment in clear cell renal cell carcinomas. Int J Cancer. 2015;136(7):1504–1514. doi: 10.1002/ijc.29137.
37. Wald G, Barnes KT, Bing MT, Kresowik TP, Tomanek-Chalkley A, Kucaba TA, Griffith TS, Brown JA, Norian LA. Minimal changes in the systemic immune response after nephrectomy of localized renal masses11This work was supported by the university of Iowa carver college of medicine/department of urology investigator start-up funds, NIH grant CA181088-01 (to L.A.N.), and NIH grant CA109446 (to T.S.G.). Urol Oncol. 2014;32(5):589–600. doi: 10.1016/j.urolonc.2014.01.023.
38. Liotta F, Gacci M, Frosali F, Querci V, Vittori G, Lapini A, Santarlasci V, Serni S, Cosmi L, Maggi L, et al. Frequency of regulatory T cells in peripheral blood and in tumour-infiltrating lymphocytes correlates with poor prognosis in renal cell carcinoma. BJU Int. 2011;107(9):1500–1506. doi: 10.1111/j.1464-410X.2010.09555.x.
39. Najjar YG, Rayman P, Jia X, Pavicic PG, Rini BI, Tannenbaum C, Ko J, Haywood S, Cohen P, Hamilton T, et al. Myeloid-derived suppressor cell subset accumulation in renal cell carcinoma parenchyma is associated with intratumoral expression of IL1β, IL8, CXCL5, and Mip-1α. Clin Cancer Res. 2017;23(9):2346–2355. doi: 10.1158/1078-0432.CCR-15-1823.
40. Kusmartsev S, Su Z, Heiser A, Dannull J, Eruslanov E, Kübler H, Yancey D, Dahm P, Vieweg J. Reversal of myeloid cell–mediated immunosuppression in patients with metastatic renal cell carcinoma. Clin Cancer Res. 2008;14(24):8270–8278. doi: 10.1158/1078-0432.CCR-08-0165.
41. Zea AH, Rodriguez PC, Atkins MB, Hernandez C, Signoretti S, Zabaleta J, McDermott D, Quiceno D, Youmans A, O'Neill A, et al. Arginase-producing myeloid suppressor cells in renal cell carcinoma patients: a mechanism of tumor evasion. Cancer Res. 2005;65(8):3044–3048. doi: 10.1158/0008-5472.CAN-04-4505.
42. Mestas J, Burdick MD, Reckamp K, Pantuck A, Figlin RA, Strieter RM. The role of CXCR2/CXCR2 ligand biological axis in renal cell carcinoma [Research support, N.I.H. extramural research support, U.S. Gov't, P.H.S.]. J Immunol. 2005;175(8):5351–5357. doi: 10.4049/jimmunol.175.8.5351.
43. Zhou N, Lu F, Liu C, Xu K, Huang J, Yu D, Bi L. IL-8 induces the epithelial-mesenchymal transition of renal cell carcinoma cells through the activation of AKT signaling. Oncol Lett. 2016;12 (3):1915–1920. doi: 10.3892/ol.2016.4900.
44. Alfaro C, Sanmamed MF, Rodríguez-Ruiz ME, Teijeira Á, Oñate C, González Á, Ponz M, Schalper KA, Pérez-Gracia JL, Melero I. Interleukin-8 in cancer pathogenesis, treatment and follow-up. Cancer Treat Rev. 2017;60:24–31. doi: 10.1016/j.ctrv.2017.08.004.
45. Cheng Y, Ma X-L, Wei Y-Q, Wei X-W. Potential roles and targeted therapy of the CXCLs/CXCR2 axis in cancer and inflammatory diseases. Biochim et Biophys Acta (BBA) - Rev Cancer. 2019;1871 (2):289–312. doi: 10.1016/j.bbcan.2019.01.005.
46. Polimeno M, Napolitano M, Costantini S, Portella L, Esposito A, Capone F, Guerriero E, Trotta A, Zanotta S, Pucci L, et al. Regulatory T cells, interleukin (IL)-6, IL-8, vascular endothelial growth factor (VEGF), CXCL10, CXCL11, epidermal growth factor (EGF) and hepatocyte growth factor (HGF) as surrogate markers of host immunity in patients with renal cell carcinoma. BJU Int. 2013;112(5):686–696. doi: 10.1111/bju.12068.
47. Scheibenbogen C, Möhler T, Haefele J, Hunstein W, Keilholz U. Serum interleukin-8 (IL-8) is elevated in patients with metastatic melanoma and correlates with tumour load. Melanoma Res. 1995;5 (3):179–182. doi: 10.1097/00008390-199506000-00006.
48. Corro C, Healy ME, Engler S, Bodenmiller B, Li Z, Schraml P, Weber A, Frew IJ, Rechsteiner M, Moch H. IL-8 and CXCR1 expression is associated with cancer stem cell-like properties of clear cell renal cancer. J Pathol. 2019;248(3):377–389. doi: 10.1002/path.5267.
49. Bakouny Z, Choueiri TK. IL-8 and cancer prognosis on immunotherapy. Nat Med. 2020;26(5):650–651. doi: 10.1038/s41591-020-0873-9.
50. Schalper KA, Carleton M, Zhou M, Chen T, Feng Y, Huang S-P, Walsh AM, Baxi V, Pandya D, Baradet T, et al. Elevated serum interleukin-8 is associated with enhanced intratumor neutrophils and reduced clinical benefit of immune-checkpoint inhibitors. Nat Med. 2020;26(5):688–692. doi: 10.1038/s41591-020-0856-x.
51. Yuen KC, Liu L-F, Gupta V, Madireddi S, Keerthivasan S, Li C, Rishipathak D, Williams P, Kadel EE, Koeppen H, et al. High systemic and tumor-associated IL-8 correlates with reduced clinical benefit of PD-L1 blockade. Nat Med. 2020;26(5):693–698. doi: 10.1038/s41591-020-0860-1.
52. Li X, Zhai J, Shen Y, Zhang T, Wang Y, He Y, You Q, Shen L. Tumor-derived IL-8 facilitates lymph node metastasis of gastric cancer via PD-1 up-regulation in CD8(+) T cells. Cancer Immunol Immunother. 2022;71(12):3057–3070. doi: 10.1007/s00262-022-03223-3.
53. Liu H, Zhao Q, Tan L, Wu X, Huang R, Zuo Y, Chen L, Yang J, Zhang Z-X, Ruan W, et al. Neutralizing IL-8 potentiates immune checkpoint blockade efficacy for glioma. Cancer Cell. 2023;41 (4):693–710.e8. doi: 10.1016/j.ccell.2023.03.004.