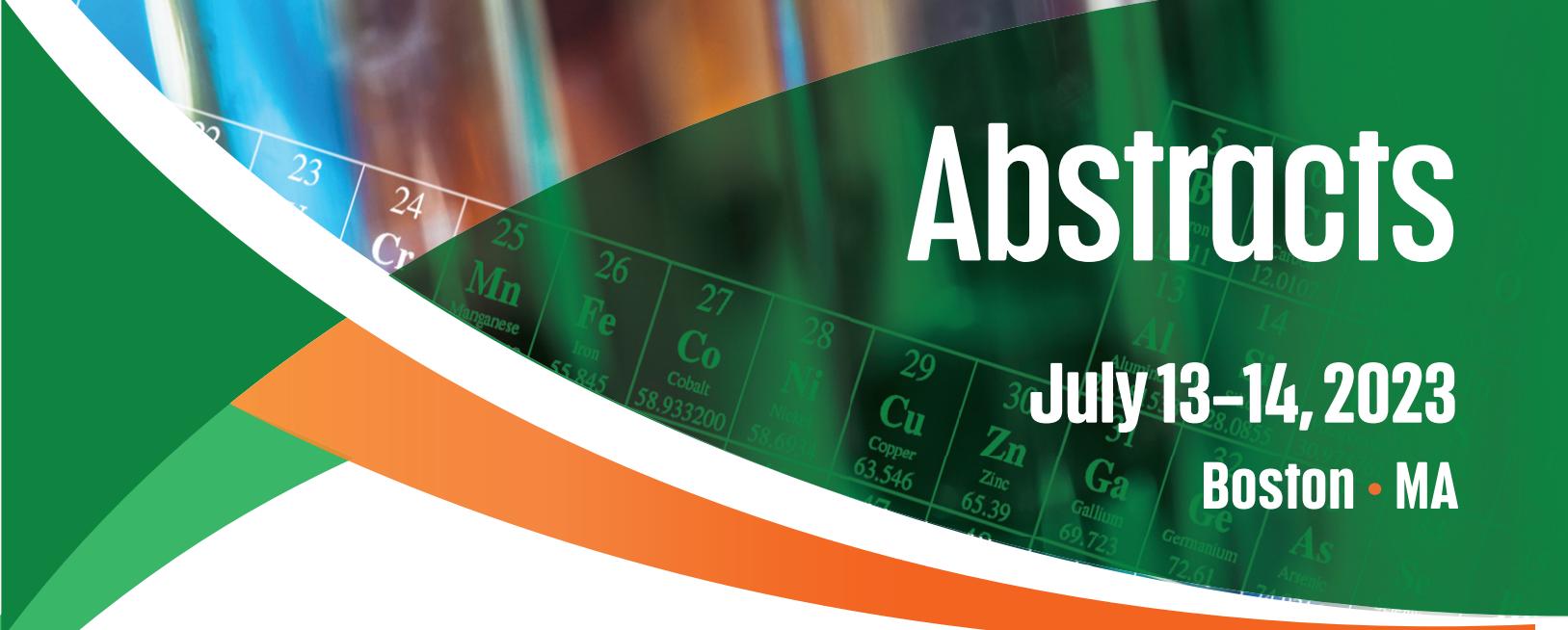


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# KCRS 23 Kidney Cancer Research Summit

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# Oral Abstract Presentations

**32**

## ORCHID: A phase II study of Olaparib in Metastatic Renal Cell Carcinoma Patients Harboring a BAP1 or Other DNA Repair Gene Mutations.

Yasser Ged MBBS, Irina Rifkind MSN, Lori Tony MSN, Keegan Daugherty MSc, Amber Michalik MSc, Hao Wang PhD, Michael Carducci MD, Mark Markowski MD, PhD

Johns Hopkins University

### Background

DNA damage repair genes alterations (DDRa) are frequent events in renal cell carcinoma (RCC), including BAP1 and other DDRa. Olaparib is a poly ADP ribose polymerase inhibitor (PARPi) that is FDA-approved for the treatment of several malignancies with DDRa. Preclinical models demonstrated synthetic lethality with PARPi in RCC cell lines including BAP1 mutant lines. Here we report an interim analysis of the ORCHID study investigating the clinical activity of single agent olaparib in patients (pts) with advanced RCC (aRCC) harboring BAP1 other select DDRa.

### Methods

We conducted a single center, single arm, investigator-initiated Phase 2 trial of olaparib in pts with aRCC. Eligible pts harbored select DDRa and had prior therapy with immune checkpoint inhibitors (ICIs) and/or VEGF-TKI. Pts were treated with olaparib at an initial dose of 150mg twice which was increased to 300mg twice daily after one month if well tolerated. The primary endpoint is disease control rate (DCR) by RECIST v1.1 (including complete response (CR), partial response (PR), and stable disease (SD) >6 months). Secondary endpoints included objective response rate (ORR), progression free survival (PFS), and safety.

### Results

Eleven pts were enrolled with a median age of 59 years (48-72) including 9 pts with clear cell RCC and 2 pts with unclassified RCC. Most pts had BAP1 mutations (Table). 36% of pts had history of brain metastasis. Median number of prior lines of therapies was 2 (1-6) and all pts received prior ICI. The study met the pre-specified Simon's 2 stage design for the first stage with 22% DCR in the evaluable pts (2/9), including deep PR (>70% reduction in tumor volume) and SD of 10 months. Both pts harbored BAP1 mutations. An additional pt with BRCA1

mutation had 20% decrease in measurable disease. There were no treatment related adverse events resulting in study discontinuation.

Total patients	Number
Mutation n (%)	
BAP1	7 (64%)
ATM	2 (18%)
PALB2*	1 (9%)
BRCA1	1 (9%)
BRCA1	1(9%)

\* one pt with BAP1 and PALB2 co-mutations

### Conclusions

This is the first study investigating single agent PARPi in RCC with the interim trial analysis indicating promising activity of olaparib in aRCC pts with BAP1 mutations including one pt with deep PR. These results support further development of PARPi in this setting.

### Keywords

PARP, DDR, BAP1

**40**

## Functional and translational consequences of immunometabolic coevolution in ccRCC

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### Background

Tumor cell phenotypes and anti-tumor immune responses are shaped by local metabolite availability, but intratumoral metabolite heterogeneity (IMH) and its phenotypic consequences remain poorly understood. In vitro mechanistic studies have demonstrated that the anti-tumor activity of lymphoid and myeloid cell populations is mediated by

metabolite availability and signaling in the TME, raising the possibility that the immune response and metabolism of ccRCC tumors coevolve and jointly influence the likelihood that a patient responds to therapy.. However, both the broad patterns of coordination between metabolite abundance and TME cellular composition, as well as the precise cell populations producing metabolic phenotypes of interest, remain unknown.

## Methods

To study IMH, we multiregionally profiled the metabolome, transcriptome, and genome of 187 tumor/normal regions from 31 clear cell renal cell carcinoma (ccRCC) patients. Using these measurements and additional multimodal metabolomic/transcriptomic profiling of ccRCC and other diseases, we developed computational models that can be used to understand RNA-metabolite covariation and ultimately impute metabolite levels from RNA sequencing data.

## Results

Analysis of intratum metabolite-RNA covariation revealed that the immune composition of the microenvironment, and especially the abundance of myeloid cells, drove intratumoral metabolite variation. Motivated by the strength of RNA-metabolite covariation and the clinical significance of RNA biomarkers in ccRCC, we deployed and benchmarked a machine learning method (MIRTH) to impute metabolite levels directly from RNA sequencing data of primary and metastatic ccRCC tumors. We inferred metabolomic profiles from RNA sequencing data of ccRCC patients enrolled in 6 clinical trials, ultimately identifying specific metabolite biomarkers associated with response to anti-angiogenic agents.

## Conclusions

Local metabolic phenotypes therefore emerge in tandem with the immune microenvironment and associate with therapeutic sensitivity.

## Keywords

Immunometabolism; Machine learning; Metabolomics

## CDMRP DOD Funding

yes

46

## Circulating KIM-1 is a minimally invasive biomarker correlated with treatment response to nivolumab in patients with metastatic renal cell carcinoma

**Wenxin Xu, Sai V. Vermula, Samuel M. Niman, Xiaowen Liu, Ayumi Takakura, Zimo Huang, Toni K. Choueiri, Matthew L Freedman, Paul J Catalano, Joseph V Bonventre, Saurabh Gupta, David F McDermott, Rupal S Bhatt**

Dana-Farber Cancer Institute

## Background

There are currently no circulating biomarkers used for clinical monitoring of clear cell renal cell carcinoma (ccRCC). Such a biomarker could facilitate individualized treatment decisions and minimize exposure to ineffective therapies. Prior studies have suggested that circulating KIM-1 is a potential minimally invasive biomarker for ccRCC, but the utility of KIM-1 for identifying early response to nivolumab therapy is not known.

## Methods

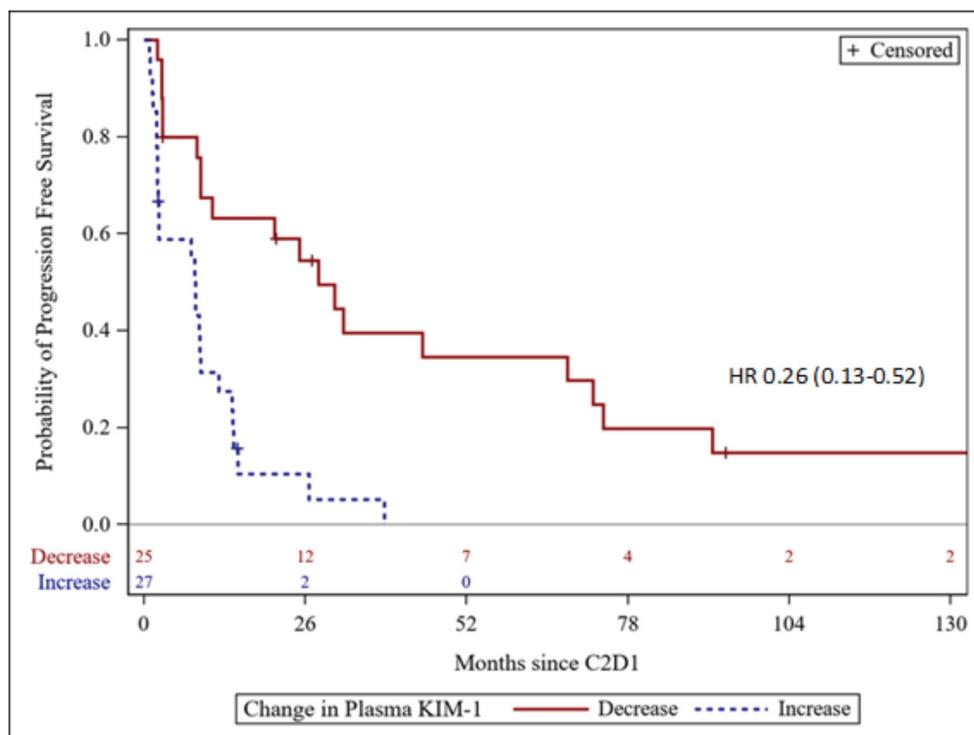
CheckMate-009 was a prospective trial investigating nivolumab (every 3 weeks at 0.3, 2, or 10 mg/kg) in patients with metastatic clear cell RCC. We measured serum KIM-1 at baseline and after 3 weeks of treatment (prior to cycle 2) using a custom sandwich immunoassay using the R-PLEX platform. Human KIM-1 antibody (R&D systems, #AF1750) was used to prepare biotin conjugated antibodies and detection antibodies. The assay lowest limit of detection for KIM-1 was 4.88 pg/mL. We assessed the association between early changes in serum KIM-1 and treatment related clinical outcomes.

## Results

Clinical data and serum KIM-1 was analyzed in 54 patients. KIM-1 was high in all patients at baseline (median serum KIM-1 5913 pg/mL, IQR 2137-25101 pg/mL). 25 patients (48%) had a decrease in KIM-1 at 3 weeks after a single dose of nivolumab. Decrease in KIM-1 at 3 weeks was associated with improved PFS (univariable HR 0.26, 95% CI 0.13-0.52; multivariable HR 0.22, 95% CI 0.097-0.50 after adjustment for sex, prior nephrectomy, nivolumab dose, and IMDC risk factors).

## Conclusions

Serum KIM-1 is elevated in patients with metastatic ccRCC and is associated with clinical outcomes. Among patients



treated with nivolumab in the CheckMate-009 trial, early decrease in KIM-1 from baseline to 3 weeks was predictive for PFS.

#### Keywords

Blood biomarkers, metastatic renal cell carcinoma, checkpoint inhibitors

#### CDMRP DOD Funding

yes

**62**

## Spatial proteomics enables identification of prognostic biomarkers in papillary renal cell carcinoma

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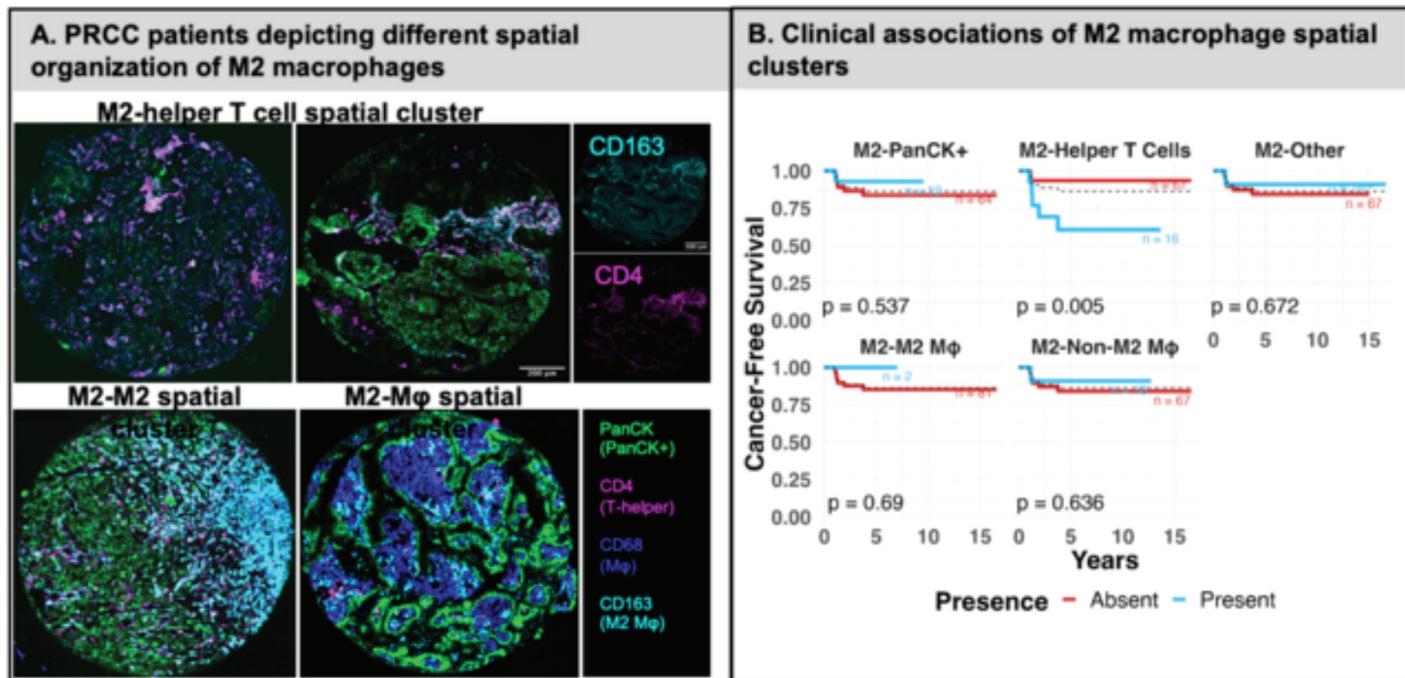
<sup>1</sup>Vanderbilt University Medical Center. <sup>2</sup>Vindhya Data Science

#### Background

Papillary renal cell carcinoma (PRCC) is the second most common adult kidney cancer histology, constituting 15-20% of cases. While some patients may have indolent PRCC tumors that grow slowly, other tumors rapidly metastasize. Some PRCC tumors respond to immune checkpoint inhibitors. Thus, understanding the immune tumor microenvironment and how it correlates with patient outcomes in this relatively rare disease is a critical need. Spatial interrogation of patient samples has the potential to offer novel insights into the tumor-immune axis and provide avenues for enhanced diagnosis and treatment. Addressing questions of spatial arrangement within tumors has remained a technical and biological challenge, but emerging spatial biology technologies provide molecular data at single cell resolution. We hypothesized that the spatial interaction of immune, stromal and tumor cells would be prognostic for PRCC patients.

#### Methods

A tissue microarray was assembled from an archive of ~100 patients presenting with PRCC. This dataset was assayed with PhenoCycler/CODEX(Akoya Biosciences) using a 31-antibody panel with immune- and cancer-related proteins. We have developed methodology and novel algorithms to perform signal normalization, cell segmentation, and cell typing. We computed neighborhoods for each of 2.5 million cells and



performed network analysis to identify spatial clusters. This method allowed us to identify clusters consistently present across multiple TMA spots from the same patient and across multiple patients.

## Results

Using spatial neighborhood analysis, we have identified diverse spatial clusters of potential clinical relevance, including five distinct M2 macrophage spatial clusters. We have described one of the clusters as being physically associated with helper T cells, which is visualized on a PRCC spot and shows co-occurrence of M2-macrophages (CD163) and helper T cells (CD4). This pattern was replicated in additional TMA spots from the same patient. In contrast, other clusters of M2-macrophages (M2-M2 spatial cluster and M2-M1 macrophages) are visualized to show vastly different cellular neighborhoods. Clinical associations show that the patients with presence of the M2-T helper cluster have a poor cancer-associated survival ( $p=0.005$ ). In comparison, the total proportion of M2 macrophages is not associated with survival

( $p=0.4$ ), highlighting the importance of characterizing spatial interactions beyond cell type quantitation.

## Conclusions

In summary, we highlight the utility of spatial biology to explain the heterogeneity in patients' tumors and to uncover novel correlates of clinical phenotypes, establishing a platform for future discovery in this field and the identification of additional spatial correlates of patient outcome and clinical response.

## Keywords

Spatial biology, tumor microenvironment, immune checkpoint inhibitors, papillary renal cell carcinoma, data science, bioinformatics

## CDMRP DOD Funding

yes

65

## Final database lock results of the phase 2 cohort of lenvatinib + pembrolizumab for progressive disease after a PD-1/PD-L1-containing therapy in metastatic clear cell renal cell carcinoma

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### Background

Lenvatinib + pembrolizumab is approved for the first-line treatment of advanced renal cell carcinoma (RCC). In the CLEAR trial, the combination showed statistically significant/clinically meaningful improvements in overall survival (OS), progression-free survival (PFS), and objective response rate (ORR) vs sunitinib (Motzer R et al. NEJM. 2021). As immune checkpoint inhibitors (ICIs) have become standards of care as first-line treatment of RCC, an unmet need exists for patients who have disease progression after treatment with ICIs.

A phase 1b/2 trial of lenvatinib + pembrolizumab was performed in multiple tumor types enrolling patients who had or had not received prior therapy; the primary analysis of the RCC cohort has been reported previously (Lee C-H et al. Lancet Oncol. 2021). Here, we report the final results of this RCC cohort with an additional 24 months of follow-up.

### Methods

Eligible patients ≥18 years old with measurable disease were treated with lenvatinib 20 mg daily + pembrolizumab

200 mg once every 3 weeks. The primary endpoint (ORR at week 24 [ORRwk24] per immune-related [ir] RECIST by the investigators) has been previously reported (Lee C-H et al. Lancet Oncol. 2021). Secondary endpoints included ORR, duration of response (DOR), PFS (each by the investigators), OS and safety. In the analyses reported herein, efficacy data were grouped by prior therapy patients had received, including ICI-pretreated, treatment-naïve, and previously treated but ICI-naïve. Tumors were assessed by the investigators per irRECIST and RECIST v1.1 (modified to allow assessment of ≤10 target lesions [ $\leq$ 5 per organ]).

### Results

145 Patients were enrolled, of whom 105 were ICI-pretreated, 23 were treatment-naïve, and 17 were previously treated ICI-naïve. By the final database lock date (August 24, 2022), the median duration of follow-up for OS was 37.7 months (95% CI 35.2–42.7). Efficacy analyses included 104 ICI-pretreated patients and 22 treatment-naïve patients; 1 patient in either group was excluded as they were negative for clear cell disease.

Per irRECIST by investigator assessment, ORR was 62.5% for ICI-pretreated patients (median DOR: 14.1 months), 77.3% for treatment-naïve patients (median DOR: 24.2 months), and 52.9% for previously treated ICI-naïve patients (median DOR: 9.0 months) (Table). Notably, 18-month PFS rates were 38.0% for ICI-pretreated patients, 70.5% for treatment-naïve patients, and 36.1% for previously treated ICI-naïve patients (Table). The median OS was 32.1 months for ICI-pretreated patients, 55.8 months for treatment-naïve patients, and 30.3 months for previously treated ICI-naïve patients; 24-month OS rates were >50% in all groups. Additional efficacy data are included in the Table.

For those who had their dose of lenvatinib reduced (per recommended algorithms for standard adverse event [AE] management) the median time to first dose reduction was 2.10 months (range 0.1–16.6) for ICI-pretreated patients (n=75), 6.47 months (range 0.3–33.1) for treatment-naïve patients (n=18), and 2.89 months (range 1.0–12.5) for previously treated ICI-naïve patients (n=10).

Overall, treatment-related (TR) AEs were reported in 99.3% of all 145 patients; grade ≥3 TRAEs were reported in 66.2%. The most common TRAE was diarrhea (n=88). Treatment-emergent, clinically significant (CS) AEs and AEs of special interest for pembrolizumab (AEOSIs) were reported in 95.2% and 55.9% of patients, respectively. Hypertension was the most common grade ≥3 CSAE (n=37), severe skin reactions were the most common grade ≥3 AEOSI (n=6). The median time to first onset of CSAEs was 0.64 months; the median time to first onset of AEOSIs was 2.07 months.

	ICI-pretreated <sup>a,b</sup> (n=104)		Treatment-naïve <sup>b</sup> (n=22)		Previously treated ICI-naïve <sup>c</sup> (n=17)	
	irRECIST	RECIST v1.1	irRECIST	RECIST v1.1	irRECIST	RECIST v1.1
<b>Objective response, n (%) [95% CI]</b>	65 (62.5) [52.5–71.8]	61 (58.7) [48.6–68.2]	17 (77.3) [54.6–92.2]	17 (77.3) [54.6–92.2]	9 (52.9) [27.8–77.0]	9 (52.9) [27.8–77.0]
CR, n (%)	1 (1.0)	1 (1.0)	0	0	0	0
PR, n (%)	64 (61.5)	60 (57.7)	17 (77.3)	17 (77.3)	9 (52.9)	9 (52.9)
<b>Median DOR, months (95% CI)<sup>d</sup></b>	14.1 (9.7–18.2)	14.1 (10.6–18.4)	24.2 (10.3–40.4)	24.2 (10.3–40.4)	9.0 (3.5–NE)	9.0 (3.5–NE)
<b>Responders by response duration, n (%)<sup>e</sup></b>						
≥18 months	21 (32.3)	21 (34.4)	10 (58.8)	10 (58.8)	3 (33.3)	3 (33.3)
≥24 months	15 (23.1)	15 (24.6)	8 (47.1)	8 (47.1)	2 (22.2)	2 (22.2)
≥30 months	5 (7.7)	5 (8.2)	7 (41.2)	7 (41.2)	2 (22.2)	2 (22.2)
≥36 months	0	0	6 (35.3)	6 (35.3)	1 (11.1)	1 (11.1)
<b>Median PFS, months (95% CI)</b>	13.6 (9.5–17.7)	11.6 (7.6–14.1)	24.1 (11.7–38.8)	22.1 (11.6–31.7)	11.8 (5.5–21.9)	11.8 (5.5–18.6)
PFS rate at 18 months, % (95% CI)	38.0 (28.2–47.7)	34.5 (25.1–44.1)	70.5 (45.7–85.6)	67.2 (43.1–82.8)	36.1 (13.2–59.8)	28.8 (8.9–52.7)
<b>Median OS, months (95% CI)</b>	32.1 (26.4–NE)		55.8 (31.4–NE)		30.3 (28.7–NE)	
OS rate at 18 months, % (95% CI)	70.3 (60.3–78.2)		90.9 (68.3–97.6)		80.4 (50.6–93.2)	
OS rate at 24 months, % (95% CI)	64.2 (54.0–72.7)		90.9 (68.3–97.6)		80.4 (50.6–93.2)	

<sup>a</sup>The ICI regimens most commonly received as prior therapy included: nivolumab monotherapy (n=41) and nivolumab + ipilimumab (n=39).

<sup>b</sup>Excluded 1 patient who had non-clear cell renal cell carcinoma.

<sup>c</sup>Previous therapy included anti-vascular endothelial growth factor receptors received by 16 patients, of which sunitinib (n=9) was most commonly received.

<sup>d</sup>Among responders.

<sup>e</sup>Percentage of responders.

CI, confidence interval; CR, complete response; DOR, duration of response; ICI, immune checkpoint inhibitor; ir, immune-related; NE, not estimable; OS, overall survival; PFS, progression-free survival; PR, partial response.

## Conclusions

Lenvatinib + pembrolizumab demonstrated promising and durable antitumor activity with a manageable safety profile in patients with metastatic RCC, including in those who were ICI-pretreated. To our knowledge, this represents the largest cohort of ICI-pretreated patients with RCC prospectively treated in a clinical trial.

## Keywords

Renal cell carcinoma, immune checkpoint inhibitor, PD-1, PD-L1

## CDMRP DOD Funding

no

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## Host immune signatures as predictors of response to immunotherapy-based regimens in patients with metastatic renal cell carcinoma (mRCC)

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### Background

Treatment options for mRCC have evolved to include VEGF targeted therapies (VEGF-TT), immune checkpoint inhibitors (ICIs), or combinations of both. However, clinical responses to systemic therapies in mRCC remain largely unpredictable and robust biomarkers are still lacking. The interaction between the tumor and its immune microenvironment has been shown to influence clinical outcomes in patients treated with ICI-based regimens. The aim of this study was to characterize the T-cell and B-cell immune repertoires in patients with mRCC treated with VEGF-TT, ICI or a combination of both, and evaluate their associations with clinical outcomes.

### Methods

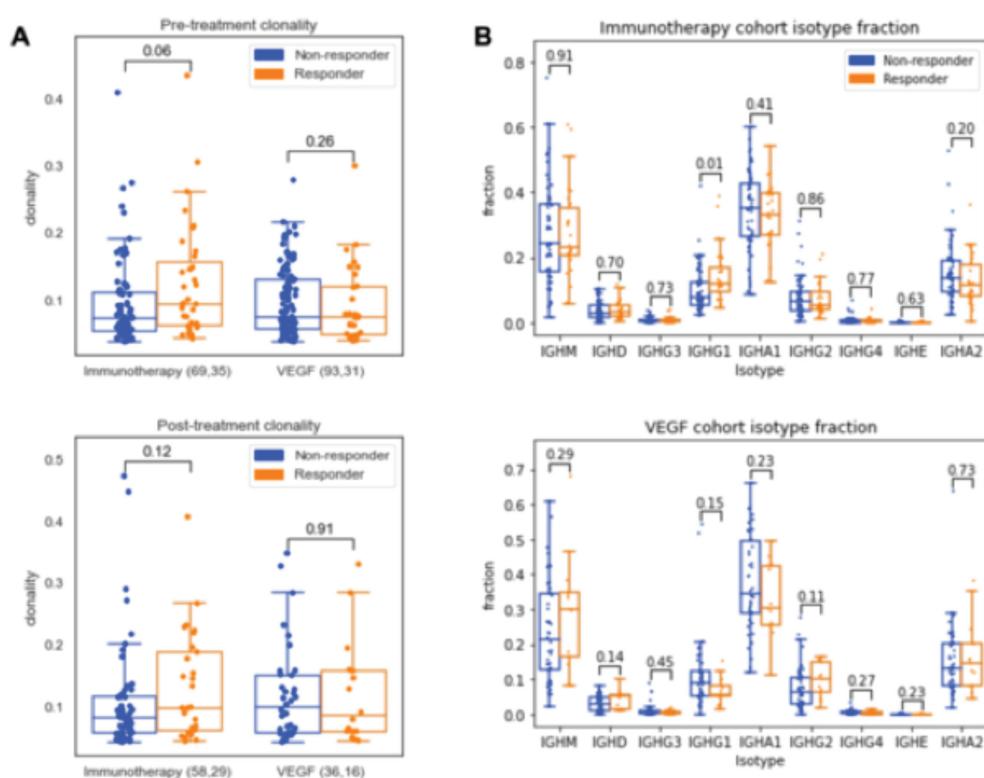
We identified patients with mRCC at Dana-Farber Cancer Institute treated with VEGF-TT, ICI or both, and for whom tumor and/or blood samples were available. T-cell receptor sequencing (TCR-seq) was performed on peripheral blood mononuclear cells (PBMCs), collected before and during therapy. Bulk RNA-sequencing (RNA-seq) was performed on available pre-treatment PBMCs and primary tumor samples. Immunoglobulin heavy chain (IgH) isotypes were inferred from bulk RNA-seq data using TRUST4. Parameters of the T-cell and B-cell repertoires were evaluated in responders vs. non-responders to systemic therapies, and between pre- and on-treatment samples within patient subgroups.

### Results

In total, blood (PBMC) samples from 386 patients were available across all treatment cohorts (186 VEGF-TT, 126 ICI and 74 ICI+VEGF-TT).

Following quality-control, TCR-seq data were available for 367 patients (228 pre-treatment and 139 on-treatment), while RNA-seq data were available for 105 PBMC and 17 tumor-derived pre-treatment samples.

In the TCR-seq analysis, responders to ICI-based regimens (ICI or VEGF-TT+ICI) presented a trend towards an increased baseline (pre-treatment) TCR clonality as compared to non-responders ( $p=0.06$ ) (Fig. 1A), corresponding to a less



**Figure 1:** (A) Evaluation of pre-treatment and post-treatment TCR clonality in responders vs. non-responders across treatment cohorts. (B) Differences in the fraction of IGH isotypes between responders and non-responders across treatment cohorts.

polyclonal T cell repertoire in responders at baseline. No significant changes in clonality were seen between pre- and on-treatment samples among responders to ICI regimens ( $p=0.14$ ), as opposed to non-responders where a significant increase was identified ( $p=0.001$ ). Therefore, responders to ICI-based regimens seem to have a more oligoclonal TCR repertoire (increased clonality) at baseline with no treatment-induced changes, whereas non-responders to ICI-based regimens seem to evolve from a more polyclonal to a more oligoclonal TCR repertoire in response to treatment.

The analysis of IgH isotypes in baseline blood samples showed higher fraction of IgG1 in responders (vs. non-responders) to ICI regimens ( $p=0.01$ ) (Fig. 1B). This was further confirmed in the analysis of IgH isotypes inferred from tumor samples ( $p=0.04$ ). No significant differences in IgH isotype fractions were identified between responders and non-responders to VEGF-TT. Furthermore, while shared clonotypes were detected between blood and tumor samples, there were no differences in the Jaccard similarity index between responders and non-responders to ICI-based or VEGF-TT regimens.

## Conclusions

We were successfully able to characterize T-cell receptor and B-cell IgH repertoires in a large cohort of patients with mRCC, and evaluate their associations with ICI response. Our results show that baseline TCR clonality and IgG1 antibody fraction are associated with the response to ICI regimens, suggesting a potential role for immune biomarker development.

## Keywords

renal cell carcinoma, immunotherapy, biomarkers, T cells, immunoglobulins

## CDMRP DOD Funding

yes

# Rapid Abstracts Presentations

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## The impact of insurance status on progression-free survival (PFS) and overall survival (OS) in patients with metastatic renal cell carcinoma (mRCC)

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### Background

In addition to the previously studied socioeconomic factors associated with health disparities, insurance status can serve as a prognostic factor associated with overall survival in RCC patients (Zhang et al., Future Oncol 2019). Moreover, within this population, having access to health insurance has been reported to result in earlier detection of disease (Javier-DesLoges et al., JAMA Netw Open 2021). In this study, we explored the impact of primary and secondary insurance status on PFS and OS in patients receiving first line systemic therapy for mRCC.

### Methods

Patients with mRCC from two NCI-designated comprehensive cancer centers diagnosed between 1990 and 2022 with available insurance information were retrospectively identified using institutional databases. Primary insurance information was categorized into three groups—Medicare, private insurance, and Medicaid/no insurance—while secondary insurance was defined by the presence or absence of secondary coverage. PFS and OS were estimated by Kaplan-Meier method and compared based on insurance status using log-rank tests. Univariate and multivariate Cox proportional hazard regression models were used to examine the impact of insurance status on PFS and OS.

### Results

In total, 645 patients with mRCC had accessible information and were included in our analysis. Of these, 344 (53.3%), 250 (38.8%), and 51 (7.9%) had primary Medicare, private insurance, and Medicaid/no insurance, respectively. Overall, most patients were male (73.0%), with a median age of 60.0 (22.0-94.0) at time of diagnosis. The most commonly rendered first-line treatments were monotherapy with targeted agents (66.4%), dual immunotherapy (13.6%), and targeted/immunotherapy combinations (10.2%). Median PFS for the entire cohort was 6.6 months (95% CI, 5.9-7.7). Median PFS for patients with primary Medicare was 7.7 months (95% CI 7.0-9.0), 5.5 months (95% CI, 4.0-7.0) for patients with private insurance, and 4.9 months (95% CI, 3.8-8.1) for Medicaid/uninsured patients. Using an overall log-rank test, a significant difference in PFS among the three groups with different primary insurance was observed ( $p<0.0001$ ). The median PFS for patients with secondary insurance was 8.1 months (95% CI, 6.6-11.3) compared to 6.1 months (95% CI, 5.5-7.4) for patients without secondary insurance. A multivariate Cox model with adjustment for other factors (such as age, gender, and ethnicity) revealed a statistically significant difference in PFS between patients with and without secondary insurance ( $p=0.0281$ ). Median OS for the entire cohort was 36.8 months (95% CI, 32.4-44.3). Median OS for patients with primary Medicare, private insurance, and Medicaid/no insurance was 49.0 months (95%, CI 41.8-55.3), 28.5 months (95% CI, 24.1-35.7), and 21.6 months (95% CI, 17.5-42.3), respectively. By an overall log-rank test, a significant difference in OS across the three groups of primary insurance was observed ( $p=0.0003$ ). No statistically significant differences in OS were observed between patients with and without secondary insurance. Overall, patients with primary Medicare had superior median PFS ( $p=0.0327$ ) and OS ( $p=0.0004$ ) compared to those with Medicaid/no insurance; although patients with private insurance had a lower risk of progression and death compared to those with Medicaid/no insurance, the result was not statistically significant.

### Conclusions

In this real-world study, we investigated the impact of insurance status on clinical outcomes in patients with mRCC receiving systemic therapy. mRCC patients with primary Medicaid/no insurance or private insurance had inferior median PFS and OS compared to those with primary Medicare. Moreover, patients with secondary insurance had superior PFS over those with primary insurance alone. Overall, our findings suggest that insurance status may serve as a determinant of clinical outcomes. These hypothesis-generating data warrant external validation in prospective studies.

**Keywords**

insurance status; clinical outcomes; disparities; social determinants of health

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## Dissection of tumor-intrinsic and tumor-extrinsic features of MiT/TFE translocation renal cell carcinoma via single-cell RNA sequencing

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**Background**

Translocation renal cell carcinoma (tRCC) is a rare and aggressive subtype of renal cell carcinoma driven by oncogenic gene fusions involving MiT/TFE family transcription factors, most commonly TFE3. Currently, there are no molecularly tailored treatments for tRCC, and standard-of-care therapies utilized for other RCC subtypes are typically less effective in tRCC. Emerging data suggest that tRCC is molecularly distinct from more common RCC subtypes. However, an incomplete understanding of both tumor-intrinsic drivers and the tumor microenvironment (TME) features of tRCC presents barriers to developing effective therapeutics for this cancer.

**Methods**

We used single-nucleus RNA sequencing (snRNA) to profile ten samples of tRCC tumors and one adjacent normal tissue. Five of the samples also underwent multiome profiling with snATAC-seq while nine of the tumor samples underwent whole genome sequencing and RNA-sequencing. We preprocessed and obtained the raw fasta files using cellranger and extracted RNA counts and chromatin accessibility peaks using the Seurat/Signac algorithm. Scrublet was used to exclude data from droplets containing more than one cell by performing doublet detection and removal on gene-barcode matrices. Cells with fewer than 200 genes detected or more than 5% of counts attributed to mitochondrial-encoded transcripts were removed before across-sample integration. We also excluded genes detected in fewer than three cells across all samples. The combined cohort was analyzed using the MergeData function in Seurat, and cancer cells were selected based on known tRCC gene markers and inferred transcriptional copy number variations estimated via the

inferCNV package and verified from WGS data. Non-tumor cell types were determined through manual annotation via known marker genes. We analyzed patterns of gene expression at the single-cell level using the Seurat V4 package and module score functions. Differential expression analysis comparing cells from different clusters or treatment exposure groups was performed using a two-sided Wilcoxon rank-sum test with Bonferroni FDR correction.

**Results**

Following quality control and integration, 71,124 single-cells were used for analysis (67,762 from tumor samples) – representing 43,214 tumor cells, 8,352 monocytes, 4,692 T cells, 4,614 endothelial/stromal cells, and normal kidney cells. The normal-adjacent sample represented multiple cell types from the normal kidney, including cells from distal connecting tubule, connecting duct, proximal tubule, thick ascending limb, podocytes, and endothelial cells. Analysis of chromatin accessibility profiles from snATAC-seq and snRNA-seq data of tumor cells was employed to identify unique cell states and a putative cell of origin for tRCCs. Tumor cell analysis also demonstrated intra- and inter-tumoral transcriptional variability in genetically similar tumor subclusters, each with distinct regulon activity. Immune subpopulation analyses revealed higher proportions of resting T cells in treatment-naïve tRCC samples and higher proportions of progenitor-exhausted and terminal-exhausted populations in an immune checkpoint inhibitor (ICI) treated sample, indicating immune reprogramming in response to ICI in this mutationally quiet tumor.

To further understand the role of, and factors permissive to, anti-tumor T cell responses in tRCC (which is characterized by a low tumor mutational burden) we explored the hypothesis that MiT/TFE fusions may constitute tumor neoantigens. We computationally identified fusion-associated neoantigens corresponding to these tRCC samples, as well as across a larger aggregate dataset of tRCC samples profiled by bulk RNA-Seq. We predicted binding affinities between fusion-associated neoantigens and HLA types and identified multiple peptide candidates with high HLA-binding affinity, which will be validated for their immunogenicity in vitro.

**Conclusions**

Overall, our results highlight tumor-intrinsic and tumor-extrinsic determinants of immunogenicity in tRCC and may guide the development of immunotherapeutic strategies with strong mechanistic rationale in tRCC.

**Keywords**

Translocation RCC, Neoantigens, Immunology, Single-nucleus

**CDMRP DOD Funding**

yes

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## A modified IL-18 drug in combination with CTLA-4 blockade enhances anti-tumor efficacy in preclinical models of renal cell carcinoma

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### Background

Cytokine-based drugs are currently being explored as alternative cancer immunotherapies. While the cytokine interleukin-18 (IL-18) has immunostimulatory effects, it is negatively regulated by a secreted high-affinity binding protein, IL-18BP, that functions as an immune checkpoint that limits IL-18's efficacy as a cancer therapeutic. A modified version of IL-18, termed "decoy-resistant" or DR-18, that can avoid trapping by IL-18BP while still maintaining its immune signaling potential, has recently been developed. DR-18 has shown promising preclinical activity in melanoma and colorectal murine models, including potential synergy with anti-PD-1 therapy, and is currently in Phase I trials. In this study, we aim to test the efficacy and determine the cellular mechanism of action of DR-18 in combination with immune checkpoint inhibitors (ICIs) in immunocompetent preclinical models of renal cell carcinoma (RCC), with the goal of establishing the basis for testing these combinations in early phase clinical trials.

### Methods

We engrafted tumors subcutaneously using two different syngeneic, immunocompetent murine RCC models: Renca and RAG. Mice were treated with single-agent DR-18 and combinations of DR-18 with single- and dual-agent anti-PD-1 and anti-CTLA-4. Tumor growth and survival were monitored. In the Renca model, plasma was collected at early time-points and cytokine/chemokine levels were profiled using a 31-plex discovery assay. Single-cell RNA and TCR sequencing was also performed on Renca tumors. Additionally, immune cell depletion studies were conducted in the Renca model with antibodies targeting CD8, CD4, NK cells, and interferon-gamma.

### Results

In the Renca model, DR-18 monotherapy modestly inhibited tumor growth and prolonged survival. The effects were comparable to single- and dual-agent ICIs. Adding PD-1

blockade to DR-18 did not enhance efficacy whereas the addition of anti-CTLA-4 to DR-18 significantly increased anti-tumor effects. Triple-therapy (DR-18 plus anti-PD-1 plus anti-CTLA-4) did not further inhibit tumor growth or prolong survival compared to the doublet (DR-18 plus anti-CTLA-4). The RAG model was more sensitive to ICIs but produced similar results, again showing modest anti-tumor activity of single-agent DR-18 and enhanced benefit of combining with anti-CTLA-4 but not anti-PD-1. Cytokine/chemokine profiling revealed significantly elevated levels of IP-10 (CXCL10) and MIG (CXCL9) after one cycle of DR-18 plus anti-CTLA-4 compared to control and single-agent treatments, suggesting that these chemokines may be key early mediators of the anti-tumor immune response. Single-cell transcriptomic analysis demonstrated changes in intra-tumoral T cell, macrophage, and granulocyte populations with DR-18 plus anti-CTLA-4 relative to other regimens, including enrichment of CD8+ precursor and terminally exhausted T cells and a neutrophil population associated with interferon signaling. Additionally, single-cell TCR analysis showed a reduction in intra-tumoral clonotype diversity with DR-18 plus anti-CTLA-4 compared to other treatments. Immune cell depletion studies identified CD8+ T cells, NK cells, and interferon-gamma, but not CD4+ T cells, as equally required for efficacy of DR-18 plus anti-CTLA-4.

### Conclusions

In this study, we identify DR-18, an IL-18-based drug engineered with resistance to a secreted decoy-receptor protein, in combination with anti-CTLA-4 as having enhanced anti-tumor activity in preclinical models of RCC. This regimen was associated with a more pro-inflammatory immune microenvironment. Further investigation is ongoing to elucidate the cellular mechanism of action of this regimen more fully and lay the groundwork for clinical testing of DR-18-based combination therapy in RCC. In the future, testing novel partner agents outside of anti-PD-1/CTLA-4 and using RCC models of ICI-resistance could be particularly informative and clinically relevant.

### Keywords

IL-18, cytokines, immune-checkpoint inhibitors, anti-CTLA-4, preclinical models, RCC

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## Characterization of the Cellular Origin and Oncogenic Mechanisms of Chromophobe Renal Cell Carcinoma (ChRCC) and Renal Oncocytic Neoplasms

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### Background

ChRCC is a rare form of kidney cancer and presents with a poor prognosis in the metastatic setting, with limited response to immune checkpoint inhibitors (ICI) and targeted therapy. While previous studies suggested that ChRCC originates from intercalated cells of the kidney, its exact cellular origin has not been clearly defined. We therefore investigated the cellular origin of ChRCC and renal oncocytic neoplasms at single-cell resolution.

### Methods

ChRCC, renal oncocytoma (RO) and low-grade oncocytic tumor (LOT) samples with matched normal kidney specimens were analyzed using single-cell RNA sequencing (scRNA-seq). Epithelial cells from normal samples were clustered and annotated into the distinct known cellular types found in the adult healthy human kidney. A logistic regression model (Young M.D. et al., *Science*, 2018) was trained on the normal epithelial clusters, using a comprehensive set of 74 marker genes (features). Subsequently, the model was tested on tumor clusters from ChRCC, RO and LOT to investigate their cellular origin through the identification of the highest predicted probabilities of similarity between normal epithelial cellular

types and tumor cells. Differential gene expression and pathway analyses between ChRCC and its cell of origin were then conducted to investigate mechanisms of oncogenesis.

### Results

Following quality-control, 46,817 cells from 5 tumors (ChRCC: n=3, RO: n=1 and LOT: n=1) and 4 normal samples were isolated for scRNA-seq analysis. Among normal samples, 784 epithelial cells were clustered and annotated into the following cellular entities: proximal tubule (PT), loop of Henle – distal tubule (LOH-DT), principal cells (PC),  $\alpha$ -intercalated cells (ICA) and  $\beta$ -intercalated cells (ICB). Across all ChRCC and oncocytic tumors, 7,573 tumor cells were identified, among which 7,425 corresponded to ChRCC. For ChRCC, RO, and LOT neoplasms, the highest predicted probability of similarity was consistently identified with ICA cells (ranging from 0.60 to 0.80). This finding was validated using an external and previously published (Zhang Y. et al., *Proc Natl Acad Sci USA*, 2021) scRNA-seq dataset from a patient with ChRCC (n=2,853 cells). Additionally, evaluation of previously defined ChRCC-specific markers (i.e. FOXI1, RHCG, KIT, CLCNKA, and CLCNKB) in scRNA-seq data of normal kidney epithelial cells consistently showed the highest expression in ICA cells. Differential gene expression between ChRCC and ICA cells revealed a significant downregulation of MHC class I genes (i.e. HLA-A, HLA-B, and HLA-C) and HSP70 (family A) genes (i.e. HSPA1A, HSPA1B, HSPA6, and HSPA8) in ChRCC. Differential pathway analysis between ChRCC and ICA cells showed a significant enrichment of the ferroptosis, glutathione metabolism, mTOR signaling and IL-15 pathways in ChRCC.

### Conclusions

Renal oncocytic tumors, including ChRCC, appear to originate from the  $\alpha$ -intercalated cells of the normal human kidney. As compared to  $\alpha$ -intercalated cells, ChRCC downregulates MHC class I genes, which may be associated with its poor response to immunotherapy. Further, the selective downregulation of HSP70 genes may in part explain ChRCC's increased sensitivity to ferroptosis.

### Keywords

chromophobe RCC ; renal oncocytoma ; cellular origin

### CDMRP DOD Funding

yes

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## Epigenomic profiling nominates master transcription factors (TFs) driving sarcomatoid differentiation (SD) of renal cell carcinoma (RCC)

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### Background

Sarcomatoid differentiation of RCC (sRCC) is associated with poor survival. Recent studies showed marked response of sRCC to immune checkpoint blockade (ICB). While distinctive patterns of gene expression in sRCC have been identified, the gene regulatory programs and TFs that drive SD remain unknown. The aim of this study is to nominate TFs responsible for SD and to investigate their association with the clinical outcomes of patients with RCC.

### Methods

Chromatin immunoprecipitation and sequencing (ChIP-seq) for H3K27ac – a histone modification associated with active regulatory elements – was performed on pathologically reviewed sRCC and non-sRCC samples collected at the Dana-Farber Cancer Institute. Regulatory elements that were differentially active between the two groups were identified based on levels of H3K27ac (Benjamini-Hochberg  $q < 0.01$ , log-fold change (LFC) threshold=1). Enrichment of specific TF binding motifs at activated regulatory elements in sRCC was assessed using HOMER. Differential gene expression analysis of TFs was performed using DESeq2 on RNA-seq data from TCGA. A Mann-Whitney U test was performed on RNA-seq data from the IMmotion151 and Javelin Renal 101 clinical trials to compare mean expression level of TFs in transcript per million (TPM) in these trials. Patients with non-sRCC enrolled in the IMmotion151 trials were divided into quartiles based on gene expression levels of candidate TFs. Progression-free survival (PFS) was compared between non-sRCC patients stratified by expression quartiles as well as patients with sRCC using a multivariable Cox proportional-hazards model accounting for age and IMDC risk score. To validate these findings, a similar analysis was performed in the Javelin Renal 101 trial.

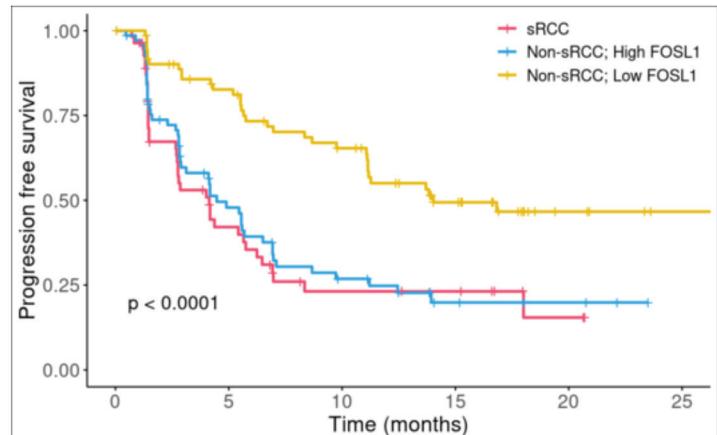


Figure: Kaplan-Meier curves of progression-free survival (PFS) in patients with the sunitinib arm of Javelin Renal 101 by sarcomatoid differentiation and FOSL1 expression levels.

### Results

We obtained high-quality H3K27ac ChIP-seq profiles for 9 sRCC and 17 non-sRCC samples. We identified 278 candidate regulatory elements with increased H3K27ac levels in sRCC vs. non-sRCC. These regulatory elements were enriched for nucleotide motifs bound by the TFs FOSL1 and E2F7. Differential expression analysis between 48 sRCC vs. 493 non-sRCC samples showed that FOSL1 and E2F7 were significantly upregulated in sRCC vs. non-sRCC (LFC=1.7,  $q=5e-11$ ; LFC=1.8,  $q=1.3e-20$ ; resp.). Mean TPMs of FOSL1 and E2F7 were significantly increased in sRCC vs. non-sRCC in IMmotion151 cohort and Javelin Renal 101 (all  $p<0.001$ ). Among patients who received sunitinib, those with the highest quartile of FOSL1 and E2F7 expression showed significantly shorter PFS in IMmotion151 patients compared to patients with the lowest quartile of expression (HR=1.6, 95%CI=1.3-2.2,  $p=0.008$  & HR=2.6, 95%CI=1.8-3.7,  $p<0.001$ ; resp.). Furthermore, patients with highest quartile of expression showed similar PFS compared to patients with sRCC ( $p=0.56$  and  $p=0.64$ ; resp.). These results were validated in the sunitinib arm of the Javelin Renal 101 cohort (Figure).

### Conclusions

This is the first study to characterize the epigenomic landscape of sRCC by integrating ChIP-seq and RNA-seq data. Our findings implicated FOSL1 and E2F7 as transcriptional regulators of SD with prognostic relevance. These TFs seem to be associated with aggressive behavior in non-sRCC as well. Further studies are underway to functionally validate these results.

### Keywords

Sarcomatoid differentiation; Transcriptional regulators; Differential gene expression

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## Examining Trends in Kidney Cancer Mortality by Gender and Race in the United States: A 20-Year Analysis

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### Background

Kidney cancer (KC) is a significant contributor to cancer mortality worldwide, with gender, racial, and ethnic disparities impacting outcomes. While previous studies have investigated the outcomes of diverse patient populations, gaps in knowledge remain. The incidence of KC has been rising steadily, and it is essential to understand the patterns of mortality to identify and address disparities in healthcare. Therefore, this study aimed to investigate trends in age-adjusted mortality rates (ASMR) by gender, race, and ethnicity in the US at national levels.

### Methods

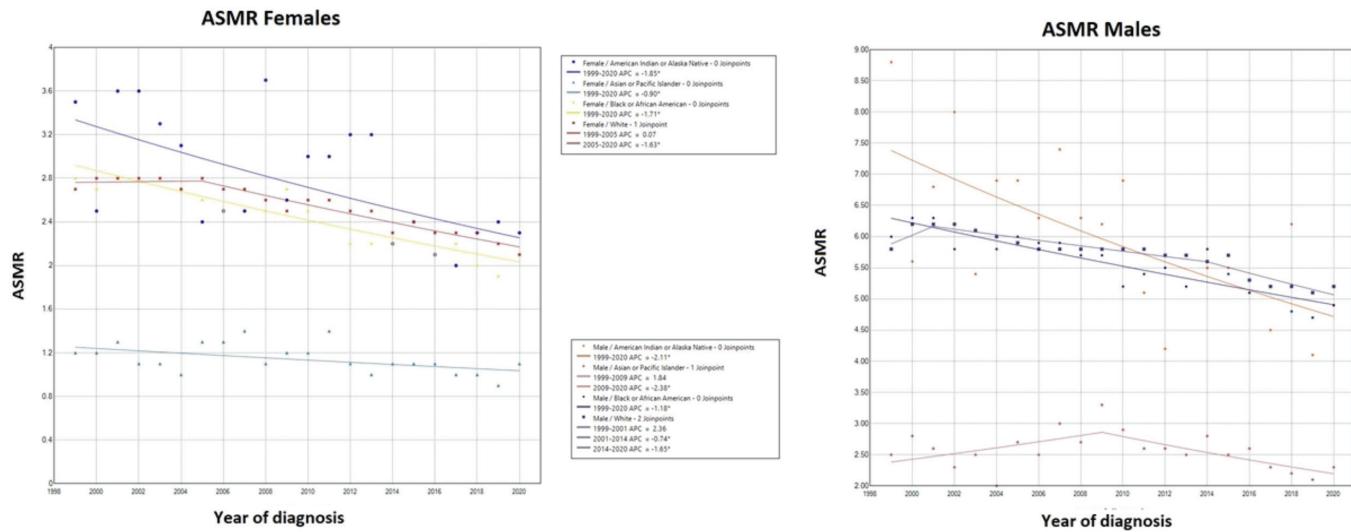
The Center for Disease Control Wonder database was used to extract national KC mortality data (ICD-10 C64) from 1999-2020. ASMR and 95% confidence intervals (CI) were extracted

based on gender and race reported per 100,000 population. Absolute percentage changes were calculated. Joinpoint Command Line Version 4.5.0.1 was used to apply a Joinpoint regression analysis and calculate Average Annual Percentage Change (AAPC). IRB approval was not needed as CDC is an open-source database with de-identified information.

### Results

Over twenty years, 284,224 KC deaths were reported, with an overall decrease in ASMR in recent years. Males had significantly higher ASMR throughout the study period, with a lower decrease than females. Female mortality rates for all racial and ethnic groups declined over the study period, with the largest decrease observed in American Indian/Alaskan Native females (-34.3%). However, American Indian/Alaskan Native females still had the highest mortality rates in 2020. Asian females had the lowest mortality rates throughout the study period, but they only decreased slightly (-8.3%). African American and white females had similar mortality rates that decreased by 25% and 22.2%, respectively. Among males, there was a general decline in mortality rates across all racial and ethnic groups, with the largest decrease observed in American Indian/Alaskan Native males (-44.3%). Despite having lower mortality rates than most other groups, White males had the highest age-adjusted mortality rate (ASMR) of 5.7 in 2020 due to the lack of significant decline. When considering the average annual percentage change (AAPC) over the entire period, the decline in mortality rate was statistically significant for all groups except Asian females. The AAPC ranged from -2.1% for American Indian/Alaskan Native females to -0.4% for Asian females. The steepest decline was observed in American Indian/Alaskan Native males (-2.1%), followed by American Indian/Alaskan Native females (-1.8%) and African American females (-1.7%).

ASMR; 95% CI	1990	2020	Percentage change	AAPC
Overall	4.0; 3.9-4.1	3.4; 3.4-3.5	-15.0	
Female	2.7; 2.6-2.7	2.1; 2.0-2.1	-22.2	
American Indians/Alaskan Natives females	3.5; 2.3-5.2	2.3; 1.7-3.1	-34.3	-1.8 (-2.9 - -0.8)
Asian females	1.2; 0.8-1.6	1.1; 0.9-1.2	-8.3	-0.9 (-1.6 - -0.2)
African Americans females	2.8; 2.5-3.1	2.1; 1.9-2.3	-25.0	-1.7 (-2.1 - -1.3)
White females	2.7; 2.6-2.7	2.1; 2.0-2.2	-22.2	-1.1 (-1.5 - -0.8)
Male	5.8; 5.7-5.9	5.0; 4.9-5.1	-13.8	
American Indians/Alaskan Natives males	8.8; 6.3-12.0	4.9; 3.9-6.1	-44.3	-2.1 (-3.1 - -1.1)
Asian males	2.5; 2.0-3.2	2.3; 2.0-2.6	-8.0	-0.4 (-1.7 - 0.9)
African Americans males	6.0; 5.5-6.5	4.9; 4.5-5.2	-18.3	-1.2 (-1.4 - -0.9)
White males	5.8; 5.7-6.0	5.7; 5.7-5.7	-1.7	-0.7 (-1.3 - -0.1)



## Conclusions

In conclusion, our study highlights significant gender, racial, and ethnic disparities in kidney cancer mortality rates in the US. Our study underscores the importance of targeted interventions to reduce mortality rates among American Indian/Alaskan Native populations, who consistently had the highest mortality rates in kidney cancer throughout the study period. Additionally, our findings highlight the need for continued attention to male mortality rates at the gender level. The results of this study can inform healthcare policies and practices that aim to address the disparities in kidney cancer mortality rates and ultimately improve the health outcomes of all populations.

## Keywords

kidney cancer, mortality, racial disparity, gender disparity

## CDMRP DOD Funding

no

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## Impact of Time to Metastasis (Synchronous vs. Metachronous) on Outcomes in Metastatic Renal Cell Carcinoma Patients Treated with First Line Immune-Checkpoint Inhibitors (ICI)-based Combinations

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## Background

Metastatic renal cell carcinoma (RCC) can present as synchronous metastatic disease, where primary metastases are detected at the time of diagnosis, or metachronous disease, where metastases appear during follow-up after initial RCC diagnosis. The effect of time to metastasis on patient outcomes is not well characterized in the era of immune checkpoint inhibitor (ICI)-based combinations. Herein, we assess the differences in clinical outcomes between patients with synchronous and metachronous mRCC.

## Methods

Data for mRCC patients treated with first line ICI-based combination therapies between 2014 and 2023 were retrospectively collected from two NCI-designated comprehensive cancer centers. Patients were categorized as having synchronous or metachronous disease based on the time of presentation of metastases. Synchronous disease was defined as the presence of metastases at the time of RCC diagnosis or within 3 months thereafter. The study endpoints were time to treatment failure (TTF), overall survival (OS), and disease control rate (DCR). TTF and OS were estimated by Kaplan-Meier method and compared based on the time of presentation of metastases using log-rank tests. Univariable and multivariable Cox proportional hazard models and logistic regression models were used to examine the impact of sex, IMDC risk score, histology, and age at treatment start on TTF, OS and DCR.

## Results

A total of 223 patients (126 synchronous and 97 metachronous) diagnosed with mRCC were included in the analysis. No significant difference was seen in gender distribution or age at first line treatment between these two groups. Although not statistically significant, the synchronous group had a higher proportion of patients with non-clear cell histology compared to those with metachronous disease (21% vs. 11%,  $P = 0.057$ ). The median TTF was shorter in patients with synchronous disease but did not reach statistical significance (9 vs. 19.8 months for synchronous and metachronous, respectively, HR 1.37, 95% CI [0.98, 1.92],  $P = 0.064$ ). Median OS was significantly shorter in patients with synchronous disease (28.0 vs. 50.9 months, adjusted HR 2.23, 95% CI [1.36, 3.65],  $P = 0.001$ ). Similarly, patients with synchronous mRCC had a lower DCR than patients with metachronous (58.7% vs 78.4%, adjusted odds ratio (OR) 0.29, 95% CI [0.13, 0.64],  $P = 0.002$ ). In the multivariable analysis, synchronous disease remained an independent factor associated with worse OS and DCR.

## Conclusions

Patients with metastatic disease at the time of RCC diagnosis who were treated with first line ICI-based combinations have a poorer OS, and worse DCR than those who develop metastatic disease during follow-up. Although further validation is needed, these findings could be valuable in designing clinical trials and selecting patients for treatment escalation and closer clinical follow-up.

## Keywords

mRCC; immune checkpoint inhibitors; prognosis; synchronous; metachronous; renal cell carcinoma

## CDMRP DOD Funding

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# The identification of a novel orally available ferroptosis inducer for the treatment of clear cell renal carcinoma

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## Background

Clear cell renal cell carcinoma (ccRCC), the most common form of kidney cancer, is typically initiated by inactivation of the von Hippel Lindau (VHL) gene resulting in constitutive activation of the hypoxia inducible factors, HIF-1 $\alpha$  and HIF-2 $\alpha$ . HIF-2 $\alpha$  is a central driver of kidney cancer progression and is a well validated therapeutic target for ccRCC. Additionally, VHL loss and HIF activation promote lipid accumulation that increases the sensitivity of ccRCC cells to ferroptosis, a form of cell death characterized by iron dependent lipid peroxidation. However, classical inducers of ferroptosis, which induce ferroptosis in vitro, show limited activity in vivo due to possible redundancies in the extracellular space, suggesting a need for novel molecular targets for the induction of ferroptosis in vivo for therapeutic benefit.

## Methods

We performed a high-throughput screen to identify small molecule inhibitors of HIF-2 $\alpha$  and performed additional validation and structure activity relationship studies to identify compounds with improved potency of HIF-2 $\alpha$  inhibition. The molecular target of these inhibitors was determined by mass spectrometry using the Drug Affinity Responsive Target Stability (DARTS) assay, and target validation was confirmed using siRNA knockdown and NMR. In vivo anti-tumor efficacy was verified using syngeneic RENCA cells injected into Balb/C mice. Exposure to KD061 was verified using LC-MS/MS detection of KD061 in the plasma and tumors of treated mice.

## Results

We identified a novel molecule, KD061, which decreases levels of HIF-1/2 $\alpha$  and induces ferroptosis in vivo through oral administration by targeting Iron Sulfur Cluster Assembly 2 (ISCA2). ISCA2 is a component of the late mitochondrial Iron Sulfur Cluster (L-ISC) assembly complex and ISCA2 inhibition either pharmacologically or using siRNA triggers the iron starvation response, resulting in iron/metals overload and death via ferroptosis. Cell death induced by KD061 was decreased by re-expression of VHL, suggesting synthetic lethality in the context of VHL loss. ISCA2 inhibition also decreased HIF-2 $\alpha$  protein levels by blocking iron-responsive element (IRE)-dependent translation, and at

higher concentrations, also decreased HIF-1 $\alpha$  translation through unknown mechanisms. Treatment of tumor-bearing RENCA-Balb/C mice with KD061 significantly reduced tumor growth *in vivo*, decreased HIF- $\alpha$  levels and increased lipid peroxidation, suggesting increased ferroptosis *in vivo*. Treatment with KD061 was well tolerated at the therapeutic dose with no detectable effects on blood chemistry and blood counts.

## Conclusions

Here, we describe the first orally available inducer of ferroptosis with anti-tumor efficacy *in vivo*. The targeting of ISCA2 using KD061 is a promising therapeutic strategy to inhibit HIF-1/2 $\alpha$  and to induce ferroptosis in pVHL deficient cells.

## Keywords

Ferroptosis, HIF, clear cell renal cell carcinoma

## CDMRP DOD Funding

yes

# Biomarkers in Kidney Cancer Abstracts

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## CT-based radiomics model for the prediction of genomic alterations in renal cell carcinoma (RCC)

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### Background

Radiogenomics is an emerging tool with applications in screening for molecular biomarkers in diagnostic and prognostic assessment through the extraction of quantitative data from medical images (Shui et al., *Front Oncol* 2021). In this study, we aim to develop machine learning (ML) models to predict the most common genomic alterations present in our subset of renal cell carcinoma patients (pts).

### Methods

Retrospectively, pts with tissue genomic testing from CT-guided biopsy samples were identified. Genomic testing was done via GEM ExTra assay, a CAP-accredited, CLIA-certified test encompassing tumor whole exome sequencing and whole transcriptome sequencing (TGen; Phoenix, AZ). Biopsy sample collection sites were identified from pre-biopsy contrast CT images, and the lesions were segmented with ITK-SNAP software, from which 510 radiomic features were extracted with Pyradiomics. The Least Absolute Shrinkage and Selection Operator (LASSO) regression was used to select the most relevant features. Logistic regression (LR) and support

vector machine (SVM) classifiers were built for the prediction of PBRM1, VHL, and SETD2 gene alterations. Multiple metrics were used to evaluate the predictive performance via leave-one-out cross-validation, including the area under the receiver operating characteristic (AUROC) and the area under the precision-recall curve (AUPRC). Feature importance was evaluated with Shapley additive explanations (SHAP) method.

### Results

A total of 14 RCC pts (10:4 M:F) with genomic testing from CT-guided biopsies were identified. The majority of pts were White (85.7%) and had clear cell histology (71.4%). The most common locations for the CT-guided biopsy were lung (18.2%), soft tissue (13.6%), kidney (9.1%), and bone (9.1%). The most common alterations were seen in PBRM1 (50%), VHL (43.9%), and SETD2 (35.7%) genes. The PBRM1 gene was predicted with the highest AUROC (0.84) and AUPRC (0.88) with the SVM classifier, followed by the SETD2 gene (AUROC=0.78 and AUPRC=0.66) with LR classifier and VHL gene (AUROC=0.56 and AUPRC=0.65) with SVM classifier. Notably, all three models showed good sensitivity in classifying gene mutation status (PBRM1: 0.86; VHL: 0.88; SETD2: 0.89). Among all radiomic features, first-order features, Gray Level Size Zone Matrix features, and Gray Level Dependence Matrix features were found to be the most important features for predictions.

### Conclusions

Using a CT-based radiomics analysis of the biopsy area, we showed that SVM and LR prediction models could predict PBRM1, VHL, and SETD2 mutations with high accuracy. These models may assist in identifying potentially actionable alterations and yield ease in treatment selection for RCC. Further extensive studies are warranted to validate our findings and improve our model.

### Keywords

Renal Cell Carcinoma, Radiogenomics, PBRM1, VHL, Machine learning

### CDMRP DOD Funding

no

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## Circulating and intratumoral immune determinants of response to atezolizumab plus bevacizumab in patients with variant histology or sarcomatoid renal cell carcinoma

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### Background

Renal cell carcinoma of variant histology (vRCC) encompasses approximately 20% of RCC diagnoses. Due to a poor understanding of the different biologies of vRCCs, there is currently no standard of care for this type of tumor and guidelines are extrapolated from clear-cell RCC trials. A phase II trial of atezolizumab plus bevacizumab in patients with vRCC or sarcomatoid differentiation had an acceptable safety profile and evidence of clinical activity, particularly in tumors with positive PD-L1 expression or sarcomatoid differentiation. To better understand the determinants of immunotherapy response in this population, we characterized blood- and tissue-based immune markers for patients with vRCC, or any RCC histology with sarcomatoid differentiation, enrolled in this phase II trial of atezolizumab and bevacizumab.

### Methods

We used patient data and biospecimens (blood and tumor) from a prospective phase-II clinical trial of the anti-PD-L1 antibody atezolizumab and the anti-VEGF-A antibody bevacizumab in patients with metastatic RCC with variant histology and/or sarcomatoid differentiation (NCT02724878). Blood samples were collected at baseline, prior to therapy (C1D1: cycle one day one), and on-treatment, following two cycles of therapy (C3D1: cycle three day one). Plasma was assayed for the levels of soluble factors (e.g., cytokines and growth factors), and peripheral blood mononuclear cells (PBMCs) were immunophenotyped using flow cytometry. In addition, formalin-fixed, paraffin-embedded (FFPE) archival or pre-treatment study tumor biopsies were analyzed by immunohistochemistry and immunofluorescence for enrolled subjects with available biospecimen. Baseline demographics, clinical characteristics, and treatment outcomes, including progression-free survival (PFS), overall survival (OS), and clinical benefit, were collected, and analyzed from the trial

database. This study was approved by each participating institution's institutional review board, and all patients provided written informed consent. We used R (version 4.1.1) to carry out our analyses. We assessed the relationships between biomarker variables were analyzed using Pearson correlations and hierarchical clustering using Euclidean distance. All biomarker variables were Z-score normalized. We calculated an inflammatory module, the systemic inflammation score (SIS), which corresponds to the average score of the five highly correlated inflammatory cytokines: MIP-1b, IL-1, MCP-1, IL-6, and IL-13. We applied pair-wise Wilcoxon tests to evaluate the association of biomarkers with clinical benefit groups and log-rank-tests and univariate Cox regression models to evaluate the relationship of biomarkers with PFS and OS.

### Results

With the exception of two angiogenic cytokines (VEGF-A and Angiopoietin 2) that were more abundant in patients with non-sarcomatoid tumors, baseline circulating cytokines (plasma) had similar distributions between sarcomatoid and non-sarcomatoid subgroups. Baseline circulating inflammatory cytokines were highly correlated with one another, forming an "inflammatory module" that was increased in IMDC-poor risk patients and was associated with worse PFS ( $p=0.028$ ) and with resistance to therapy ( $p=0.033$ ). At baseline, an elevated circulating VEGF-A level was associated with a lack of response ( $p=0.03$ ) and worse PFS ( $p=0.021$ ). However, a larger increase in on-treatment levels of circulating VEGF-A was associated with clinical benefit ( $p=0.01$ ) and improved overall survival ( $p=0.0058$ ). Among peripheral immune cell populations, an on-treatment decrease in circulating PD-L1+ T cells was associated with improved outcomes, with a reduction in CD4+ PD-L1+ (HR:0.62[0.49-0.91],  $p=0.016$ ) and CD8+ PD-L1+ T cells (HR:0.59[0.39-0.87],  $p=0.009$ ) correlated with improved PFS. Within the tumor itself, a higher percentage of terminally exhausted (PD-1+ and either TIM-3+ or LAG-3+) CD8+ T cells was associated with worse PFS ( $p=0.028$ ). We observed similar trends for all of these results in different subgroups according to sarcomatoid status and histology.

### Conclusions

Overall, these findings support the value of tumor and blood-based immune assessments in determining therapeutic benefit for patients with RCC receiving atezolizumab plus bevacizumab. Furthermore, they provide a foundation for future biomarker studies for patients with variant histology RCC receiving immunotherapy-based combinations.

### Keywords

metastatic renal cell carcinoma, variant histology RCC, non-clear cell RCC, sarcomatoid, immune checkpoint inhibitors, vascular endothelial growth factor inhibitor.

# Posters

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## Inhibition of the mitochondrial chaperone TRAP1 disrupts kidney cancer cell survival

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### Background

The molecular chaperone TNF-receptor-associated protein 1 (TRAP1) is a master regulator of mitochondrial metabolism and apoptosis and is often overexpressed in cancer. Insensitivity to apoptotic signaling underlies the pathogenesis of cancer, though the impact of TRAP1 on kidney cancer survival has not been explored. TRAP1 activity is modulated by post-translational modifications, which are often deregulated in disease. The proto-oncogene tyrosine kinase c-Abl is hyperactive in ccRCC, though the mitochondrial targets of c-Abl are unknown.

### Methods

We have used established clear cell renal cell carcinoma (ccRCC) cell lines to determine the impact of TRAP1 on ccRCC survival. We further evaluated TRAP1 phosphorylation using transient expression of phosphomutants and observed the impact of targeted disruption of TRAP1 phosphorylation on cell survival in HAP1, MEF and ccRCC cells.

### Results

We observed elevated TRAP1 expression in the most common subtype of kidney cancer, ccRCC, and inhibition of TRAP1 in ccRCC induced apoptosis. We found that c-Abl-mediated phosphorylation of TRAP1 antagonized apoptosis by promoting TRAP1 binding to the apoptosis effector CypD. Inhibitor-sensitive c-Abl-mediated TRAP1 phosphorylation was enriched in cancers, suggesting a direct link to cancer cell survival. Co-targeting TRAP1 and c-Abl displayed drug synergy, suggesting a potentially viable therapeutic strategy for ccRCC.

### Conclusions

Here we observed that elevated TRAP1 expression and increased c-Abl-mediated phosphorylation drove the pro-survival role of TRAP1 in ccRCC. Therefore, combination c-Abl and TRAP1-specific inhibition merits continued evaluation.

### Keywords

chaperone, apoptosis, mitochondria, ccRCC

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## Nucleotide excision repair deficiency is a hypoxia regulated, targetable therapeutic vulnerability in clear cell renal cell carcinoma.

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### Background

Due to a demonstrated lack of DNA repair deficiencies, clear cell renal cell carcinoma (ccRCC) has not benefitted from targeted synthetic lethality-based therapies. We investigated whether nucleotide excision repair (NER) deficiency is present in an identifiable subset of ccRCC cases that would render those tumors sensitive to therapy targeting this specific DNA repair pathway aberration.

### Methods

We used functional assays that detect UV-induced 6-4 pyrimidine-pyrimidone photoproducts to quantify NER deficiency in ccRCC cell lines. We also measured sensitivity to irofulven, an experimental cancer therapeutic agent that specifically targets cells with inactivated transcription-coupled nucleotide excision repair (TC-NER). In order to detect NER deficiency in clinical biopsies, we assessed whole exome sequencing data for the presence of an NER deficiency associated mutational signature previously identified in ERCC2 mutant bladder cancer.

### Results

ccRCC cell lines showed various degrees of NER deficiency by functional assays under normoxic conditions. In hypoxia, NER activity was reactivated. Some of these cell lines also showed increased sensitivity to irofulven. This sensitivity was also

correlated with the expression of prostaglandin reductase 1 (PTGR1), an enzyme required for the activation of irofulven. Next generation sequencing data of the cell lines indicated the presence of NER deficiency associated mutational signatures. The same signature as well as significant expression levels of PTGR1 was detected in a significant subset of ccRCC patients.

## Conclusions

ccRCC cell line based analysis showed that NER deficiency is likely present in this cancer type. Approximately 10% of ccRCC patients in the TCGA cohort showed mutational signatures consistent with ERCC2 inactivation associated NER deficiency and also substantial levels of PTGR1 expression. These patients may be responsive to irofulven, a previously abandoned anticancer agent that has minimal activity in NER-proficient cells.

## Keywords

DNA repair, nucleotide excision repair, cancer genomics, biomarker discovery, kidney cancer.

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## A novel combination treatment to overcome c-Met-induced and RUBICON-Nrf2-mediated therapeutic resistance in renal cancer.

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## Background

Although there are some progresses in the treatment of renal cell carcinoma (RCC), tumors almost inevitably develop therapeutic resistance. Therefore, new target molecules and combination therapies need to be explored. Receptor tyrosine kinases (RTKs) play a critical role in the growth RCC. The RTK, c-Met, is overexpressed in renal cancer cells and mediates tumor-promoting pathways and therapeutic resistance through the regulation of anti-oxidant transcription factor, Nrf2. The tumor cells utilize the cytoprotective role(s) of Nrf2 to overcome the oxidative stress generated by therapeutic agents. Depending on context, the process of autophagy can play both pro- and anti-tumorigenic role(s). Apart from apoptosis, the process of autophagy can also promote tumor cell death following therapeutic treatments. RUBICON (a RUN domain containing protein) is a component of the class

III PI-3K complex and a novel Beclin-1-binding partner, which can negatively regulate canonical autophagy. p62 is an autophagy adaptor protein, which can stabilize Nrf2 (through degradation of Keap-1) and facilitate its nuclear translocation. Honokiol (C18H18O2) is a phenolic compound (originally isolated from Magnolia obovata) with both anti-inflammatory and anti-tumorigenic properties. We found that Honokiol can markedly downregulate c-Met-induced and Ras-mediated tumor-promoting pathways in renal cancer cells; and it can inhibit Nrf2 effector molecules. The c-Met/RTK inhibitor, cabozantinib, has been approved for the treatment of RCC; however, most of the patients develop therapeutic resistance. We wanted to explore the effectiveness of a combination treatment (cabozantinib + Honokiol) to overcome c-Met-induced and Nrf2-mediated therapeutic resistance in RCC through the regulation of RUBICON and p62.

## Methods

We used human RCC cell lines, 786-0 (clear cell types) and ACHN (papillary type) from ATCC. Protein expression was measured by Western blot. RUBICON knockout stable cells were generated using CRISPR-Cas9. The total reactive oxygen species (ROS) was measured utilizing a kit (Enzo Life Sciences). Cellular apoptosis was measured by flow cytometry (Annexin-V and Propidium Iodide staining). Cellular autophagy was measured using a kit from Enzo Life Sciences. Cell proliferation was studied by using CCK-8 kit (Abcam).

## Results

We found that RUBICON, p62 and Nrf2 are markedly expressed in all tested renal cancer cell lines; and activation of c-Met (following treatment with its ligand, HGF) further induced the expression of these three proteins in renal cancer cells. The combination treatment with cabozantinib + Honokiol significantly inhibited cancer cell proliferation compared to cells treated separately with individual agents (either cabozantinib or Honokiol). On the basis of cell proliferation data, we calculated the synergistic effect of cabozantinib and Honokiol (using SynergyFinder). We obtained a high synergy score, which indicates that Honokiol can potentiate the effect of cabozantinib. Interestingly, we found that cabozantinib + Honokiol combination significantly reduced the expression of RUBICON, p62 and Nrf2 in RCC cells; and it also increased the total cellular ROS level and induced autophagy, which can promote cell death. Next, we checked the effect of this combination treatment in RUBICON knockout stable renal cancer cells. The knockout of RUBICON reduced the expression of p62 and Nrf2; and this was further downregulated following cabozantinib + Honokiol combination treatment. When we knocked down p62 in RCC cells using gene-specific siRNA, Nrf2 was markedly downregulated; and this suggests that p62 can regulate Nrf2 in these cells. Finally, we found that cabozantinib + Honokiol combination markedly downregulated the nuclear translocation of Nrf2.

## Conclusions

Our findings suggest that c-Met induced RUBICON can downregulate the oxidative stress in renal cancer cells through increased expression of p62 and Nrf2, and mediate therapeutic resistance. However, a combination treatment with cabozantinib + Honokiol can effectively inhibit the expression of RUBICON, p62 and Nrf2 to induce oxidative stress and cancer cell death.

## Keywords

Receptor Tyrosine Kinase, c-Met, Therapeutic Resistance, Oxidative stress, Combinartion Therapy, Cell Death

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## Immune Restoring (IR) CAR-T Cells Display Superior Antitumor Activity in a Humanized ccRCC Orthotopic Mouse Model via Reversing Immunosuppressive TME

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Dana Farber Cancer Institute

## Background

Chimeric antigen receptor (CAR) T cells are routinely used in the treatment of hematologic malignancies, but there are several barriers that have restricted their successful use in the treatment of solid tumors. It has been hypothesized that one of the major barriers is an unfavorable tumor microenvironment (TME).

## Methods

To provide a more favorable TME, we engineered CAR-T cells targeting carbonic anhydrase IX (CAIX) G36 to secrete PD-L1 monoclonal antibody, which we refer to as immune restoring (IR) CAR G36-PDL1. We tested IR CAR-T cells in a humanized clear cell renal cell carcinoma (ccRCC) orthotopic mouse model (termed hccRCC-NSG-SGM3) with reconstituted human leukocyte antigen (HLA) partially matched human leukocytes derived from fetal CD34+ hematopoietic stem cells (HSCs) and bearing human ccRCC skrc-59 cells under the kidney capsule.

## Results

CD45+ tumor infiltrating leukocytes (TILs) in hccRCC-NSG-SGM3 reconstituted most CD45+ cell types including NK cells, dendritic cells, macrophages, exhausted CD8 T cells, and regulatory T cells that are observed in the TME of

advanced ccRCC patients from clinical trials of the anti-PD-1 monoclonal antibody nivolumab. We found that G36-PDL1 CAR-T cells, haploidential to the tumor cells, had a potent antitumor effect, while those without immune restoring effect had limited ability to control tumor growth. Analysis of the TME revealed that G36-PDL1 CAR-T cells restored active antitumor immunity by promoting tumor killing cytotoxicity, reducing immunosuppressive cell components such as M2 macrophages and exhausted CD8 T cells, and enhancing Tfh-B cell crosstalk.

## Conclusions

These findings highlight that the hccRCC-NSG-SGM3 serves as a powerful tool for ccRCC TME study, and anti-CAIX G36-PDL1 IR CAR-T cell secreting anti-PD-L1 mAb holds promise to achieve ccRCC cures by rewiring local TME.

## Keywords

Chimeric antigen receptor (CAR) T, clear cell renal cell carcinoma (ccRCC), carbonic anhydrase IX (CAIX), single cell RNA sequencing (scRNAseq)

## CDMRP DOD Funding

yes

**42**

## Development of an experimental toolkit to assess T cell function in renal cell carcinoma (RCC)

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## Background

With the rise of immune checkpoint inhibitors (ICIs) as the primary treatment options for metastatic RCC, the investigation of T cells within the tumor microenvironment (TME) has emerged as a critical component of understanding immunotherapeutic response and resistance. Prior efforts, including single-cell transcriptomic approaches, have provided an important landscape of T cell transcriptional phenotypes. However, such immunoprofiling efforts are hypothesis-generating and will require validation through functional interrogation of patient T cells to facilitate the development of novel immunomodulatory therapies. Thus, we established a toolkit to directly assess patient T cell function including capacity to proliferate, produce effector cytokines, and exert cytotoxic effects on target RCC cells. In this initial proof-of-concept study, we evaluated the change in T cell function in hypoxic conditions to simulate the RCC TME.

## Methods

T cells were isolated from healthy donor peripheral blood mononuclear cells. To assess proliferation, T cells were labeled with CellTrace Violet division-tracking dye, stimulated with anti-CD3/CD28 Dynabeads, and assessed by flow cytometry (FC) to quantify the number of cell divisions undergone by CD4+ and CD8+ T cells. To assess cytokine production, T cells were stimulated with Dynabeads for 8 hours total and treated with Brefeldin A for the final 4 hours to inhibit secretion and retain cytokines intracellularly. Intracellular cytokine staining was then performed and the production of IFN- $\gamma$ , TNF $\alpha$ , and IL-2 by each T cell was quantified via FC. In both assays, hypoxic conditions (1% O<sub>2</sub>) were additionally assessed to simulate the RCC TME. To assess the cytotoxic activity of T cells, we designed an antigen-specific killing system utilizing NY-ESO-1 (CTAG1B) as a model antigen. Human RCC cell lines A498 (HLA-A\*02:01+), UOK127 (HLA-A\*02:01+), and 769P (HLA-A\*02:01-) were used as target cells. CTAG1B and luciferase genes were introduced into the RCC cell lines, and primary human T cells were engineered to express the cognate NY-ESO-1 antigen-specific T cell receptor (NY-ESO-1 T cells). Genetically-engineered RCC cell lines and T cells were co-cultured for 18 hours with varying ratios of effector and target cells, and luminescence was quantified using Bright-Glo and SpectraMax M3. Target cells alone were plated to determine the maximal luciferase expression (relative light units; RLUmax), and specific lysis was determined as  $(1 - (RLU_{\text{sample}})/(RLU_{\text{max}})) \times 100$ .

## Results

We successfully optimized experimental workflows for the quantification of proliferation and cytokine production. Through dose-response analysis, we observed that stimulation with 1 Dynabead for every 8 T cells in each protocol yielded significant but non-saturating levels of proliferation and cytokine production, as quantified by FC. The average number of cell divisions per sample and the percentages of cells which produced IFN- $\gamma$ , TNF $\alpha$ , and IL-2 significantly decreased upon treatment with T cell inhibitors such as tacrolimus and cyclosporine. Importantly, neither the production of effector cytokines nor T cell proliferation differed significantly under normoxic and hypoxic conditions. In our engineered system to assess antigen-specific T cell-mediated killing of RCC tumor cells, NY-ESO-1 T cells exhibited significant cytotoxic activity against A498 and UOK127 cells with the NY-ESO-1 antigen in comparison with corresponding parent cell lines. There was no difference between 769P with the NY-ESO-1 antigen and without the NY-ESO-1 antigen.

## Conclusions

Through the optimization of assays evaluating T cell proliferation and cytokine production, we were able to assess multiple axes of T cell function. Initial pilot studies suggested

that the hypoxic TME in RCC may not consistently impact T cell proliferation or cytokine production. Further, our engineered system for assessing T cell cytotoxicity successfully demonstrated antigen-specific killing of RCC tumor cells. Taken together, this "toolkit" has extensive applications in the study of RCC, including assessing the impact of ICIs on T cell function.

## Keywords

Renal cell carcinoma, T-cell function, Antigen-specific cytotoxic activity

## CDMRP DOD Funding

yes

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## SETD2 loss and ATR inhibition synergize to promote cGAS signaling and immunotherapy response in renal cell carcinoma

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## Background

Immune checkpoint blocking agents (ICB) have become a mainstay for the treatment of patients with renal cell carcinoma (RCC); however, only a minority of patients show complete responses to ICB. Targeting the S-phase DNA damage repair (S-DDR) network has been reported to convert a non-immunogenic tumor microenvironment into an immunogenic tumor microenvironment by increasing immune cell infiltration, thus improving the immunotherapy response. ATM loss confers greater sensitivity to ATR inhibition in patients with advanced solid tumors. However, ATM mutations are rarely identified in RCC; thus, identifying the genetic features that influence ATM activity might provide therapeutic strategies with ATR inhibitors and develop approaches to enhance the response to immunotherapy.

## Methods

We investigated the effects of *SETD2*/Setd2 loss on the DNA damage response pathway, the cytosolic DNA sensing pathway, the tumor immune microenvironment, and the response to ATR and checkpoint inhibition in RCC cell lines, the Renca-BALB/c immune competent murine model, and RCC patient cohorts.

## Results

Targeting the ATR-CHK1 axis with pharmacological inhibitors activated the cGAS-IRF3-dependent cytosolic DNA-sensing pathway, resulting in the expression of inflammatory cytokines and immune checkpoints. Among the common RCC genotypes, SETD2 loss is associated with preferential ATR-CHK1 activation and sensitizes cells to ATR inhibition. SETD2 knockdown promoted the cytosolic DNA sensing pathway in response to ATR-CHK1 inhibition. Treatment with the ATR inhibitor VE822 concurrently upregulated immune cell infiltration and immune checkpoint expression in *Setd2* knockdown Renca tumors, providing a rationale for ATR inhibition plus ICB combination therapy. *Setd2* deficient Renca tumors demonstrated greater vulnerability to ICB monotherapy or combination therapy with VE822 than *Setd2* proficient tumors. Moreover, SETD2 mutations were associated with a higher response rate and prolonged overall survival in ICB-treated RCC patients but not in non-ICB-treated RCC patients.

## Conclusions

SETD2 loss and ATR inhibition synergize to promote cGAS signaling and enhance immune cell infiltration in renal cell carcinoma, providing a mechanistic rationale for the combination of ATR and checkpoint inhibition in RCC patients with *SETD2* mutations.

## Keywords

RCC, SETD2, ATR inhibitor, cGAS, Immunotherapy

## CDMRP DOD Funding

yes

**48**

## Novel inhibitor of pro-survival protein phosphatase 5 (PP5) leads to extrinsic apoptosis in VHL-null clear cell renal cell carcinoma

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## Background

Despite good survival rates for localized renal cell carcinoma a proportion of patients present with or will progress to metastatic disease, which portends worse prognosis and poor overall survival. Survival has been improved with the use of new targeted therapies and immunotherapy regimens; many patients, however, ultimately develop resistance. There is therefore a need for identification and development of new therapeutics. Clear cell renal cell carcinoma (ccRCC), the most common histologic subtype, generally harbors loss of the tumor suppressor von Hippel Lindau (VHL). The serine/threonine protein phosphatase-5 (PP5) plays a role in the regulation of numerous signaling pathways essential for cancer growth. Previously we have shown that PP5 is targeted for ubiquitination and degradation by a VHL-containing E3 ubiquitin ligase complex in a hypoxia-independent manner. Expression and activity of PP5 is consequently increased in ccRCC, and it plays a pro-survival role. Knock-down of PP5 expression or targeting its activity through inhibition of the kinase casein kinase-1 $\delta$  (CK1 $\delta$ ) caused apoptosis in VHL-null ccRCC. The objective of this study was to develop a novel PP5 inhibitor for ccRCC and identify the mechanism of PP5 inhibition-induced apoptosis.

## Methods

An in silico docking screen using the crystal structure of the PP5 active site and a large compound library was used to screen for candidate inhibitors. A series of candidates were then evaluated using both in vitro and cell-based assays for their ability to inhibit PP5 activity and cause apoptosis in ccRCC cells. In vitro PP5 activity was assessed using specific phospho-peptide substrates and in cells by immunoblotting to assess phosphorylation levels of known substrates. Two primary candidates were conjugated to biotin for PP5 pulldown experiments to assess specificity.

## Results

Our in silico screen identified approximately 200 candidate inhibitors per structure. Further screening of analogs of the single overlapping hit identified two compounds, P5 and P13, which inhibit PP5 activity both in vitro and in ccRCC cells and were further refined based on structure. Pulldown using biotin-conjugated drugs demonstrated good inhibitor specificity. Furthermore, treatment with these inhibitors leads to apoptosis in VHL-null ccRCC cell lines. We further identified that PP5 interacts with the components of the extrinsic apoptotic complex II: FADD, RIPK1, and caspase 8. Specifically, PP5 dephosphorylates and inactivates FADD, preserving complex II integrity and regulating extrinsic apoptosis.

## Conclusions

PP5 promotes cell survival in ccRCC and knockdown or inhibition of PP5 leads to apoptosis through the extrinsic pathway. We have identified a small molecule which

specifically binds to and inhibits PP5. This novel PP5 inhibitor causes apoptosis in VHL-null ccRCC cells and may serve as a new therapeutic strategy for treatment of advanced ccRCC.

### Keywords

ccRCC, PP5, phosphatase inhibitor, VHL

### CDMRP DOD Funding

yes

**49**

## Dissecting metabolic alterations of clear cell renal cell carcinomas one cell at a time

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### Background

Pseudohypoxia-related metabolic alterations are known to exist in clear-cell renal cell carcinoma (ccRCC). Here we characterized single-cell metabolic pathways with the 10Xmultiome assay comparing metabolic and compositional shifts in tumor vs. normal-adjacent samples.

### Methods

We analyzed 57 tumor and 6 normal-adjacent samples from the Dartmouth Renal Tumors Biobank collected between 1994-2009. Samples were enzymatically disassociated and stored at -80 C until 10X multiome processing. RNA counts were extracted using Seurat, cell types were deconvolved using Azimuth, and 85 KEGG metabolic pathways were interrogated using scMetabolism scores. Linear mixed-effects models compared the metabolic scores in 10 cell lineages (juxtaglomerular apparatus, glomeruli, proximal tubule system, nephron loop, distal tubules, collecting ducts, lymphoid, myeloid, endothelia, and other stroma cells) between tumor and normal adjacent adjusting for sex, patient age, stage and grade as fixed-effects and donor, and year of collection, as random-effects. P-values were calculated using Satterthwaite's method.

### Results

The mean age at diagnosis was 61.7 yrs (SD: 12.5), and 67% were stages I/II. 79,804 tumor and 5,967 normal-adjacent cells were analyzed. 34,318 cells were nephron epithelia (juxtaglomerular, glomeruli, renal tubules), 5,947 were collecting ducts, 18,959 were immune, 13,445 were endothelial, and 13,112 were other stromal cells. Out of 85 KEGG metabolic pathways, nine were prioritized based on the kidney cancer literature; glycolysis, oxidative phosphorylation, TCA cycle, fatty acid biosynthesis, glutamine/glutamate, arachidonic acids, phenylalanine metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, and glutathione.

When comparing vs. the corresponding normal adjacent cell type, in tumor cells: 1) glutamine/glutamate expression increased in lymphoid, endothelial, and stromal cells; 2) fatty acid biosynthesis increased in myeloid and reduced in lymphoid, collecting ducts-like, and nephron loop-like cells, 3) glutathione and glycolysis increased in glomeruli-like and nephron loops-like, 4) glycolysis increased in collecting ducts-like and endothelial, 5) oxidative phosphorylation and TCA cycle genes decreased in distal tubules-like, collecting ducts-like, and endothelia, 6) arachidonic acid metabolism increased in nephron loops-like and glomeruli-like, and 7) phenylalanine, tyrosine, and tryptophan biosynthesis decreased in nephron loops-like, distal tubules-like, and collecting ducts-like cells.

### Conclusions

Cell-specific metabolic shifts were observed between tumor and normal adjacent samples that cannot be explained only by sample heterogeneity or cell lineages. Oxidative phosphorylation and TCA cycle modifications were observed in tumor endothelia and cells resembling distal tubules and collecting ducts. Similarly, glutamate and fatty acid alterations were focused on tumor microenvironment immune cells. Further dissecting these findings will provide additional therapeutic avenues for newer targets, such as glutaminase inhibitors and combinations with current therapies.

### Keywords

Clear renal cell carcinoma, single-cell RNA-seq, tumor metabolism, cell compositional analysis, cell heterogeneity.

### CDMRP DOD Funding

yes

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## Characterization of FOLH1 Expression in Renal Cell Carcinoma

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### Background

The Folate Hydrolase 1 (FOLH1) gene encodes prostate-specific membrane antigen (PSMA), a transmembrane glycoprotein that is highly expressed in prostate cancer cells and endothelial cells in the neovasculature of solid tumors, including renal cell carcinoma (RCC). PSMA has been used as a target for diagnostic imaging and therapeutic radioligand therapy. We utilized a database of molecularly profiled RCC tumors to evaluate associations with FOLH1 expression. We hypothesized that FOLH1 expression would differ by histology and site of metastasis and would be enriched in highly angiogenic tumors. Additionally, we hypothesized the tumors with high FOLH1 expression would have improved outcomes to VEGF targeted therapy.

### Methods

NextGen sequencing of DNA (592-gene/whole exome) and RNA (whole transcriptome) was performed on RCC tumor specimens (n=1765) through Caris Life Sciences (Phoenix, AZ). FOLH1-High/Low expression were defined as 75th/<25th-percentile of RNA transcripts per million (TPM). We evaluated FOLH1 expression by sites of metastasis (primary vs. metastasis) and histology (clear cell vs. non-clear cell). We describe frequent DNA alterations in FOLH1 high vs. low groups. Angiogenic, T-effector, and myeloid expression signatures were calculated using previously defined gene sets (McDermott, 2018) and FOLH1 expression was described across the signature groups. Immune cell infiltration in tumor microenvironments (TMEs) was estimated using MCP-Counter (Becht, 2016). Tumor cell PD-L1+ expression ( $\geq 2+$ ,  $\geq 5\%$ ; SP142) was assessed by IHC. Overall survival, from time of tissue collection, and time-on-treatment from time

**Table 1. Patient demographics and association with FOLH1 expression**

Cohort Characteristics	N (%)	Median FOLH1 expression (TPM)	P-value
<b>Samples</b>	1765 (100%)		---
<b>Age</b>	<b>Median (range)</b>	<b>Correlation FOLH1 expression (TPM)</b>	<b>P-value</b>
Years old at biopsy	63 (1-90+)	0.02 (Spearman)	0.42
<b>Sex</b>	<b>N (%)</b>	<b>Median FOLH1 expression (TPM)</b>	<b>P-value</b>
Male	1248 (70.71%)	11.2	0.54
Female	517 (29.29)	11.3	
<b>Histology</b>	<b>N (%)</b>	<b>Median FOLH1 expression (TPM)</b>	<b>P-value compared to ccRCC</b>
ccRCC	524 (71.10%)	19.0	
Non-ccRCC	206 (27.95%)	3.3	<0.001
Mixed	7 (0.95%)	22.9	0.78
NOS	1028 (---)	10.6	<0.001
<b>Biopsy site</b>	<b>N (%)</b>	<b>Median FOLH1 expression (TPM)</b>	<b>P-value compared to Kidney</b>
Kidney	795 (45.04%)	13.6	
Metastatic	970 (54.96)	9.9	<0.001
Lung	174 (17.94%)	10.4	0.172
Bone	165 (17.01%)	14.2	0.402
Lymph nodes	144 (14.85%)	5.3	<0.001
Soft tissue	114 (11.75%)	9.3	0.051
Liver	100 (10.31%)	9.2	0.114
Other	80 (8.25%)	9.2	0.051
CNS	62 (6.39%)	9.7	0.258
Endocrine	58 (5.98%)	16.6	0.375
Pleural	36 (3.71%)	7.7	0.258
Skin	21 (2.16%)	16.7	0.434
GI	16 (1.65%)	13.3	0.932

of treatment initiated was calculated using the Kaplan-Meier method.

### Results

FOLH1 expression was similar between sexes (71% male/29% female, 11.2 vs. 11.3 median TPM, p=0.54) and was not correlated with age at time of profiling (median 63 years, p=0.42). FOLH1 expression was significantly higher in clear cell RCC (ccRCC) (71.1% prevalence) compared to non-clear cell RCC tumors (non-ccRCC) (19.0 vs 3.3 TPM, p<0.001). Clear cell RCC showed higher expression of Carbonic Anhydrase 9 (spearman = 0.28, p<0.05) compared to non-clear cell RCC (spearman = 0.07, p=0.28). FOLH1 expression varied by specimen site (45% kidney/55% metastatic, 13.6 vs. 9.9 TPM, P<0.001), with notably lower expression in lymph nodes (5.3 TPM, p<0.001, 8.2% prevalence). FOLH1 expression was strongly correlated with angiogenic gene expression compared to T-effector and myeloid signatures (spearman = 0.76 vs 0.33 and 0.20, respectively, each p<0.001), with

similar correlation strength observed for endothelial cell abundance in TMEs (spearman = 0.76 vs 0.04-0.5 for immune cell subtypes, p<0.001). PD-LI + IHC frequency was lower, but not statistically different in FOLH1-high compared to - low tumor among clear cell RCC (10 vs 17%, p=0.07) but was similar among non-clear cell RCC (31 vs 32%, p=0.95). For patients stratified by median FOLH1 expression, no difference in overall survival from time of tissue sampling was observed for clear cell RCC (HR 1.2, P=0.57) or non-clear cell RCC cohorts (HR 0.77, P=0.59). FOLH1-high was associated with numerically longer tyrosine kinase inhibitor (TKI) time-on-treatment (223 vs. 61 days, HR 0.60, p=0.08).

## Conclusions

We observed differential patterns of FOLH1 expression by histology and tumor site. Among various tumor sites only lymph nodes were found to have lower FOLH1 expression, with no significant increase in liver or bone. FOLH1 expression was strongly correlated with angiogenic gene expression and distinct differences in TME composition, including endothelial cell abundance. FOLH1 gene expression was positively correlated with increased duration of anti-angiogenic treatment. These investigations are important for future PSMA-directed diagnostic and therapeutic modalities in RCC.

## Keywords

FOLH1, PSMA, renal cell carcinoma

**53**

# Vascular endothelial profilin-1 drives a pro-tumorigenic tumor microenvironment and tumor progression in renal cancer

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University of Pittsburgh

## Background

Overexpression of actin-binding protein Profilin-1 (Pfn1) correlates with advanced disease features and adverse clinical outcome of patients with clear cell renal carcinoma (ccRCC), the most prevalent form of renal cancer. However, unlike many other types of human cancer where Pfn1 expression is dysregulated in tumor cells, Pfn1 is predominantly (over)-expressed in tumor-associated vascular endothelial cells (EC) via transcriptional upregulation in ccRCC. The objective of the present study was to query a causal relationship between endothelial Pfn1 dysregulation and disease progression in RCC, and further engineer novel therapeutic strategies revolving around Pfn1.

## Methods

We performed orthotopic tumor model studies in fully immunocompetent syngeneic mice that involved EC-specific deletion, overexpression of Pfn1 in a kidney-localized manner, and widespread deletion of endothelial Pfn1. Vascular and immunological aspects of tumor microenvironment (TME) were assessed by a comprehensive experimental strategy utilizing immunohistochemistry (IHC), protein array for angiogenesis markers, and Luminex analyses of a broad range of immunomodulatory cytokines and chemokines. By RNA sequencing of tumors, we identified endothelial Pfn1-dependent changes in differentially expressed genes and key biological pathways in TME. Multiplexed quantitative IHC and immune deconvolution analyses of single-cell RNAseq data of clinical samples were performed to corroborate mouse model findings. Dendritic cell vaccines were generated targeting Pfn1.

## Results

Our studies demonstrate that vascular endothelial Pfn1 overexpression accelerates tumor progression, and conversely, endothelial Pfn1 depletion dramatically inhibits tumorigenicity, progressive growth and metastasis of RCC cells regardless of the VHL expression status. We established an important requirement for endothelial Pfn1 in tumor angiogenesis, and further identified endothelial Pfn1-dependent regulation of several pro-(VEGF, SERPINE1, CCL2) and anti-angiogenic factors (platelet factor 4) in vivo. Endothelial Pfn1 overexpression does not further augment tumor angiogenesis but increases tumor infiltration by macrophages and concomitantly diminishes tumor infiltration by CD8+ T cells in vivo. These data correlate with the pattern of endothelial Pfn1-dependent changes in tumor abundance of several prominent immunomodulatory cytokines. Guided by Upstream Regulator Analysis of tumor transcriptome data, we further established endothelial Pfn1-induced Hif1 $\alpha$  elevation and suppression of STAT1 activation. Finally, to develop novel Pfn1-centric immunotherapy agents, we have generated dendritic cell Pfn1 peptide vaccines that elicit strong CD4- and CD8- T cell responses.

## Conclusions

In conclusion, this study demonstrates for the first time a direct causal relationship between vascular endothelial Pfn1 dysregulation, immunosuppressive TME, and disease progression with mechanistic insights in kidney cancer. Our study also provides a conceptual basis for targeting Pfn1 for therapeutic benefit in kidney cancer.

## Keywords

clear cell RCC, profilin, tumor microenvironment, vaccine

## CDMRP DOD Funding

yes

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## PDL1 expression is inherently tied to the epithelial to mesenchymal transition in renal cell carcinoma: implications for immunotherapy

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### Background

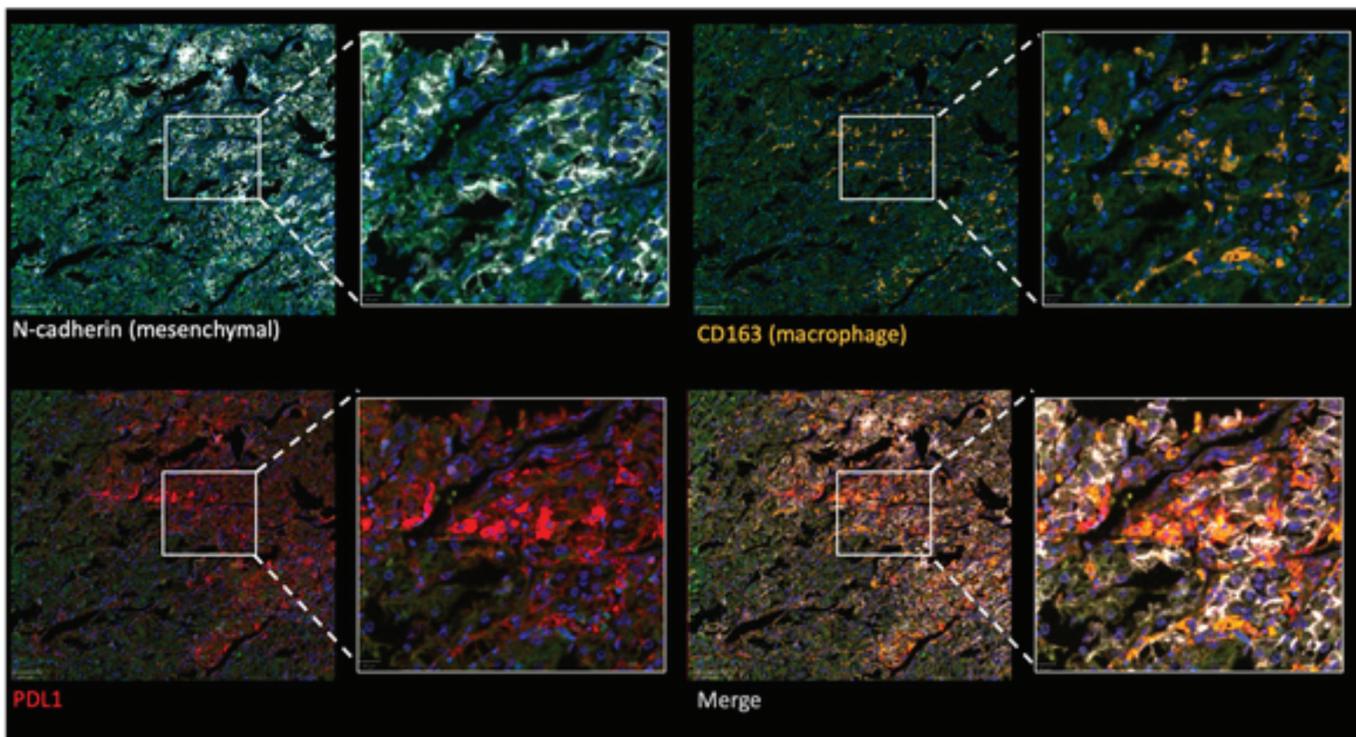
Sarcomatoid dedifferentiation in renal cell carcinoma (RCC) occurs when the parental cell type, such as clear cell RCC (ccRCC), undergoes an epithelial mesenchymal transition (EMT). The mesenchymal sarcomatoid cells have increased ability to invade and metastasize, and thus sarcomatoid RCC (sRCC) has a very poor prognosis. However, there is encouraging evidence that sRCC responds at higher rates to PD-1/PD-L1 directed immunotherapy than ccRCC and sRCC is also known to exhibit higher expression of PDL1 than ccRCC. We aim to test the hypothesis that EMT directly upregulates PDL1 and that PDL1 may have positive reciprocal feedback on EMT, potentially explaining the high response of sRCC to immunotherapy.

### Methods

To assess correlation of EMT and PDL1 expression in tumor cells and immune cells, multiplex immunofluorescent staining was performed on 29 human RCC specimens with areas of clear cell and sarcomatoid histology using the epithelial marker, E-cadherin, mesenchymal marker N-cadherin, and several immune cell markers. Areas of N-cadherin high and N-cadherin low staining were compared for PDL1 expression as well as areas of clear cell versus sRCC. In vitro, baseline expression of EMT markers, EMT transcription factors and PD-L1 were measured using Western Blot and qPCR in two clear cell RCC lines (Caki-1 and 786-O) and five sarcomatoid RCC lines (RCJ-41T1, RCJ-41T2, RCJ-41M, UOK-276, UOK-127). EMT was induced in the cells through treatment with multiple agents including TGF $\beta$ , IFN $\gamma$ , HGF, and SPP1 and by over expression of Snail or Zeb1. Effect of EMT on PD-L1 expression was measured. To evaluate the role of PD-L1 on EMT, PD-L1 expression was decreased using siRNA and its effect on EMT related protein expression was measured using Western Blot. RCC cells were co-cultured with THP-1 cells, a macrophage precursor cell line, to assess effect on macrophage PDL1 expression.

### Results

In RCC, N-cadherin expression was highly positively associated with PDL1 expression (Figure 1; >7 fold as many



**Figure 1.** Representative section from mIF staining of human clear cell and sarcomatoid tumor. Mesenchymal marker, N-cadherin (white), is strongly positively associated with PDL1 (red) in both tumor cells as well as macrophages (CD163 – orange) in the N-cadherin high areas.

cells with high PDL1 expression in N-cadherin high areas compared to N-cadherin low,  $p<0.001$ ). Notably, PDL1 expression increased on tumor cells themselves as well as drastically increased on macrophages in those areas. In vitro, baseline PD-L1 expression positively correlated with increasing EMT state in all of the 2ccRCC and 5sRCC cell lines tested. Multiple different approaches for EMT induction were performed in vitro, including exposure to cytokines TGFB or IFNg, treatment with recombinant proteins SPP1 or HGF which have shown to be of interest in other preliminary work, and overexpression of EMT transcription factors Snail or Zeb1. All methods resulted in increased EMT state as measured by EMT marker protein expression, and interestingly, all methods showed a concurrent strong upregulation of PDL1. Co-culture of EMT-high sRCC cells with macrophages also lead to enhanced expression of PDL1 in the macrophages. To explore the intrinsic role of PDL1 in EMT regulation, PDL1 was silenced in RCC cells which led to a decreased EMT state in the tumor cells.

## Conclusions

These data demonstrate EMT is associated with increased PDL1 in RCC cells and EMT in tumor cells may also enhance PDL1 expression in macrophages in the microenvironment. Furthermore, we demonstrate a reciprocal intrinsic role of PDL1 in facilitating EMT in tumor cells. These findings help explain the enhanced response of sRCC to immunotherapy and suggest that EMT markers may be important biomarkers for immunotherapy response in other RCC tumors. This study also provides rationale to explore EMT targeted therapy in combination with immunotherapy for RCC.

## Keywords

sarcomatoid renal cell carcinoma; epithelial to mesenchymal transition; PDL1; immunotherapy

**55**

## The Clinical Utility of Circulating Tumor Cells (CTC) Detection and Profiling to Predict Outcomes in Advanced Renal Cell Carcinoma (RCC).

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## Background

No genomic mutations have been shown to predict therapeutic outcome in kidney cancer. In RCC, the selection of immune checkpoint inhibitors (ICI) or targeted anticancer agents is not guided by any biomarkers. We evaluated the utility of profiling of CTCs via multiplexed fluorescence immunocytochemistry (ICC) to identify biomarkers linked to treatment response (or resistance) in advanced RCC patients. Transcriptome analysis for 20802 genes from exosomal RNA was planned to evaluate novel prognostic and predictive signatures.

## Methods

Patients with either untreated or pretreated metastatic RCC were eligible. IRB approved written informed consent was obtained. Serial blood samples for CTC detection were collected at baseline, and longitudinal collections at 3, 6, 12 and 24 months and at progression. The changes in CTC detection and immunotyping is correlated with response and clinical outcomes. This will enable exploration of biomarkers of resistance that emerge on therapy. Primary endpoint of the study is to detect the proportion of patients with RCC in whom CTCs can be detected and utilized to recommend individualized anticancer therapies. Secondary endpoints are to evaluate the response rate, progression free survival and overall survival in the patient cohort tested and compare the outcomes in biomarker positive and negative patients. The study will meet its primary endpoint if for at least 12 patients the assay provides a therapeutic recommendation. With an overall sample size of 50 the width of a 95% confidence interval for the rate of providing a therapeutic intervention is guaranteed to be less than 26%. Exploratory endpoint is to evaluate the serial changes in CTC detection and immunotyping of the samples collected at months 3, 6, 12 and 24 and at progression, and compare to the baseline sample. This will be correlated with clinical outcomes.

## Results

47 patients, 25 untreated and 22 pretreated have been enrolled: 10 females, 37 males. 38 white patients, 3 black patients and 6 of other ethnicities have been enrolled. Median age was 64 years (range 40-85 years). IMDC Risk Category revealed 30 patients with Intermediate, 9 patients with poor and 8 patients with favorable. 24 received immunotherapy-based regimen in the untreated group and 17 received VEGF therapy alone and 6 received immunotherapy in pretreated group. 42 of the 44 (95.5%) patients had detectable CTCs in the baseline sample. 53 of 65 samples (81.5%) demonstrated detection of at least one biomarker by ICC. VEGFA was the most commonly detected biomarker in ICC (detected in 28 of 63 samples). Correlation of CTC biomarker data with clinical response will be reported. 25 patients were untreated, and 22 were pretreated. 41 patients had clear cell histology, 1 non clear cell, 3 papillary and 2 unclassified histology. 42 of the 44 (95.5%) patients had detectable CTCs in the baseline sample.

21 on treatment samples have been collected to date, with detectable CTCs in all except one sample. 53 of 65 samples (81.5%) demonstrated detection of at least one biomarker by ICC. VEGFA expression was the most commonly detected biomarker on ICC (detected in 28 of 63 samples).

## Conclusions

Feasibility of the test was demonstrated with 95% of the baseline samples showing CTC detection and 81.5% showing biomarker expression. This blood-based, non-invasive liquid biopsy demonstrated high sensitivity for detection of cancer cells and presents a potential opportunity for biomarker profiling to predict therapeutic efficacy of conventional RCC therapeutic agents. Transcriptome analysis is under evaluation for novel prognostic and predictive signatures.

## Keywords

liquid biopsy; circulating tumor cells; kidney cancer; predictive biomarkers

## CDMRP DOD Funding

yes

**57**

## Stratifying Patients Based on Abundance of Sarcomatoid Features Reveals Differential Transcriptomes and Survival Outcomes in Sarcomatoid Renal Cell Carcinoma

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## Background

Sarcomatoid renal cell carcinoma (sRCC) is highly aggressive, with an average patient survival of less than one year. sRCC tumors are fundamentally heterogenous, typically containing nests of sarcomatoid regions defined by spindle-cell morphology embedded within background epithelioid RCC histologies. Despite this intratumoral heterogeneity, sRCCs are pathologically defined homogenously: regardless of overall abundance of sarcomatoid features, all are uniformly classified as sRCC. This homogeneity also applies to biological analyses, which have associated mesenchymal and proliferative processes with sRCC. While the presence of sarcomatoid features is an independent predictor of survival,

correlation between sarcomatoid abundance and prognosis has been limited. Therefore, we sought to identify pertinent clinical and biological differences between sRCC tumors based on overall abundance of sarcomatoid features.

## Methods

Patients with sRCC were retrospectively identified from a prospectively maintained nephrectomy database at a comprehensive cancer center. The abundance of sarcomatoid features for each case was extracted from pathology reports. For cases with inconclusive sarcomatoid percentages in primary reports, formalin-fixed paraffin embedded (FFPE) blocks were reviewed by a genitourinary pathology team and sarcomatoid abundance was calculated as the average of blinded reviews. Youden's index criterion was employed to establish an optimal sarcomatoid threshold to differentiate between survival status and presence of metastatic disease. An optimal cut-off value of greater than five but no greater than ten percent was identified. Cases were stratified into sarcomatoid-lo (<10%) and sarcomatoid-hi ( $\geq 10\%$ ) cohorts. Kaplan-Meier survival analysis was performed to assess differences in overall and metastasis free survival between cohorts. RNA was extracted from a subset of FFPE blocks and underwent bulk RNA sequencing. Hallmark Gene set enrichment analysis (GSEA) was performed using GSEA v4.3.2 software. Transcriptomes were classified into one of seven previously defined RCC microenvironmental subgroups (Motzer et al. *Cancer Cell*. 2020). Statistical comparisons were performed using the Mann-U Whitney and Fisher's exact test for continuous and categorical variables, respectively.

## Results

In total, 104 patients with sRCC were identified; 52 (50%) were classified as sarcomatoid-hi and 52 (50%) were sarcomatoid-lo. Patients were predominantly male (65%), had clear cell epithelioid histology (71%), with pathologic T3 disease (63%). No significant differences were observed across demographic, clinical, and pathologic variables between cohorts. Patients with sarcomatoid-lo tumors experienced significantly prolonged overall survival (median: 62.86 vs 14.54 months,  $p=0.0005$ ). Significance was retained even when controlling for tumor size and pathologic T stage using a multivariate Cox linear regression model (Hazard Ratio=2.483,  $p=0.0007$ ). Among patients with localized disease at time of nephrectomy ( $n=31$  sarcomatoid-lo,  $n=29$  sarcomatoid-hi), metastasis free survival was also significantly prolonged in the sarcomatoid-lo cohort (median: Not Reached vs. 38.60 months,  $p=0.029$ ). A total of 45 cases were selected for bulk RNA sequencing: 24 sarcomatoid-hi and 21 sarcomatoid-lo. Twelve Hallmark pathways were significantly enriched in sarcomatoid-hi tumors, including G2M ( $q=0.001$ ), Epithelial-to-Mesenchymal Transition ( $q=0.002$ ), and MYC Targets V1 ( $q=0.018$ ). Sarcomatoid-lo specimens were significantly enriched for hypoxia signaling ( $q=0.016$ ). Sarcomatoid-hi tumors were

significantly enriched for proliferative, stromal/proliferative, and T-effector/proliferative microenvironmental phenotypes relative to sarcomatoid-lo tumors.

## Conclusions

These findings define a stratification of clinical outcomes and underlying biology between sarcomatoid-hi and sarcomatoid-lo tumors. Patients with sarcomatoid-hi tumors experience significantly diminished survival outcomes. Transcriptomes of sarcomatoid-hi tumors were more congruent with previously described biology of sRCC: enriched for mesenchymal, proliferative, and immune programs. Sarcomatoid-lo tumors were enriched for pathways canonically associated with clear cell RCC: hypoxia and angiogenesis. Further studies will investigate the predictive value of this stratification on response to immune checkpoint blockade in patients with metastatic sRCC. Together, these data promote the clinical utility of stratifying sRCC patients based on intratumoral abundance of sarcomatoid features while providing novel insight into the biological differences underlying such stratification.

## Keywords

Sarcomatoid; RNA Sequencing; Transcriptomics

**60**

## 89Zr-DFO-girentuximab for PET/CT imaging of clear cell renal cell carcinoma - results from phase 3 ZIRCON study

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## Background

The increasing detection of renal masses presents a significant dilemma as conventional tools (e.g., CT, MRI, biopsy) have limitations. Accurate noninvasive techniques to risk stratify patients with renal mass remains an unmet need.

Girentuximab is a chimeric monoclonal antibody with high affinity for carbonic anhydrase IX (CAIX), which is highly expressed in clear cell renal carcinoma (ccRCC); thus, 89Zr-DFO-girentuximab may aid differentiation of ccRCC and other renal lesions. ZIRCON (NCT03849118) was an open label, multicenter clinical trial evaluating the performance of 89Zr-DFO-girentuximab PET/CT for detection of ccRCC.

## Methods

300 patients with an indeterminate renal mass ( $\leq 7$  cm; cT1) who were scheduled for partial or radical nephrectomy within 90 days from planned 89Zr-DFO-girentuximab administration received a single dose of 89Zr DFO girentuximab IV (37 MBq $\pm$ 10%; 10 mg girentuximab) on Day 0 and underwent abdominal PET/CT imaging on Day 5 ( $\pm 2$  days). Blinded central histology review determined ccRCC status and CAIX expression; PET positivity was assessed by 3 independent blinded central readers. The coprimary objectives were to evaluate both the sensitivity and specificity of 89Zr-DFO-girentuximab PET/CT imaging for ccRCC detection, using histology as the standard of truth. Key secondary objectives included both sensitivity and specificity in the subgroup of patients with IDRM  $\leq 4$  cm (cT1a). Other secondary objectives included CAIX expression, safety, and tolerability.

## Results

300 patients received 89Zr-DFO-girentuximab (mean age  $62\pm 12$  y; 71% Male). Of 288 patients with central histopathology assessment, 67% had ccRCC, and 62% had CT1a. The primary analysis included 284 evaluable patients (central histology and readable PET). Across all 3 readers, the average [95% CI] sensitivity and specificity was 86% [80%, 90%] and 87% [79%, 92%] respectively for coprimary endpoints, and 85% [77%, 91%] and 90% [79%, 95%] resp. for key secondary. For all evaluable patients, positive and negative predictive values were  $\geq 91.7\%$  and  $\geq 73.7\%$ , respectively, and accuracy  $>85.6\%$ . The optimal SUVmax threshold to distinguish the ccRCC from other lesions was  $\geq 24.1$ , based on Youden index. PET+ ccRCC had higher mean CAIX expression compared with PET ccRCC patients ( $p<0.05$ ). Of 11 PET+ patients who were ccRCC negative on central histopathology assessment, 9 had papillary RCC, and 1 each had sarcoma and oncocytoma. Of 263 adverse events (AEs) in 124 patients, 2 AEs of mild intensity were treatment related.

## Conclusions

ZIRCON study confirms 89Zr-DFO-girentuximab PET/CT is a well-tolerated and accurate modality for noninvasive detection and characterization of ccRCC, with promising clinical utility.

**Disclosures:** Telix Pharmaceuticals sponsored this study.

## Keywords

clear cell renal cell carcinoma; 89Zr-girentuximab; PET/CT imaging

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## Statins inhibit the collaborative metastasis mediated by VHL heterogeneity in clear cell renal cell carcinoma (ccRCC) via a non-cholesterol pathway

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### Background

Despite the advancement of targeted therapies and immunotherapies, the outcome of clear cell renal cell carcinoma (ccRCC) remains poor at the metastatic stage, with a median survival of mere 7 months. This devastating outcome is partially due to an inadequate understanding of the metastatic mechanism of ccRCC. Previously, we found that two distinct cell populations co-exist in the malignancy: one with the functional von-Hippel Lindau (VHL) gene (VHL(+)) and the other without (VHL(-)). This heterogeneity in VHL plays an essential role, where the VHL(-) cells serve as the metastatic driver and promote VHL(+) cells to disseminate to distant organs such as the lungs. In this study, we sought to find small molecules that can selectively inhibit the VHL(-) cells' growth and survival. We hypothesize that these molecules will effectively inhibit ccRCC metastases by targeting the metastatic driver population.

### Methods

CRISPR knockout was conducted to generate VHL(-) cells from a murine RCC cell line, RENCA. Both VHL(-) and (+) cells were then fluorescently labeled and went through high-throughput screening (HTS) with 2,530 FDA-approved drugs. We followed a ratiometric black-box approach to identify selective modulators of cell growth and survival of VHL(-)

cells without affecting VHL(+) counterparts. CRISPR knockin was conducted on human ccRCC cells, as well, generating VHL(+) and (-) pairs of 786O, RCC4, and a patient-derived primary cell line.

### Results

HTS yielded 9 hits that selectively inhibited the survival of the metastatic drivers VHL(-) cells. 4 out of 9 hits belonged to the same drug family called statins. Statins bind to HMG-CoA reductase (HMGCR) and help lower cholesterol, thus are widely prescribed to high-risk patients for cardiovascular diseases. Interestingly, not all statins showed the VHL(-) suppressing effect, resulting in IC<sub>50</sub> varying from 0.6uM to >50mM. Atorvastatin and rosuvastatin, the two most potent and prescribed statins for cholesterol-lowering with the highest HMGCR affinity, did not show as much efficacy as other statins. This observation also held true in human ccRCC cell lines, suggesting an involvement of a non-cholesterol/HMGCR pathway in statins' inhibitory effect on VHL(-) cells. Animal studies were conducted using fluvastatin, which had the lowest IC<sub>50</sub> in our model. Intraperitoneal injection of 15mg/kg fluvastatin Q.O.D for 2 weeks successfully suppressed the growth of primary tumors, especially by eliminating VHL(-) cells, and most importantly, prevented lung metastases in the treatment group, whereas all of the control group developed metastases.

### Conclusions

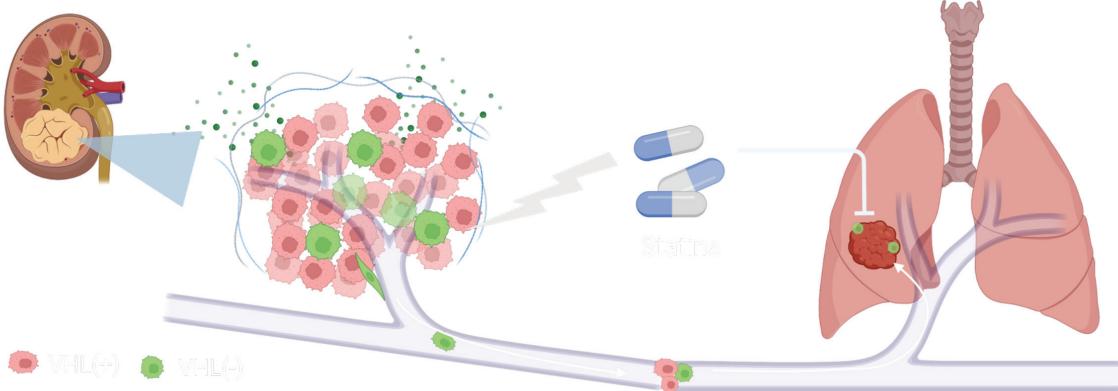
Translational potential of this project is enormous. By investigating the mechanism of action further, we could potentially reposition the FDA-approved, cheap, and widely available small molecules as a novel anti-metastatic therapeutic in ccRCC.

### Keywords

Metastasis, Heterogeneity, VHL, Small molecule drugs

### CDMRP DOD Funding

yes



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## Characterization of tumor response with lenvatinib plus pembrolizumab in patients with advanced renal cell carcinoma: final overall survival analysis of the CLEAR study (4-year median follow up)

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### Background

In the phase 3 CLEAR trial of lenvatinib plus pembrolizumab or everolimus versus sunitinib for the treatment of advanced renal cell carcinoma (aRCC), lenvatinib plus pembrolizumab showed clinically/statistically significant benefit versus sunitinib in progression-free survival (PFS per independent review using RECIST v1.1, primary endpoint; HR 0.39 [95% CI 0.32-0.49]; P<0.001), overall survival (OS; HR 0.66 [95% CI 0.49-0.88]; P=0.005), and objective response rate (ORR; relative risk 1.97 [95% CI 1.69-2.29]) (Motzer NEJM 2021). Efficacy benefits were maintained at the final prespecified OS analysis (data cutoff date: July 31, 2022; median OS follow-up: lenvatinib plus pembrolizumab, 49.8 months; sunitinib, 49.4 months). Herein we characterize patients with an objective response in the lenvatinib plus pembrolizumab arm of the CLEAR study at the final analysis with a median follow-up of approximately 4 years.

### Methods

Treatment-naïve patients (n=1069) who had aRCC with a clear-cell component were randomized (1:1:1) to receive: lenvatinib 20 mg orally per day (QD) plus pembrolizumab 200 mg IV every 3 weeks; or lenvatinib 18 mg plus everolimus 5 mg orally QD; or sunitinib 50 mg orally QD (4 weeks on/2 weeks off). Stratification factors were region and MSKCC prognostic risk group. Tumor responses were assessed per independent review using RECIST v1.1. Patients with complete response (CR), partial response (PR) with maximum tumor shrinkage ≥75% from baseline (ie, near-CR), or PR, were characterized by baseline characteristics (IMDC risk group [derived programmatically], programmed cell death ligand-1 [PD-L1] status, and prior nephrectomy). Median OS and duration of response in responders were calculated using the Kaplan-Meier method; 95% CIs were estimated with a generalized Brookmeyer and Crowley method. OS-rates and 95% CIs were calculated using the Kaplan-Meier product-limit method and Greenwood formula.

### Results

In the final OS analysis dataset, 253 (71.3%) of 355 patients in the lenvatinib + pembrolizumab arm had an objective response; 65 (18.3%) patients had a best response of CR, and 188 (53.0%) had a best response of PR, including 59 (16.6%) who had a near-CR. Median duration of objective response (95% CI) for patients with a CR, near-CR, or PR was 43.7 months (39.2-not estimable [NE]), 30.5 months (22.4-NE), and 20.4 months (17.0-25.7), respectively (Table). Among patients with CR, near-CR, and PR, 55 (84.6%), 35 (59.3%), and 80 (42.6%) patients, respectively, had a duration of response ≥18 months.

For patients with a CR or near-CR, median OS (95% CI) was not reached (NE); for patients with PR, median OS (95% CI) was 53.7 months (46.9-NE). OS-rates (95% CI) at 36 months were 96.9% (88.1-99.2) for CR, 86.0% (74.0-92.8) for near-CR, and 69.4% (62.1-75.5) for PR (Table).

Patients with CR, near-CR, and PR were distributed across baseline IMDC risk groups (CR/near-CR/PR in favorable: 38.5%/33.9%/27.1%; intermediate: 56.9%/57.6%/61.7%; poor: 4.6%/6.8%/10.6%) (Table).

Tumor responses (CR/near-CR/PR) were observed across patients, irrespective of baseline PD-L1 status (PD-L1 positive: 40.0%/30.5%/28.2%; PD-L1 negative: 29.2%/30.5%/36.7%; PD-L1 not available: 30.8%/39.0%/35.1%). Prior nephrectomies had occurred in 93.8% of CR patients, 86.4% of near-CR patients, and 70.7% of PR patients.

Overall median duration of treatment was 36.5 months (range 4.4-59.1) among patients with a CR; median duration of treatment was similar in patients with near-CR (26.6 months

	ICI-pretreated <sup>a,b</sup> (n=104)		Treatment-naïve <sup>b</sup> (n=22)		Previously treated ICI-naïve <sup>c</sup> (n=17)	
	irRECIST	RECIST v1.1	irRECIST	RECIST v1.1	irRECIST	RECIST v1.1
<b>Objective response, n (%) [95% CI]</b>	65 (62.5) [52.5–71.8]	61 (58.7) [48.6–68.2]	17 (77.3) [54.6–92.2]	17 (77.3) [54.6–92.2]	9 (52.9) [27.8–77.0]	9 (52.9) [27.8–77.0]
CR, n (%)	1 (1.0)	1 (1.0)	0	0	0	0
PR, n (%)	64 (61.5)	60 (57.7)	17 (77.3)	17 (77.3)	9 (52.9)	9 (52.9)
<b>Median DOR, months (95% CI)<sup>d</sup></b>	14.1 (9.7–18.2)	14.1 (10.6–18.4)	24.2 (10.3–40.4)	24.2 (10.3–40.4)	9.0 (3.5–NE)	9.0 (3.5–NE)
<b>Responders by response duration, n (%)<sup>e</sup></b>						
≥18 months	21 (32.3)	21 (34.4)	10 (58.8)	10 (58.8)	3 (33.3)	3 (33.3)
≥24 months	15 (23.1)	15 (24.6)	8 (47.1)	8 (47.1)	2 (22.2)	2 (22.2)
≥30 months	5 (7.7)	5 (8.2)	7 (41.2)	7 (41.2)	2 (22.2)	2 (22.2)
≥36 months	0	0	6 (35.3)	6 (35.3)	1 (11.1)	1 (11.1)
<b>Median PFS, months (95% CI)</b>	13.6 (9.5–17.7)	11.6 (7.6–14.1)	24.1 (11.7–38.8)	22.1 (11.6–31.7)	11.8 (5.5–21.9)	11.8 (5.5–18.6)
PFS rate at 18 months, % (95% CI)	38.0 (28.2–47.7)	34.5 (25.1–44.1)	70.5 (45.7–85.6)	67.2 (43.1–82.8)	36.1 (13.2–59.8)	28.8 (8.9–52.7)
<b>Median OS, months (95% CI)</b>	32.1 (26.4–NE)		55.8 (31.4–NE)		30.3 (28.7–NE)	
OS rate at 18 months, % (95% CI)	70.3 (60.3–78.2)		90.9 (68.3–97.6)		80.4 (50.6–93.2)	
OS rate at 24 months, % (95% CI)	64.2 (54.0–72.7)		90.9 (68.3–97.6)		80.4 (50.6–93.2)	

<sup>a</sup>The ICI regimens most commonly received as prior therapy included: nivolumab monotherapy (n=41) and nivolumab + ipilimumab (n=39).  
<sup>b</sup>Excluded 1 patient who had non-clear cell renal cell carcinoma.  
<sup>c</sup>Previous therapy included anti-vascular endothelial growth factor receptors received by 16 patients, of which sunitinib (n=9) was most commonly received.  
<sup>d</sup>Among responders.  
<sup>e</sup>Percentage of responders.

CI, confidence interval; CR, complete response; DOR, duration of response; ICI, immune checkpoint inhibitor; ir, immune-related; NE, not estimable; OS, overall survival; PFS, progression-free survival; PR, partial response.

[2.8–56.7]) and PR (23.8 months [2.8–56.7]). Additional dosing data will be reported.

## Conclusions

Objective responses were durable across complete and partial responders, including patients with near-complete responses. Median OS was not yet reached in both patients with CRs and near-CRs. Tumor response was observed regardless of PD-L1

status and IMDC risk group. These results corroborate data from the primary analysis with deep and durable responses in patients with aRCC, and further support lenvatinib plus pembrolizumab as a standard-of-care treatment in 1L aRCC.

## Keywords

lenvatinib, pembrolizumab, final analysis, responders, kinase inhibitor, immuno-oncology

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## A Phase 1 multicenter study (TRAVERSE) evaluating the safety and efficacy of ALLO-316 following conditioning regimen in pts with advanced or metastatic clear cell renal cell carcinoma (ccRCC)

Samer Srour

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### Background

While doublet therapies provide important first-line treatment options in metastatic renal cell carcinoma (mRCC), novel and mechanistically distinct agents are needed for pts who are not cured by/intolerant of such therapies. Due to the high incidence (~80%) of CD70 antigen expression in primary and mRCC, yet limited expression in normal tissue, ccRCC is an attractive proof-of-concept tumor for CD70 directed allogeneic CAR T. TRAVERSE (NCT04696731), a first-in-human trial, seeks to identify a maximum tolerated dose (MTD) of ALLO-316 after conditioning with fludarabine/cyclophosphamide with/without ALLO-647 in pts with advanced or metastatic ccRCC. ALLO-316 is an anti-CD70 allogeneic CAR T cell product that utilizes TALEN® gene editing to knock out TCR $\alpha$  constant gene to reduce the risk of graft-versus-host disease (GvHD), and knock out CD52 gene to permit use of ALLO-647 (a humanized anti-CD52 mAb) to selectively deplete host T cells without affecting allogeneic CAR T cells.

### Methods

This multicenter, single-arm, open-label, 3+3 dose-escalation trial enrolls adults with advanced or metastatic ccRCC and  $\geq 1$  measurable lesion and ECOG Performance Status 0 or 1. Prior treatment with an immune checkpoint inhibitor and

a vascular endothelial growth-factor targeted therapy was required, with evidence of progression on/after treatment or discontinuation due to toxicity. ALLO-316 is administered at escalating doses (40 - 240 X 10<sup>6</sup> allogeneic CAR+ cells IV) on Day 0 after conditioning. The primary endpoint is a target incidence rate for dose-limiting toxicities (DLTs) <33% in the first 28 days after infusion of ALLO-316.

### Results

By 12/3/2022, 18 pts with ccRCC (median age: 63 yrs; 82% male) were enrolled; all (100%) 17 pts who received ALLO-316, had metastatic disease with 3 lines (median) of prior therapy. Eleven (65%) of these pts experienced CRS, all low Gr except one (6%) Gr 3. No ICANS or GVHD was observed. One (6%) DLT (elevated LFT) was observed and required dose expansion. MTD has not yet been reached. Three pts achieved best overall response of PR at all time points with two PRs confirmed at subsequent visits; ORR = 12% and disease control rate (DCR) = 71%. In pts with confirmed CD70+ tumors (n=9), confirmed ORR = 22% (unconfirmed ORR = 33%) and DCR = 100%. High CAR T cell expansion was observed in peripheral blood (median Cmax > 35,000 copies/ $\mu$ g) and high VCN in available tumor aspirates (n=3).

### Conclusions

ALLO-316, an allogeneic CAR T cell product targeting CD70 in advanced mRCC, is demonstrating encouraging antitumor activity and a manageable safety profile. A single administration of ALLO-316 could be an effective treatment for pts with CD70+ solid tumors, including RCC, and hematologic malignancies. The MTD for ALLO-316 in TRAVERSE will support Phase 2 trial design. Enrollment of pts with CD70+ tumors is ongoing.

### Keywords

TRAVERSE, ALLO-316

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## Adjuvant pembrolizumab for renal cell carcinoma across UCLA Integrated Staging System risk groups and disease stage: Subgroup analyses from the KEYNOTE-564 study

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### Background

Adjuvant pembrolizumab prolonged disease-free survival (DFS) for patients with renal cell carcinoma (RCC) at increased risk of recurrence after nephrectomy in the phase 3 KEYNOTE-564 study (NCT03142334). This post hoc exploratory analysis evaluated efficacy of adjuvant pembrolizumab in patient subgroups based on UCLA Integrated Staging System (UISS) risk groups and disease stage.

### Methods

Patients with histologically confirmed clear cell RCC (pT2, Grade [G] 4 or sarcomatoid, N0, M0; pT3 or pT4, any G, N0,

M0; any pT, any G, N+, M0; or M1 evidence of disease [NED]) were randomly assigned 1:1 to receive pembrolizumab 200 mg intravenously or placebo every 3 weeks for up to 17 cycles (approximately 1 year). DFS was assessed by investigator. UISS risk groups were derived retrospectively from TNM stage, Fuhrman nuclear grade, and ECOG performance status score. UISS groups were intermediate risk (pT2, G4, N0, M0; pT3, G1, N0, M0; or pT3, G2-4, N0, M0, ECOG 0), high risk (pT3, G2-4, N0, M0, ECOG PS 1; pT4, any G, N0, M0; or N1, M0), or M1 NED. Other subgroups were evaluated based on American Joint Committee on Cancer (AJCC) disease stage, which classifies cancers by TNM status, and by TNM status and Fuhrman nuclear grade.

### Results

Baseline characteristics were balanced within subgroups. Median follow-up, defined as time from randomization to the data cutoff date (June 14, 2021), was 30.1 months (range, 20.8–47.5). Of 994 enrolled patients, most had UISS intermediate risk (n=732, 73.6%; pembrolizumab n=359; placebo n=373); 195 patients (19.6%; pembrolizumab n=100; placebo n=95) had UISS high risk, and 58 patients (5.8%; pembrolizumab and placebo n=29 each) had M1 NED. In the UISS intermediate risk group, the hazard ratio (HR) for DFS was 0.65 (95% CI, 0.48–0.88); the 24-month rates were 81.5% with pembrolizumab and 72.4% with placebo. In the UISS high-risk group, HR for DFS was 0.77 (95% CI, 0.49–1.20); the 24-month rates were 65.0% with pembrolizumab and 55.9% with placebo. In the M1 NED group, HR for DFS was 0.28 (95% CI, 0.12–0.66); the 24-month rates were 78.4% with pembrolizumab and 37.9% with placebo. DFS by other subgroups are in the Table.

### Conclusions

Consistent with the results of the intention-to-treat (ITT) population, adjuvant pembrolizumab prolonged DFS compared with placebo for all subgroups. Results of this exploratory analysis further support use of adjuvant pembrolizumab after nephrectomy as standard of care for patients with RCC at increased risk of recurrence.

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### Keywords

Adjuvant therapy; immunotherapy; pembrolizumab; renal cell carcinoma

<b>DFS HR and 24-month rates</b>	<b>Pembrolizumab</b>	<b>Placebo</b>
<b>ITT</b>	N=496 HR (95% CI): 0.63 (0.50-0.80) 24-month rate: 78.3%	N=498 - 24-month rate: 67.3%
<b>Disease stage:</b> T2, G4, N0, M0 and T3, G1-2, N0, M0 (33.0% of ITT)	n=161 HR (95% CI): 0.79 (0.49-1.27) 24-month rate: 83.5%	n=167 - 24-month rate: 78.3%
T3, G3-4, N0, M0 (52.6% of ITT)	n=258 HR (95% CI): 0.63 (0.45-0.88) 24-month rate: 79.3%	n=265 - 24-month rate: 68.0%
T4, N0, M0 (2.2% of ITT)	n=11 HR (95% CI): 0.63 (0.17-2.36) 24-month rate: 63.6%	n=11 - 24-month rate: 54.5%
N1, M0 (5.4% of ITT)	n = 29 HR (95% CI): 0.57 (0.29-1.12) 24-month rate: 42.8%	n=25 - 24-month rate: 27.4%
M1 NED (5.8% of ITT)	n=29 HR (95% CI): 0.28 (0.12-0.66) 24-month rate: 78.4%	n=29 - 24-month rate: 37.9%
<b>AJCC TNM stage:</b> Stage 2: pT2, N0, M0 (4.1% of ITT)	n=22 HR (95% CI): 0.74 (0.30-1.83) 24-month rate: 57.3%	n=19 - 24-month rate: 47.4%
Stage 3: pT3, N0, M0 or pT1-3, N1, M0 (87.5% of ITT)	n =432 HR (95% CI): 0.67 (0.52-0.88) 24-month rate: 79.9	n=438 - 24-month rate: 70.7%
Stage 4: pT4, Any N, M0 or M1 NED (8.2% of ITT)	n=41 HR (95% CI): 0.37 (0.19-0.74) 24-month rate: 72.4%	n=41 - 24-month rate: 41.5%

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## CTX130 allogeneic CRISPR-Cas9-engineered chimeric antigen receptor (CAR) T cells in patients with advanced clear cell renal cell carcinoma: results from the phase 1 COBALT-RCC study

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### Background

Clear cell renal cell carcinoma (ccRCC) is the most common subtype of renal tumors. Patients with ccRCC who fail checkpoint inhibitors (CPIs) and tyrosine kinase inhibitors (TKIs) have a poor prognosis. Preclinical studies identified substantial expression of CD70 in ccRCC tumor samples. Here, we report results from the Phase 1 dose-escalation study of CTX130™, a CD70-targeting allogeneic CAR T cell therapy in patients with ccRCC.

### Methods

COBALT-RCC (NCT04438083) is a Phase 1, open-label, multicenter, global study evaluating safety and efficacy of CTX130 in patients ≥18y with advanced (unresectable or metastatic), relapsed, or refractory (R/R) ccRCC and prior exposure to both CPIs and TKIs. Patients received standard lymphodepleting chemotherapy with fludarabine 30mg/m<sup>2</sup> and cyclophosphamide 500mg/m<sup>2</sup> for 3 days, followed by CTX130 infusion.

### Results

As of May 2, 2022, 14 patients with a median age of 64.5y (range, 51-77) were treated with CTX130. All patients had stage IV disease, and received a median of 3 (range, 1-6) prior treatments. Six patients had documented refractory disease at study entry. Median CD70 expression level on the tumors was 100% (range, 1-100%). Patients received CTX130 at dose

levels (DLs) ranging from 3x10<sup>7</sup> to 9x10<sup>8</sup> CAR T cells. Overall, expansion occurred across all DLs. An emerging relationship between higher CAR T exposure and disease control was observed. CTX130 had an acceptable safety profile; there were no dose-limiting toxicities across all DLs. Seven (50%) patients experienced grade (Gr) 1-2 cytokine release syndrome (CRS); there was no Gr≥3 CRS. Three patients experienced serious adverse events (SAEs) related to CTX130; all were episodes of CRS. Three patients had SAEs of infections, all unrelated to CTX130, including a Gr5 pneumonia with Gr4 dyspnea resulting in death. No patients experienced immune effector cell associated neurotoxicity syndrome, graft versus host disease, or hemophagocytic lymphohistiocytosis. One patient (7.7%) had a durable complete remission (CR) maintained at 18+ months and 9 (69.2%) patients had stable disease (SD) with 4 patients (30.8%) in SD at 4 months. The disease control rate (CR + partial response + SD) was 76.9%.

### Conclusions

This first-in-human clinical trial exploring CD70 CAR T-cell therapy in ccRCC showed an excellent safety profile with no unexpected on-target off-tumor toxicities and encouraging antitumor activity. To our knowledge, this durable CR is the first to be achieved with allogeneic CAR T cell therapy in patients with R/R solid tumors and represent a proof-of-concept for further exploration of CD70-targeted CAR T cells in ccRCC and other CD70+ malignancies.

### Keywords

CD70; CAR T

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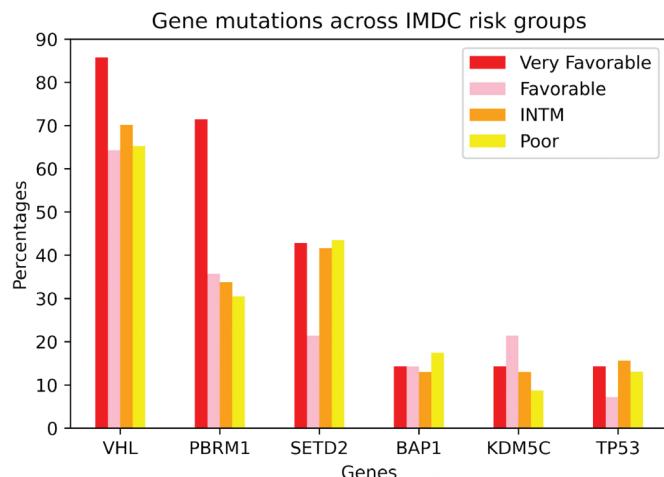
## A genomic analysis of the very favorable risk group (grp) in metastatic renal cell carcinoma (mRCC): a study from the International Metastatic RCC Database Consortium (IMDC)

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### Background

The IMDC criteria stratify patients (pts) with mRCC into 3 prognostic risk grp. The aim of this study was to identify a subset of patients with improved clinical outcomes within the favorable risk group, known as the “very favorable” risk group, and investigate their genomic characteristics.



**Figure 1:** Distribution of common gene mutations across the different IMDC risk groups.

## Methods

In the IMDC database, predictors of overall survival (OS) were assessed among pts from the favorable risk grp, using a multivariate Cox regression model. For a subset of pts from the Dana-Farber Cancer Institute (DFCI) with a clear cell histology and available targeted panel sequencing data, genomic characterization was performed among the different risk grps, including single nucleotide variant (SNV) and copy number variants (CNV), as well as tumor mutational burden (TMB) estimates. We compared the frequency of each gene alteration between grps using the Fisher's exact test.

## Results

Our analysis included 1638 pts with favorable risk, most of whom (91%) received targeted therapy in the first line setting. Predictors of OS on multivariate analysis were: time from primary diagnosis to systemic therapy (<3 vs ≥3yr), Karnofsky Performance Status (80 vs >80), and presence of brain, liver, or bone metastasis (HR = 1.4-1.5, p-values<0.05). Using these variables, 454 pts were classified as having very favorable risk (absence of all 3 risk factors). The genomic analysis included 97 patients from DFCI (6 very favorable, 10 favorable, 63 intermediate and 18 poor risk). When examining SNVs, pts in the very favorable risk grp, had a higher proportion of PBRM1 mutations vs. other risk grps [83% (n=5/6) vs. favorable: 50% (n=5/10), intermediate: 39% (n=25/63) and poor: 27% (n=5/18), p=0.07]. CNVs and TMB estimates were not significantly different across the risk groups.

## Conclusions

We identified a “very favorable” subset of patients with mRCC with improved overall survival. In the exploratory genomic analysis, PBRM1 mutations were more prevalent in this grp. Validation in larger cohorts is currently ongoing.

## Keywords

pts=patients, grp=group, grps=groups, IMDC=International mRCC Database Consortium

## CDMRP DOD Funding

yes

75

## HHLA2 Regulation in *in vivo* kidney cancer models

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## Background

The immune checkpoint HERV-H LTR-associating 2 (HHLA2) is expressed in many tumors, including clear cell renal cell carcinoma (ccRCC). Multiple therapeutics directed against HHLA2 or its inhibitory receptor KIR3DL3 are about to enter clinical trials in metastatic RCC; however, little is known about the regulation of HHLA2 expression. Better understanding the regulation of HHLA2 in tumor cells and within the tumor microenvironment will facilitate translation of these therapeutic agents into the clinic by assisting in patient selection and development of rational drug combinations.

## Methods

Using flow cytometry, immunohistochemistry (IHC), and bulk / single cell RNA sequencing, HHLA2 expression was examined in monocytes, primary nephrectomy specimens and the established ccRCC cell lines, A498 and 786-0. A panel of cytokines was screened for the induction of HHLA2 in vitro. *In vivo* expression of HHLA2 in A498 and 786-0 human kidney cancer xenograft mouse models was examined. Tumor cells obtained from these xenograft models (either freshly isolated or cultured ex vivo) were characterized by RNASeq and flow cytometry.

## Results

Analysis of HHLA2 expression in monocytes shows that among a wide range of cytokines tested, only IL-10 and BMP4, and to a lesser extent, IL-1b and IL-6, modestly enhanced HHLA2 mRNA and protein expression. These combinations of cytokines failed to induce HHLA2 in both A498 and 786-0.

HHLA2 was frequently expressed in primary ccRCC tumors by IHC. Interestingly, analysis of HHLA2 by flow cytometry in primary RCC tumors showed that HHLA2 is progressively lost over 4 weeks when cells are cultured ex vivo. Hypoxia alone did not change HHLA2 expression. While HHLA2 was not expressed on either A498 or 786-O cells in vitro, both A498 and 786-O cells expressed HHLA2 when grown as subcutaneous xenografts in NSG immunodeficient mice. A498 xenografts express significantly more HHLA2 than 786-O xenografts by IHC. Furthermore, single cell RNASeq analysis of A498 and 786-O xenografts identified differences in the tumor cell population transcriptional programs between tumor types. Of note, BMP4 and HHLA2 reads were elevated in the A498 compared to 786-O, while IL10 were essentially undetected.

## Conclusions

HHLA2 expression is differentially regulated in monocytes and kidney cancer epithelial cells. High concentrations of cytokines, particularly IL10, that induce HHLA2 expression in monocytes fail to upregulate HHLA2 expression in ccRCC. However, the tumor microenvironment in NSG mice significantly induced HHLA2 expression in both A498 and 786-O xenografts. Unlike IL10, BMP4 is associated with HHLA2 expression in xenografts, but is not sufficient for induction of HHLA2 in vitro. Complementary whole genome CRISPR knock out and gain-of-function screens are underway to assess by an unbiased means both nonimmune and immunologic regulators or repressors that may be responsible for inducing HHLA2 in kidney cancer in vivo. Furthermore, the A498 xenograft mouse model is an excellent humanized HHLA2 model for testing regulation of HHLA2 and HHLA2-directed therapeutics.

## Keywords

HHLA2, A498, 786-O, mouse model

## CDMRP DOD Funding

yes

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## Intratumoral heterogeneity of VHL and HIF1A drives lung metastasis in clear cell renal carcinoma

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## Background

Loss of function in the von Hippel-Lindau (VHL) tumor suppressor gene is a hallmark of clear cell renal cell carcinoma (ccRCC). However, continuous efforts to establish transgenic mouse model by single or combined knockout of VHL do not produce prominent metastasis as seen in clinic, in which distant metastasis takes up 20-30% of all cases. However, studies found patients with primary ccRCC tissue harboring subclonal VHL mutations or wildtype VHL cancer cells have much worse prognosis than others with only VHL null cancer cells or clonal VHL mutations. Study to elucidate the possible role of VHL in ccRCC metastasis is required.

## Methods

Immunohistochemistry (IHC), multiplex immunofluorescence (MIF) staining as well as single and bulk RNAseq, whole exosome sequencing were used to evaluate tissue VHL mRNA expression and genetic mutation profiles. HALO analysis was used to examine cancer cell distribution with different marker combinations (VHL, POSTN, Ki67) in the IHC and MIF sections. VHL gene knockout (VHL-ko) was implemented in VHL expressing mouse and human cell lines including RENCA, ACHN, Caki-1 and a metastatic patient primary tumor derived cell line #22. Intra-renal injection of tumor cells immediately under the renal capsule in Balb/c nude mice was used to establish the orthotopic kidney cancer model as in-vivo assessment of primary tumor and distant metastasis. Live microscope videotaping for 24 hours to 48 hours on cell scratch assay was adopted as in-vitro assessment of cell motility. MTS assay was for cell proliferation evaluation.

## Results

1) IHC anti-VHL staining of tumor samples in a 26 patients' cohort showed VHL positive cancer cells are present in ccRCC patients. 2) Bulk and single cell sequencing, whole exome sequencing of a metastatic ccRCC patient #22 confirmed the presence of VHL expressing cancer cells. HALO analysis on the cancer cell distribution of the metastatic case #22 showed high resemblance to our metastatic ccRCC mouse model. 3) Orthotopic co-implantation of VHL-ko and VHL expressing parental cancer cells produced prominent lung metastasis in nude mice. Despite that two cell types were co-implanted equally, the VHL expressing cancer cells eventually evolved as the majority cell subpopulation in lung metastasis. 4) Mechanistic study revealed that VHL-ko cancer cells can secrete Periostin protein, which is driven by HIF1A but not HIF2A, to promote the motility of VHL expressing parental cancer cells in both 2D and 3D scratch assay. 5) VHL-ko cancer cells induced vascular endothelial cell apoptosis thereby demolishing the vascular barrier facilitating the entry of more proliferative VHL expressing cells into systemic circulation.

## Conclusions

VHL expressing cancer cells are highly underestimated in ccRCC patients. The proliferative VHL expressing cancer cell population can be driven by cancer cells lacking VHL which is upregulated in HIF1A but not HIF2A to form distant lung metastasis.

## Keywords

VHL; clear cell renal cell carcinoma; ccRCC; distant metastasis; tumor heterogeneity

## CDMRP DOD Funding

yes

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# Pathological Response and Outcomes of Patients with Metastatic Renal Cell Carcinoma (mRCC) Undergoing Cytoreductive Nephrectomy after Immunotherapy (IO) Regimens

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## Background

Immunotherapy (IO), mainly given in combination (IO-IO or IO-VEGF TKI), has improved outcomes for patients with first-line treatment of metastatic or advanced RCC. After CARMENA, the role and timing of cytoreductive nephrectomy are harder to define. In this study, we characterized the pathological and survival outcomes for patients with metastatic RCC undergoing cytoreductive nephrectomy after IO-based peri-operative treatments.

## Methods

We conducted a retrospective analysis of patients with mRCC receiving IO-based therapies and undergoing cytoreductive nephrectomy at the University of Texas Southwestern. An IRB-approved and HIPAA-compliant registry was used to collect data from the electronic medical record. The primary endpoint was pathologic tumor size reduction and downstaging of the primary tumor; secondary endpoints included progression-free survival (PFS), and overall survival (OS) using Kaplan-Meier analyses.

## Results

From 4/2016 to 10/2022, 51 patients with mRCC were included (median age 62 years, range 36-86; 76.5% male), with a median follow-up of 21 months. 63% (n=32) patients received IO-IO and 21.6% (n=11) received IO-VEGF combinations, with the remainder receiving IO monotherapy. 38 (74.5%) patients received treatment prior to surgery while 13 (25.5%) patients underwent up-front cytoreductive surgery. 86% of patients were treatment-naïve at the start of IO therapy and mean IO cycles before surgery was 6.7 (range 1-39). Neoadjuvant IO-based treatment reduced median tumor size from pre-treatment 10cm to 7.5cm post-treatment. Pathologic T downstaging occurred in 42% (n=16)

of patients, 11% (n=4) of whom had pT0 disease. Thrombus downstaging occurred in 13% (n=5) patients. PFS (HR=0.7, 95% CI 0.29-1.98, p=0.58) and OS (HR 0.4, 95% CI 0.13-1.57, p=0.21) were not statistically significant, though trended better for patients treated with neoadjuvant therapy. Two patients with synchronous bilateral renal masses underwent staged radical nephrectomy and partial nephrectomy after neoadjuvant treatment.

### Conclusions

Neoadjuvant IO treatment resulted in reductions in tumor size and pathologic necrosis at the time of nephrectomy. In

this small cohort, PFS and OS were similar for patients who received either upfront IO-based therapy or cytoreductive nephrectomy prior to systemic treatment. Randomized patient cohorts are ongoing to investigate the timing and outcomes of cytoreductive nephrectomy for patients with mRCC.

### Keywords

Renal Cell Carcinoma, Immunotherapy, Nephrectomy

# Virtual Posters Only

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## Using Infrared Light to Improve the Diagnosis and Treatment of Patients with Kidney Cancer

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### Background

People who are suspected of having kidney tumors often require a biopsy to be taken to confirm the presence of tumor, the extent of the disease and to further classify the tumor type. This involves taking a small piece of tissue from the kidney and then having a specialized doctor, a pathologist, examine the tissue using special stains and immunohistochemistry and examining the tissue using a light microscope. This process is expensive, laborious, time-intensive and requires the subjective visual analysis of the tissue to make a diagnosis. This can lead to delayed and sometimes inaccurate diagnoses being made. Furthermore, the prognostic information that can be derived from these kidney tissues are often limited which prevents accurate prediction of disease progression or the most appropriate course of treatment.

### Methods

We are developing a new label-free, automated and objective technique to improve diagnosis and prediction of kidney disease outcome based on how tissues absorb and modify light. We are developing methodologies using mid-infrared (IR) light to examine renal biopsy tissues for biochemical changes observed in patients with diabetes and kidney cancers. Mid-IR imaging is an attractive technique to interrogate cells and tissues as different regions of the mid-IR are absorbed by key biomolecules such as proteins, lipids, DNA, collagen, and glycosylation. This leads to the acquisition of an IR spectral signature or "biochemical fingerprint" which we have shown is unique to different cell types and disease states. Furthermore, we have shown in the kidney that the biochemical fingerprint of cells and tissue can be altered before there are any indications of disease using conventional medical techniques.

### Results

Using a combination of image analysis, spectral multivariate analysis, and artificial intelligence, we have demonstrated that IR imaging can identify biochemical changes in an objective fashion in patients with renal tumors including cell carcinoma

(RCC) compared to normal tissues. Furthermore, we have demonstrated that there are biochemical changes in RCC in patients with ( $n=30$ ) and without ( $n=30$ ) diabetes, highlighting biochemical differences between these two populations. We have also demonstrated that we can discriminate based upon the spectral signatures between two kidney cancers tumors that appear histologically similar, chromophobe RCC and renal oncocytomas. We have also been using model tissue engineered systems to replicate the effects on renal cells in response to high glucose levels (to replicate diabetes) and to the presence of cisplatin (to replicate nephrotoxicity).

### Conclusions

Using mid-IR technologies coupled with advanced computational techniques, such as artificial intelligence, there is the exciting potential to improve kidney disease diagnosis, better understand a patient's disease course, and identify renal diseases earlier allowing for more timely treatment. Furthermore, this work builds towards the idea of using precision medicine for kidney disease patients with personalized treatment based upon their individual biochemical factors that may relate to disease progression and outcome.

### Keywords

Cancer, Imaging, spectroscopy, diagnostic, prognostic

**41**

## Creation and implementation of an institutional workflow for systematic collection of fresh renal cell carcinoma tumor biospecimens for laboratory investigative studies

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### Background

There is a growing need for fresh specimen collection in molecular oncology research. Fresh tumor tissue can be utilized for advanced immune profiling such as single-cell RNA-seq analysis, and for patient-derived model systems required for functional investigation. Due to their larger size,

renal cell carcinoma (RCC) tumor specimens provide a unique opportunity to perform functional analyses on primary human tumors. Furthermore, concomitant blood collection allows for comparison of systemic immune function to that of the tumor microenvironment. For fresh collection, biospecimens must be received by researchers in a timely and efficient manner. Therefore, a workflow of this process must be incorporated to coordinate between the clinical setting and research laboratory. Remaining tissue can be stored and organized for further use in translational research studies.

## Methods

Potential subjects are identified, screened, and tracked using the electronic medical record system, through manual review of outpatient clinic and operating room schedules with pre-specified criteria such as a potential surgery and confirmed or suspected RCC diagnosis. Informed consent for an institutional review board (IRB)-approved protocol is obtained, either in person at an appointment or remotely using an IRB-approved eConsent form. Communication is required between surgical, pathology (including the Yale Pathology Tissue Services), and research teams to obtain fresh samples rapidly. Pathology receives the tissue and allocates a portion (not affecting clinical diagnosis) to a research team member in chilled DMEM medium. The specimen is transported to the research laboratory on ice in a biosafety container for immediate use in fresh tissue studies. Any remaining tissue is stored using 4 different methods: formalin-fixed paraffin-embedded (FFPE), frozen in optimal cutting temperature (OCT) media, fresh frozen, and cryopreservation of viable pieces of tumor. Cryopreservation produces viable cells that keeps the tissue structurally intact and preserves the genetic material for studies such as single cell RNA sequencing. Blood is also collected at the time of surgery. Samples are centrifuged to store plasma and peripheral blood mononuclear cells (PBMCs) are isolated by a Ficoll density gradient. Patients are tracked post-operatively for future blood collections. De-identified clinical data for each subject is encoded into a clinical RedCap database.

## Results

After initial pilot studies, we initiated a workflow for collection of fresh tumor material beginning September 2022. In the time period of September 2022 through April 2023, clinical data from 54 patients were added to the RedCap Database. From these patients, we have collected tumor tissue from 43, adjacent normal tissue from 40, and blood from 49 and registered 63 patients to our protocol.

There were 37 males (69%) and 17 females (31%). The median age was 63.5, with a range of 30-87. For self-reported race, 48 (88.9%) patients were White or Caucasian, 4 (7.4%) were Black or African American, 1 (1.9%) was Hispanic or Latino, and 1 (1.9%) was Asian. For self-reported ethnicity, 6 patients (11.1%) self-reported as Hispanic or Latino and 48 did not.

31 patients had Clear Cell RCC (57%), 7 with Chromophobe RCC (13%), 7 with Papillary RCC (13%), 9 considered "Other" (17%) (includes benign oncocytomas, mucinous tubular and spindle cell RCC, and eosinophilic RCC). There were 38 stage I RCC patients (58%), 7 stage II (15%), 10 stage III (21%), and 3 stage IV (6%).

## Conclusions

We have successfully implemented and utilized a workflow to collect and distribute fresh specimen for research, including single-cell RNA-sequencing, the creation of ex vivo patient-derived tumor-models, the generation of primary patient-derived RCC tumor cell lines, and the isolation and functional interrogation of tumor-infiltrating immune cells. This work demonstrates the feasibility of implementing a multidisciplinary fresh biospecimen collection protocol for improving our understanding of RCC specifically and human tumor immunology more generally.

## Keywords

Renal cell carcinoma, fresh tissue, biospecimen collection, patient-derived models

## CDMRP DOD Funding

no

**44**

## Efficacy of immune checkpoint inhibitor (ICI) combination therapy as first-line (1L) treatment in metastatic renal cell carcinoma (mRCC).

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## Background

The efficacy of ICI-combination as 1L therapy across different subgroups remains unclear for patients with mRCC. Therefore, we assessed the efficacy of ICI-combination by different clinically relevant subgroups using evidence from up-to-date clinical trials.

## Methods

MEDLINE and EMBASE were systematically searched to identify phase II/III randomized controlled trials(RCTs) assessing ICI-combination therapies in 1LmRCC and reporting efficacy by age (< 65 y; >65 y), gender ,PDL1 receptor status,

Subgroup	Overall survival		Progression-free survival		Objective response rate	
	HR [95% CI]	P-value*	HR [95% CI]	P-value*	OR [95% CI]	P-value*
<b>IMDC Risk Category</b>						
Favorable	0.98 [0.76-1.27]	0.01	0.76 [0.49-1.17]	0.33	1.61 [0.63-4.14]	0.33
Intermediate/Poor	0.67 [0.60-0.75]		0.59 [0.47-0.75]		2.63 [2.05-3.37]	
<b>Age</b>						
< 65 years	0.64 [0.57-0.73]	0.02	0.53 [0.41-0.69]	0.28	1.85 [0.66-5.18]	0.76
≥ 65 years	0.84 [0.69-1.01]		0.66 [0.49-0.89]		2.23 [1.17-4.24]	
<b>Gender</b>						
Male	0.77 [0.69-0.86]	0.09	0.56 [0.41-0.74]	0.54	2.71 [2.06-3.58]	0.93
Female	0.64 [0.53-0.77]		0.63 [0.48-0.83]		2.76 [1.99-3.84]	
<b>PDL1 Status</b>						
Positive	0.74 [0.64-0.85]	0.84	0.57 [0.45-0.72]	0.53	2.63 [1.64-4.22]	0.73
Negative	0.72 [0.62-0.85]		0.65 [0.47-0.91]		2.39 [1.83-3.11]	
Abbreviations: HR - hazard ratio; OR: odds ratio; CI: confidence interval *P-value of interaction						

and international mRCC database consortium (IMDC) risk categories (favorable;intermediate/poor [IP]). Main efficacy outcomes included overall survival (OS), progression-free survival (PFS),and objective response rate (ORR). A random-effects meta-analysis was conducted. A p-value of interaction <0.1 indicated statistical significance.

## Results

In a metanalysis of 6 trials with 5121 patients, ICI-combination improved OS compared to sunitinib in the overall population. ICI-combination therapy significantly improved OS compared to sunitinib in IP risk (hazard ratio: 0.67; 95% CI: 0.60-0.75) but not in the favorable risk (0.98;0.76-1.27). There was statistically significant effect modification by IMDC risk (P:0.01). Younger patients were observed to derive a significant OS benefit with ICI-combination compared to older (< 65 years: 0.64; 0.57-0.73; >65 years: 0.84;0.69-1.01; P:0.02). Females appeared to derive greater benefit with ICI-combination therapy (females: 0.64;0.53-0.77; males: 0.77;0.69-0.86; P:0.09). No statistically significant difference

in benefit was observed for PDL +ve compared to PDL -ve (PDL +ve: 0.74;0.64-0.85; PDL1-ve: 0.72;0.62-0.85; P:0.84). The results for PFS and ORR are provided in Figure. Post-hoc sensitivity analyses excluding CheckMate 274 trial showed a consistent direction of results. However, no significant effect modification was observed by age and sex.

## Conclusions

Factors such as intermediate-poor risk disease, younger age (< 65 years), and female gender may predict a higher magnitude of benefits for patients with mRCC contemplating treatment with immunotherapy combination with tyrosine kinase inhibitors.

## Keywords

mRCC , ICI Combination therapy in mRCC

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## Mapping the distribution of brain metastases in renal cell carcinoma (RCC) and association with clinical features

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### Background

Brain metastases (BrM) are frequently observed in patients with metastatic RCC (mRCC), often presenting with hemorrhagic BrM. However, the distribution patterns of BrM in RCC and their associations with clinical outcomes are not well-understood. Here we present a detailed analysis of the distribution of BrM in a surgically resected BrM RCC cohort, with a focus on clinically relevant associations.

### Methods

A retrospective analysis was performed on patients with mRCC who underwent craniotomy for BrM at our institution. We reviewed gadolinium-enhanced MRI brain and CT head (if available) at the time of BrM diagnosis. We assessed the presence of hemosiderin on MRI or hemorrhage on CT in the BrM lesion and marked each at their corresponding anatomic location. Public brain atlases were used for distribution analysis. We calculated overall survival (OS) from the date of BrM diagnosis and CNS progression-free survival (CNS-PFS) from the date of craniotomy.

### Results

A total of 67 BrMs were analyzed from 46 patients. Of these, 23 had synchronous BrM, 9 had two, and 6 with three concurrent BrM. Most patients were male (n=35, 76%) with a median age of 63 years at diagnosis. Primary histology was clear cell in 42 patients (91%), and 10 patients (22%) had renal vein thrombosis (RVT) at primary diagnosis. IMDC scores at the time of BrM diagnosis were favorable in 20% of the cases, intermediate in 60%, and poor in 20%. Hemosiderin (MRI) or hemorrhage (CT) was seen in 82% and 80% of BM, respectively. 55% of lesions were left-sided, 45% frontal, 21% parietal, 18% occipital, 7% temporal, and 6% cerebellar. Posterior circulation and posterior cerebral artery territories contained 42% and 34% of BrM despite supplying only 30% and 16% of the brain volume, respectively. Patients with solitary BrM had higher Karnofsky performance status

(p=0.006) and lower disease burden (p=0.004) than those with multiple BrM. Shorter CNS-PFS was seen in association with hemosiderin on MRI (log-rank p=0.04) and RVT in the primary specimen (log-rank p=0.002). IMDC risk scores (log-rank p<0.001) predicted OS. On multivariable OS analysis, BrM lateralized to the right (HR 2.6, p=0.06), hemosiderin in BrM (HR 6.5, p=0.02) and IMDC risk groups (intermediate HR 2.9, poor risk HR 25.6, p<0.04) were independent prognostic factors for shorter OS.

### Conclusions

The above-presented lesion mapping method shows clinically relevant findings in RCC BrM. Tumoral hemosiderin deposits appear to be a potential parameter to predict outcomes in RCC BrM, which deserve further study and validation.

### Keywords

renal cell carcinoma, brain metastases

56

## Single Cell Spatial Analysis Identifies Enriched Transcriptomic and Spatial Patterns Among Immunotherapy Resistant Clear Cell Renal Cell Carcinoma Tumors

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### Background

Immunotherapy (IO) treatments for clear cell renal cell carcinoma (ccRCC) have been developed to leverage patients' immune system against malignant tissues and have yielded improvements in patient survival. However, many tumors become resistant following IO. Hence, understanding how IO

changes the tumor immune microenvironment (TIME) leading to resistance in ccRCC is critical.

## Methods

To identify differences in the ccRCC TIME after IO treatment, we collected tissue samples from patients with primary treatment naive ccRCC (pre-IO) ( $N = 6$ ) and resistant ccRCC tissue (post-IO) ( $N = 4$ ) after IO treatment. Tumor tissue samples were assayed using NanoString's CosMx spatial transcriptomics platform. Cells with fewer than 20 identified transcripts were removed while others were phenotyped using InSituType based on Kidney Cell Atlas. Analyses were completed to explore the cell type abundance and spatial architecture differences in the TIME between pre- and post-IO samples.

## Results

Investigation of cell type abundance found increased numbers fibroblast cells and a decreased number of non-classical monocytes following IO ( $p = 0.016$  and  $p = 0.018$ , respectively). Collapsing gene expression at the FOV level, we found no expression difference of ADORA2A (adenosine receptor; FDR = 0.4) which is being targeted for next generation therapies. Differential gene expression demonstrated enrichment of gene transcripts involved in the epithelial mesenchymal transition (EMT) gene set (FDR < 0.001) among 11 specific cell types in post-IO tumor, including tumor cells, M2 macrophages, and cytotoxic T cells. This indicates a shift in the TIME to increase tumorigenicity and cancer stem cell propagation. Additionally, we found tumor cells post-IO to be significantly enriched in genes belonging to IL-6/JAK/STAT3 (IL-6) signaling (FDR =  $8.9 \times 10^{-11}$ ) indicating transition to cell survival and further proliferation. In terms of the spatial organization, there was significant spatial aggregation of cells showing enrichment in oxidative phosphorylation, PI3K/AKT/MTOR signaling, and TGF-beta signaling in tumors after IO treatment. We also identified significant spatial aggregation of cells expressing EMT and IL-6 gene sets in post-IO tumors. In a spatial gradient analysis to detect changes in gene expression in cytotoxic T cells as their distance to M2 macrophages changed in both pre- and post-IO samples, we found that IFNA1 and ZFP36 expression gradients were significantly different post-IO compared to pre-IO ( $p = 0.003$  and  $0.004$ , respectively), whereby IO samples showed higher expression as distance decreased which is indicative of active cytotoxic T cells after antigen recognition.

## Conclusions

Significant enrichment of EMT and IL-6 gene sets indicates a potential link to ccRCC IO resistance. EMT is largely noted as being involved in the metastatic process of solid tumors and the IL-6 gene set shows evidence that ccRCC tumor cells are becoming resistant to DNA damage caused by therapy. Further, the immune cells show interactions, such as the activation of

cytotoxic T cells near M2 macrophages, providing insight into how colocalization of these cell types relate to poorer patient outcomes previously described. Further studies are needed to validate our findings and confirm associations with tumors becoming resistant to immunotherapy.

## Keywords

single cell spatial, spatial transcriptomics, clear cell renal cell carcinoma

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# In Pursuit of the IMDC: Turkish Oncology Group Kidney Cancer Consortium Database (TKCC)

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## Background

Clinical trials are the main part of evidence-based medicine. However, some concerns due to the selected patient groups in clinical trials have revealed the necessity of evaluating real-world data (RWD). Especially in oncology, the importance of RWD is increasing over time. The International Metastatic Renal Cell Carcinoma (mRCC) Database Consortium (IMDC) is one of the excellent examples of the source for the RWD.

## Methods

Turkish Oncology Group Kidney Cancer Consortium (TKCC) includes patients from 14 centers in Türkiye. It has more than 1,000 patients with mRCC. The number of patients is increasing with the inclusion of new centers. Patients' data is constantly updated.

## Results

As of December 2022, 1,001 patients were included in the TKCC database. Most of the patients were male (73.9%). The median age was 60 years (interquartile range: 54-67). Approximately three out of four patients (75.3%) had undergone nephrectomy. The rate of patients was 9.1%, 35.7%, 19.2% in the IMDC favorable, intermediate, and poor risk groups. The median overall survival was 38.4, 29.8, and 7.2 months in the IMDC favorable, intermediate, and poor risk groups, respectively. ( $p < 0.001$ ). 10.5% of all patients had sarcomatoid features. The rate of patients was 90.6%, 56.4%, 23.8%, 7.9%, and 2.5% in the 1st, 2nd, 3rd, 4th, and 5th lines of the treatment in the metastatic setting.

## Conclusions

IMDC is the role model for many researchers interested in genitourinary oncology. We created our national mRCC database using the way paved by the IMDC.

## Keywords

kidney cancer, cohort, real world data

## 86

# Identification of molecular vulnerabilities in aggressive renal cell carcinoma by a small molecule

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## Background

Among patients with renal cell carcinoma (RCC), one out of three individuals experience metastatic spread. The average survival period for metastatic RCC patients is 6 to 12 months, with a 2-year survival rate ranging from 10% to 20%. Metastatic RCC patients are treated with systemic therapies, including targeted and immunotherapy. Currently, seven FDA-approved drugs are being applied in clinical treatment as monotherapy or combination. However, despite the availability of various treatment approaches, the prognosis remains poor. Therefore, developing effective therapeutic agents is an urgent need for RCC patients.

## Methods

To identify chemical compounds that can inhibit the growth of metastatic RCC cells, we performed a small molecule compound screening with 240 small molecule compounds never tested on metastatic RCC cells. Two metastatic RCC cell lines, Caki-1 and ACHN were used, and an immortalized normal proximal tubular cell line, HK-2 was used as a control. We used a cell viability assay to select compounds specifically inhibiting the growth of metastatic RCC cells compared to HK-2 cells. Various molecular biology assays were performed to understand the molecular mechanisms of the selected compound. First, the cell cycle was analyzed using PI staining. Second, the expression of proteins related to cell cycle and death was detected by immunoblot assay. Lastly, we utilized a high-throughput sequencing to profile mRNA expression and a proteomics technique, thermal proteome profiling (TPP) assay, to find proteins that bind with the selected compound. Our approach will reveal the mechanisms of action of the selected compound and provide insight into developing more potent anti-cancer compounds.

## Results

Our compound screening effort identified a new small molecule named CBA that significantly inhibited the growth of Caki-1 or ACHN cells compared to HK-2 cells. IC<sub>50</sub> of Caki-1 or ACHN was significantly higher than it of HK-2. We found that CBA treatment significantly reduced the proliferation of Caki-1 or ACHN in colony-forming assay. The decreased viability and proliferation of RCC cells by CBA were caused

by de-regulating the cell cycle of RCC cells. We confirmed that CBA increased two cell cycle checkpoint regulators, phospho-CHK1 and phospho-CHK2. However, CBA did not affect the cell cycle of HK-2 cells. In addition, CBA increased the expression of cleaved-caspase 3 and annexin-V positive cells, suggesting induced apoptosis. Our findings suggested that CBA specifically interrupts the growth of RCC cells by decreasing proliferation and inducing apoptosis. In addition, we found that CBA can de-regulate the expression of iron-related proteins. CBA treatment can synergize the growth inhibition of metastatic RCC cells with an iron-chelating agent. Our pilot studies found that mRNA-sequencing analysis revealed significant changes in the expression of genes in DNA repair, hypoxia and G2/M checkpoint, and metabolic pathways upon treatment with CBA. Also, TPP assay demonstrated de-regulated expression of proteins associated with various metabolic pathways, including oxidative phosphorylation and ROS.

## Conclusions

In this study, a new chemical compound, CBA, which showed specific anticancer effects on metastatic RCC cell lines was discovered. The mechanism of action of CBA may tackle multiple cellular and molecular processes based on our findings, which will provide insight into discover new therapeutic vulnerabilities in metastatic RCC cells. Currently, we aim to validate the translational potential of CBA and develop/test new chemical analogs of our compound using patient-derived xenografts and cell-line derived xenografts.

## Keywords

Metastatic renal cell carcinoma, Anti-cancer therapy, Cell cycle arrest, High-throughput sequencing

## CDMRP DOD Funding

yes

**89**

## Preclinical investigation of PSMA-based theranostics for renal cell carcinoma

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## Background

Metastatic clear cell renal cell carcinoma (mccRCC) is poorly responsive to conventional therapies, including most chemotherapeutic drugs and radiation therapy. Targeted therapies against the vascular endothelial growth factor (VEGF) pathway, [e. g., tyrosine kinase inhibitors (TKIs)] and immunotherapy with checkpoint blockade have demonstrated improved overall survival, although such treatment is often at the expense of significant toxicities, and only a minority of patients have durable responses. Recently, others and we have demonstrated the high sensitivity and specificity in mccRCC of positron emission tomography (PET) compounds that target prostate-specific membrane antigen (PSMA), such as [F-18]-DCFPyL and [Ga-68]-PSMA-11. Several recent studies revealed that high levels of PSMA expression are prognostic of unfavorable clinical outcomes in patients with mccRCC. We aim to develop safe and effective alpha-particle-emitting radiopharmaceutical therapy (alpha-radiotherapy) that can be paired with PSMA PET imaging for rational selection of patients with treatment planning and therapy response monitoring of mccRCC. PSMA alpha-radiotherapy would be a novel precision medicine approach to treating this disease. The highly lethal DNA damage and immunomodulatory effect induced by alpha particles will be complementary/synergistic with currently available methods of treating mccRCC. We hypothesize that the short-range and high-linear energy transfer radiation of alpha particles from [Ac-225]-labeled PSMA-targeted compounds are ideal for killing neovascular endothelial cells of mccRCC. Many of the commonly used drugs for this disease target angiogenesis, suggesting that the neovasculature is a highly relevant target for alpha-radiotherapy for mccRCC. We have selected 225Ac-L1 based on our recent structure-activity relation studies that led to a clinical trial in patients with metastatic castration-resistant prostate cancer (NCT03490838). 225Ac-L1 has also been evaluated in one-year-long acute and chronic radiotoxicity in healthy immunocompetent mice. We have also developed a bivalent compound 225Ac-bi-L1 to enhance the effectiveness of 225Ac-L1. Here we will present the preclinical performance of the agents and relevant validation studies in primary human RCC specimens and animal models.

## Methods

An autoradiography-based assay was developed using our previous lead agent, <sup>177</sup>Lu-L1, to evaluate PSMA expression in RCC specimens and also characterized by immunohistochemistry (IHC) and histology. Next, PSMA protein and gene expression of the specimens and 3 standard RCC lines (SK-RC-52, Caki 1, and 786-O) and mouse cell line RENCA in subcutaneous and orthotopic xenografts were assayed. Cell uptake studies were done at 1 and 2 h post-injection. Immunodeficient NOD-SCID gamma (NSG) mice were used for orthotopic tumor implantation of the human RCC cell lines. For RENCA, immunocompetent Balbc mice were used. Time-dependent tissue biodistribution studies were done for up to 48 h for <sup>225</sup>Ac-L1 and <sup>225</sup>Ac-bi-L1, respectively, after intravenous administration via tail-vein. A preliminary therapy study was conducted using the RENCA model.

## Results

Low PSMA expression levels were noted in the studied cell lines and RCC specimens. The cellular uptake and blocking study confirmed the non-specificity of the PSMA-targeted probe binding to SK-RC-52, 786-O, Caki-1, and RENCA cells. In the orthotopic models, both compounds showed fast clearance from the organs. <sup>225</sup>Ac-bi-L1 displayed 2-fold

higher tumor uptake at 2 h and displayed high retention in the tumor, and was associated with high kidney uptake up to 24 h. A receptor-blocking study was done and revealed PSMA-specific uptake. The staining of tumor tissue sections with CD31- and PSMA-specific antibodies visualized the tumor-associated blood vessels and PSMA expression on endothelial cells in the orthotopic tumor tissues and human RCC tissues, confirming endothelial PSMA expression. Moderate tumor growth control was noted for the RENCA model using <sup>225</sup>Ac-L1.

## Conclusions

Our initial validation studies revealed relatively low PSMA levels in the preclinical RCC model. Based on the initial treatment studies, future studies will be focused in combination with immunotherapies and relevant other standard-of-care therapeutic options.

## Keywords

Radiopharmaceutical therapy, alpha-particle, theranostics

## CDMRP DOD Funding

yes

# Trials in Progress

**63**

## Zanzalintinib in combination with immune checkpoint inhibitors: Design of the renal cell carcinoma expansion stage cohorts in STELLAR-002

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### Background

Zanzalintinib (XL092) is a novel, potent, orally bioavailable small molecule inhibitor of several receptor tyrosine kinases, including VEGFR2, MET, and the TAM kinases AXL, and MER. These receptor tyrosine kinases are implicated in several oncogenic processes, including tumor angiogenesis, proliferation, invasion, and metastasis. MET and AXL are known to play important roles in the development of resistance to antiangiogenic therapies. In addition, drugs targeting the TAM kinases are thought to promote an immune-permissive environment, potentially enhancing responses to immune checkpoint inhibitors (ICIs). This hypothesis has been confirmed in preclinical murine tumor models in which zanzalintinib in combination with an anti-PD-1 agent showed significantly greater antitumor and immunomodulatory activity, as well as a greater survival benefit, when compared with either agent alone (Hsu J, et al. *Mol Cancer Ther* 2023). In a phase 1 first-in-human study, zanzalintinib alone or in combination with atezolizumab showed a manageable safety profile and a half-life of 16–22 hours for zanzalintinib supporting once-daily dosing. The combination also showed promising clinical activity in patients with advanced or metastatic solid tumors, including renal cell carcinoma (RCC; Sharma MR, et al. *ESMO 2022:Abs 481P*). The encouraging

preclinical and clinical activity support the evaluation of zanzalintinib in combination with ICIs in clinical studies, including STELLAR-304 (NCT05678673), a randomized study that is exploring the efficacy and safety of zanzalintinib in combination with nivolumab in patients with non-clear cell RCC (nccRCC). STELLAR-002 (NCT05176483) is another ongoing study assessing the safety and preliminary efficacy of zanzalintinib in multiple ICI combinations in patients with advanced solid tumors. Presented here is the study design of the cohort-expansion stage for patients with RCC in STELLAR-002.

### Methods

STELLAR-002 is a multicenter, open-label, phase 1b trial consisting of a dose-escalation stage and a tumor-specific cohort-expansion stage. The study is enrolling adults who have a cytologically or histologically confirmed solid tumor that is unresectable, locally advanced, or metastatic. In addition, patients with RCC enrolled in the expansion phase must have measurable disease per RECIST v1.1 as assessed by the investigator. The recommended doses of zanzalintinib plus nivolumab, zanzalintinib plus nivolumab and ipilimumab, and zanzalintinib plus nivolumab and relatlimab are being established in the dose-escalation stage of the study. The study includes three dose-expansion cohorts for patients with RCC: first-line clear cell RCC (ccRCC; Cohort 1), second-line ccRCC after 1 prior ICI-based combination therapy (Cohort 2), and first-line nccRCC (Cohort 6). The following regimens are available options for randomization at the recommended dose depending on the cohort: single-agent zanzalintinib (Regimen A), zanzalintinib plus nivolumab (Regimen B), zanzalintinib plus nivolumab and ipilimumab (Regimen C), or zanzalintinib plus nivolumab and relatlimab (Regimen D). Patients in Cohort 1 may be randomized to Regimens B, C, or D, patients in Cohort 2 to Regimens A, B, or D, and patients in Cohort 6 to Regimens A or B. The primary objectives of the cohort-expansion stage are objective response rate per RECIST v1.1 (as assessed by the investigator) and incidence and severity of adverse events, including serious and immune-mediated events. The trial is ongoing and enrolling patients from 13 countries in North America, Europe, and the Asia-Pacific region.

### Keywords

Zanzalintinib, nivolumab, relatlimab, renal cell carcinoma

**68**

## LITESPARK-024: A randomized phase 1/2 study of belzutifan with or without palbociclib for previously treated advanced renal cell carcinoma

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### Background

Immunotherapy is a standard-of-care first-line treatment for advanced clear cell renal cell carcinoma (ccRCC). However, many patients will develop resistance to first-line therapy, and effective second- and later-line treatment options are therefore needed. The von Hippel-Lindau (VHL) gene is inactivated in approximately 90% of patients with RCC and results in the constitutive activation of hypoxia-inducible factor 2α (HIF-2α), a key oncogenic driver in RCC. Belzutifan, a first-in-class HIF-2α inhibitor, has demonstrated antitumor activity with manageable safety in previously treated patients with advanced ccRCC. The cyclin-dependent kinase (CDK) pathway is also associated with poor clinical outcomes in ccRCC. In RCC cell lines, the CDK 4/6 inhibitor palbociclib inhibited cell growth. CDK 4/6 inhibition has shown synergistic antiproliferative effects with HIF-2α inhibition in HIF-2α-dependent VHL -/- RCC cell lines. Palbociclib could therefore potentially enhance the efficacy of belzutifan as combination therapy for previously treated pts with advanced RCC.

### Methods

LITESPARK-024 is a 2-part, open-label, multicenter, phase 1/2 randomized study (NCT05468697). Part 1 is intended to establish the recommended phase 2 dose (RP2D) of belzutifan plus palbociclib using a modified toxicity probability interval design. After the RP2D is established, part 2 will directly compare the safety and efficacy of belzutifan monotherapy with belzutifan + palbociclib in patients with advanced ccRCC. In both parts, patients with measurable disease per RECIST v1.1, a Karnofsky Performance Status score of ≥70%, and histologically confirmed unresectable stage IV RCC with a clear cell component and disease progressing on or after receiving at least 2 systemic treatments (both an anti-PD-1/L1 monoclonal antibody and a VEGF receptor-targeted tyrosine kinase inhibitor, in sequence or in combination) will

be enrolled. Up to 30 patients will be enrolled into 3 dose groups in part 1 and will receive belzutifan 120 mg once daily plus palbociclib (75, 100, or 125 mg) daily for 21 consecutive days followed by 7 days off. In part 2, approximately 150 patients will be randomly assigned 2:1 to receive belzutifan 120 mg once daily plus palbociclib RP2D (21 consecutive days/7 days off) or belzutifan 120 mg once daily. Patients will be stratified by International metastatic RCC Database Consortium risk (0 vs 1-2 vs 3-6) and sarcomatoid histology (yes vs no) at randomization in part 2. The primary end point for part 1 is to assess dose-limiting toxicities and adverse events and to determine the RP2D of belzutifan plus palbociclib. The primary end point for part 2 is objective response rate per RECIST v1.1 by investigator assessment. Secondary end points for part 2 are clinical benefit rate, duration of response, and progression-free survival per RECIST v1.1 by investigator assessment; overall survival, and safety and tolerability. Enrollment began in July 2022.

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### Keywords

Belzutifan; CDK 4/6 inhibitor; palbociclib; HIF-2α inhibitor; renal cell carcinoma

### CDMRP DOD Funding

no

**78**

## SAMETA: A Phase III study of savolitinib + durvalumab vs sunitinib and durvalumab monotherapy in patients with MET-driven, unresectable, locally advanced/metastatic papillary renal cell carcinoma

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### Background

Papillary renal cell carcinoma (PRCC) is the most common subtype of non-clear cell renal cell carcinoma (RCC) and accounts for 10–15% of RCCs. Many PRCC cases are MET-driven, characterized by genomic abnormalities resulting in dysregulation of the MET signaling pathway, making these abnormalities a potential therapeutic target for treatment.

The estimated prevalence of MET-driven status is 35–40% in PRCC. Savolitinib is an oral, potent and highly selective MET tyrosine-kinase inhibitor (TKI) demonstrating preliminary clinical activity in advanced solid tumors, including in MET-driven PRCC, defined as presence of any of the following molecular alterations, in the absence of co-occurring fumarate hydratase mutations: chromosome 7 gain, MET amplification, MET kinase domain variations, or hepatocyte growth factor amplification. In the Phase III SAVOIR study, in PRCC, savolitinib monotherapy showed encouraging efficacy vs the multi-targeted TKI, sunitinib. In addition, non-clinical studies suggest a possible synergistic anti-tumor effect of MET-inhibitors and programmed cell death-ligand (PD-L1) inhibitors, such as durvalumab; emerging data from the Phase I/II CALYPSO study investigating savolitinib plus durvalumab shows a notable efficacy signal in patients with MET-driven PRCC. Following these findings, the SAMETA study (NCT05043090) is designed to evaluate the efficacy and safety of savolitinib in combination with durvalumab vs sunitinib and durvalumab monotherapy in PRCC.

## Trial Schema

In this open-label, three-arm, multi-center, Phase III study, adult patients with unresectable, MET-driven and locally advanced/metastatic PRCC are eligible. An estimated 200 patients (25 countries, 165 centers) will be randomized in a 2:1:1 ratio into three treatment arms (A-C) with stratification by International metastatic RCC database consortium risk group & PD-L1 expression tumor status. Arm A: oral savolitinib 600 mg once daily plus intravenous durvalumab 1500 mg every 4 weeks; Arm B: oral sunitinib 50 mg once daily for 4 consecutive weeks, followed by a sunitinib-free interval of 2 weeks every 6 weeks; Arm C: intravenous durvalumab 1500 mg every 4 weeks. Study treatment continues until RECIST 1.1 disease progression, or another discontinuation criterion is met. The primary endpoint is progression-free survival (by Blinded Independent Central Review; Response Evaluation Criteria in Solid Tumors v1.1). Secondary endpoints include overall survival, objective response rate and duration of response. Safety (adverse events, vital signs, echocardiograms, hematology and biochemistry parameters) will also be reported.

## Current status

The first patient was enrolled onto the study on 28 October 2021. At the early enrollment stage, during the biomarker pre-screening, approximately 29% of patients with PRCC who submitted a sample had eligible MET-driven status and of these patients with MET-driven PRCC, approximately 84% also met other eligibility criteria and were randomized.

## Keywords

PRCC, metastatic, MET-driven, MET-tyrosine kinase inhibitor

80

## A Phase 1/2, Open Label Dose-escalation and Expansion Trial of NKT2152, an Orally Administered HIF2 $\alpha$ Inhibitor, to Investigate Safety, PK, PD and Clinical Activity in Patients with Advanced ccRCC

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## Background

Inactivation of the VHL gene leading to aberrant HIF2 $\alpha$  activity is nearly universal in clear cell renal cell carcinoma (ccRCC). NKT2152 is a novel, potent, selective orally available HIF2 $\alpha$  inhibitor optimized for enhanced PK exposure and sustained target inhibition which has demonstrated robust activity in both ccRCC cell line-derived and patient-derived xenograft RCC and other solid tumor models.

This is a Phase 1/2 open label, multicenter, first in human study of NKT2152 in adults with advanced clear cell renal cell carcinoma (ccRCC) (NCT05119335). In Phase 1, up to ~60 patients will be enrolled according to a 3+3 design with backfill as permitted by the Safety Review Committee. The primary objective of phase 1 is to determine the recommended dose for expansion (RDE) based on the totality of clinical data.

Phase 2 will enroll ~50 additional patients with the primary objective of determining investigator-assessed by RECIST v1.1. Key secondary objectives include safety, tolerability, PD effects, progression free survival, duration of response, and disease control rate. Exploratory objectives include evaluation of biomarkers predictive of tumor response.

Adults who have advanced ccRCC without available standard therapy), ECOG PS 0-2, with measurable disease per RECIST 1.1 are eligible. Patients who have had prior HIF2 $\alpha$  inhibitors,

require supplemental oxygen, and with significant cardiac disease are excluded. Tumor assessments by CT or MRI are conducted every 8 weeks until 48 weeks, then every 12 weeks thereafter. Adverse events will be monitored and graded in severity using CTCAE v5.0. The Phase 1 study is currently actively accruing in the United States with Phase 2 dose expansion anticipated to start in Q3, 2023.

### Keywords

HIF2alpha, clear cell, ccRCC, Phase 1

**81**

## NRG-GU012: Randomized Phase II Stereotactic Ablative Radiation Therapy (SABR) for Metastatic Unresected Renal Cell Carcinoma (RCC) Receiving Immunotherapy (SAMURAI)

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### Background

It is estimated that a significant number of patients presenting with metastatic RCC are either ineligible, not recommended for, or refuse surgery. There remains no well-established method for addressing the primary tumor in such patients. Stereotactic ablative radiotherapy (SABR) is a highly effective modality for the treatment of the primary tumor in RCC. Our study seeks to evaluate SABR as an alternative approach to treat the primary tumor in patients with metastatic RCC receiving immunotherapy, who are not recommended for surgery, or who decline surgery. The primary objective of the study is to determine whether the addition of SABR to the primary tumor in combination with immunotherapy improves outcomes compared to immunotherapy alone in patients with metastatic, unresected, renal cell carcinoma (RCC). The primary endpoint is nephrectomy and radiographic progression-free survival (nrPFS) with progression determined as per iRECIST criteria. Patients are eligible if they are not recommended for upfront surgery, have a primary tumor treatable with SABR, and are candidates for immunotherapy. Immunotherapy is physician's choice and will include either two different immunotherapy regimens (IO-IO) or immunotherapy in combination with a VEGF inhibitor (IO-VEGF).

### Methods

240 patients will be randomized in a 2:1 ratio favoring SABR plus immunotherapy (n=160) as compared to standard of care immunotherapy alone (n=80). The sample size will provide 90% power at a one-sided alpha level of 0.05 to detect a hazard ratio for nrPFS of 0.61, i.e., a 40% reduction in the rate of events. One-year PFS in the nivolumab plus ipilimumab arm was 50% (n=550, intermediate/poor risk population). Checkmate 9ER (Choueiri 2021) showed a one-year PFS rate in patients receiving nivolumab plus Cabozantinib (n=323) of 45-58% (intermediate/poor risk population). We have therefore estimated a one-year nrPFS rate of 50% in the control (immunotherapy alone) arm for SAMURAI. We expect nephrectomies to contribute only a small fraction to the total nrPRS events. To detect a hazard ratio of 0.62, which corresponds under exponential distribution assumptions to an absolute 15% improvement in one-year nrPFS from 50% in the control group to 65% in the experimental arm (SABR plus immunotherapy), a total sample size of 240 patients will be enrolled. This design will provide 90% power, based on a one-sided logrank test at the 0.05 alpha level, with three years of accrual and two further years of follow-up. The expected number of nrPFS events is E=175. The calculations allow for a 10% non-eligible/early dropout rate. The trial is currently open and accruing through the NRG oncology cooperative group (NCT05327686).

### Keywords

Radiation therapy, primary renal tumor, immunotherapy, SABR

**83**

## STARLITE 1: Phase 1b/2 study of combination 177Lu girentuximab plus cabozantinib and nivolumab in treatment naïve patients with advanced clear cell RCC

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<sup>1</sup>MD Anderson Cancer Center, <sup>2</sup>Telix Pharmaceuticals

### Background

Complete response (CR) is still a rare event in patients with advanced clear cell renal cell carcinoma (ccRCC). The combination of nivolumab plus cabozantinib was recently

approved for the first-line treatment of ccRCC based on the CheckMate 9ER phase 3 study demonstrating improved progression-free survival (PFS) and objective response rate (ORR) in comparison to sunitinib. However, the CR rate was only 9%. Since the anti-tumor effects of immune checkpoint inhibitors are dependent on the presence of activated tumor-infiltrating T cells, drugs that could synergize with T cells' anti-tumor activity can allow us to improve CR rates. Activation of the cGAS-STING pathway, the master regulator of anti-tumor immunity which is induced by radiation-induced DNA damage, is one promising mechanism that has been investigated. Many studies have shown that radiation treatment augments immune checkpoint inhibition. However, it is not always possible to radiate all metastatic lesions. Therefore, targeted peptide receptor radionuclide therapies (PRRT), have been developed by conjugating radioisotopes to receptor binding analogs targeting specific cancer cell surface proteins, thereby delivering targeted radiation to cancer cells in the body with minimal damage to surrounding healthy cells. <sup>177</sup>Lu girentuximab is the first antibody-radioisotope designed for ccRCC, targeting carbonic anhydrase IX-expressing cells, which includes >90% of ccRCC. It has been tested in metastatic ccRCC as a single agent and shown to be safe and effective in stabilizing disease in 57% of pts. In this study, we hypothesize that <sup>177</sup>Lu girentuximab-induced DNA damage will potentiate the STING pathway, and this activation will synergize with nivolumab and cabozantinib to promote trafficking and infiltration of activated T cells to tumors and achieve higher CR rates.

## Methods

Up to 100 patients with treatment naïve, biopsy-proven ccRCC with adequate organ/marrow function with  $\geq 1$  evaluable lesion by RECIST 1.1 will be enrolled. A 5-patient safety lead-in will evaluate myelosuppression. Ongoing safety, and futility monitoring will employ a Bayesian approach. The sample size was chosen for reasonable operating characteristics to distinguish a CR rate (primary endpoint) of 18% as better than 9% using a beta (0.09, 0.91) prior. Secondary endpoints are objective response, PFS by RECIST 1.1, and overall survival. <sup>177</sup>Lu-girentuximab 1480 MBq/m<sup>2</sup> (61% of single agent MTD) will be administered every 12 weeks for up to 3 treatment cycles. Starting with the second cycle, nivolumab and cabozantinib will be added at standard dose. To explore the effects of the combination therapy on inducing activated T cell infiltration, patients will undergo pre/post-treatment PET scan with [18F]F-AraG radiotracer as well as biopsies for single cell, spatial transcriptomics and proteomics studies. This is an investigator initiated trial. Telix Pharmaceuticals provided drug and funding support. Also supported by DOD grant W81XWH-22-1-0456

## Keywords

brain metastasis, renal cell carcinoma, pembrolizumab, lenvatinib

## CDMRP DOD Funding

yes

87

## Phase 1b/2 trial of Ipilimumab, Nivolumab, and Ciforadenant (INC) (adenosine A2a receptor antagonist) in first-line advanced renal cell carcinoma

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## Background

Ciforadenant is an investigational immunotherapeutic small molecule that selectively and reversibly binds adenosine 2A receptors (A2ARs) on T lymphocytes and other cells of the immune system. RCC metabolism is known to be highly glycolytic with a need to export adenosine triphosphate (ATP) to allow for continued proliferation of cancer cells. In the tumor microenvironment (TME) ATP is hydrolyzed to adenosine by CD39/CD73. Adenosine has immune suppressive effects on the TME through the A2 adenosine receptor (A2AR) including decreased T cell activation and proliferation (Ohta et al., 2009). Blocking A2AR on tumor associated myeloid cells such as macrophages, dendritic cells, and myeloid derived suppressor cells in preclinical mouse models have shown enhanced tumor killing (Cekic et al 2014). Preclinical studies show that the addition of ciforadenant to CTLA4 and PD1 blockade shows enhanced efficacy and in some cases elimination of the established tumors (Willingham et al., 2018). Recently, in a first in human study, the A2AR antagonist ciforadenant was found to be safe and showed activity as monotherapy in RCC patients with refractory disease following multiple lines of therapy showing a median progression free survival (mPFS) of 4.1 months (Fong et al., 2019). In the same study, the addition of ciforadenant to PD-L1 blockade with atezolizumab was shown to be safe and demonstrate activity with mPFS of 5.8 months and OS probability at 25 months of 90%. We

hypothesize that the addition of the A2aR antagonist, cregorafenib, to the combination of ipilimumab and nivolumab will favorably modulate metabolic adenosine signaling and the myeloid compartment to enhance patient response by reducing immunosuppression.

## Methods

INC is a Phase 1b/2 single-arm, multicenter study to assess safety and efficacy of the combination of ipilimumab, nivolumab, and cregorafenib in the frontline treatment of patients with advanced clear cell renal cell carcinoma. This study is being conducted through the Kidney Cancer Clinical Trial Consortium. Eligibility criteria include untreated advanced clear cell RCC, ECOG PS 0 or 1, measurable disease by RECIST 1.1 and adequate organ function and excludes patients who have previously received immunotherapy. The study will include a lead-in safety phase 1b portion with enrollment of four to eight patients treated with cregorafenib 100 mg BID, nivolumab 3 mg/kg and ipilimumab 1 mg/kg (IV) q3 weeks. If the rate of patients with a dose limiting toxicity is more than 45% another four patients will be enrolled at reduced dose of cregorafenib 50 mg BID, nivolumab 3 mg/kg and ipilimumab 1 mg/kg IV q3 weeks. If continuing on trial, patients will receive nivolumab 480 mg infusion and cregorafenib beginning Cycle 2, Day 1 q4 weeks. In the Phase 2 dose-expansion portion of the study, 42 additional patients (total 50) patients consisting of untreated advanced clear cell renal cell carcinoma will be treated at the RCD determined in the Phase 1b portion of the study.

The primary objective is to determine the safety and tolerability and to assess the depth of response (>50% by RECIST 1.1 Eisenhauer, 2009) based on a Bayesian design in patients with advanced RCC treated with ipilimumab, nivolumab, and cregorafenib. Secondary objectives will estimate the objective response rate (ORR), duration of response (DOR) progression free survival (PFS), progressive disease (PD) rate, and irAE rate of ipilimumab, nivolumab, and cregorafenib combination in untreated advanced RCC. Exploratory objectives include assessing gene expression signatures and pharmacodynamic parameters with outcome.

This study is open to enrollment with six patients on trial anticipating lead-in safety analysis to be completed shortly for identification of randomized phase 2 dosing and opening the expansion cohorts. The trial will be open through the Kidney Cancer Research Consortium at MD Anderson, Vanderbilt University Medical Center, Duke University, and University of Pennsylvania.

## Keywords

clinical trial, front-line, metabolism, immunotherapy

## CDMRP DOD Funding

yes

88

## Optimal Treatment by Invoking biologic clusters in renal cell carcinoma (OPTIC RCC)

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## Background

The first-line treatment for metastatic clear cell renal cell carcinoma (mccRCC) includes immune oncology (IO) based combination therapy. The current standard includes a PD-1 inhibitor plus either an anti-CTLA-4 inhibitor (IO/IO) or an anti-vascular endothelial growth factor receptor tyrosine kinase inhibitor (VEGF TKI) (IO/TKI). Currently, there is no evidence to guide a physician's choice between an IO/IO versus an IO/TKI combination. The phase III IMmotion 151 trial performed RNA-seq from 823 ccRCC tumors and established seven biologically distinct gene expression clusters of ccRCC (Motzer and Rini et al., Cancer Cell 2020). The seven clusters showed differential responses to immune checkpoint inhibitor and may serve as a predictive biomarker to select clusters to assign patients with mccRCC to either an IO/IO (ipilimumab/ nivolumab) or an IO/TKI (nivolumab/ cabozantinib) regimen.

## Methods

Patients diagnosed with mccRCC without prior systemic therapy (including in the neoadjuvant or adjuvant setting) and at least one measurable lesion as defined by RECIST 1.1 are eligible for enrollment. RNA-seq will be performed on metastatic tumor specimens and used to assign tumor clusters. Patients with cluster 1/2 tumors will be assigned to the nivolumab/cabozantinib arm. Patients with cluster 4/5 tumors will be assigned to the ipilimumab/nivolumab arm. Cluster 3/6/7 will be excluded. The primary endpoint is overall response rate (ORR) per RECIST 1.1. Key secondary endpoints include progression-free survival, depth of response >80%, and rate of immune-related adverse events (irAEs).

The hypothesis is that use of tumor clusters to assign front-line therapy to either nivolumab/cabozantinib or ipilimumab/ nivolumab will lead to a 20% greater ORR compared to unselected historical controls in CheckMate 9ER (ORR 55%) or CheckMate 214 (ORR: 40%). This trial adopts Simon's MiniMax two-stage design (power: 80%, one-sided alpha: 0.1). For the nivolumab/cabozantinib arm, stage I will enroll 12 eligible patients. If there are 7 or more responders in the

first 12 patients, stage II will enroll an additional 14 patients (n=26). For the ipilimumab/ nivolumab arm, stage I will enroll 16 eligible patients. If there are 7 or more responders in the first 16, stage II will enroll an additional 12 patients (n=28). The primary endpoint will be met if there are 15 or more responders (ORR>60%).

This trial is open and enrolling with 12 patients on study at Vanderbilt University Medical Center and additional sites to open including UT Southwestern, City of Hope, University Hospitals Seidman Cancer Center, and Cleveland Clinic.

**This trial is funded by the Department of Defense Kidney Cancer Research Program Clinical Trial Award (W81XWH-22-1-1033) (NCT05361720).**

### Keywords

clinical trial, biomarker, front-line, immunotherapy

### CDMRP DOD Funding

yes

**92**

## Phase 1/2 study of PRO1160, a CD70-directed antibody-drug conjugate, in patients with advanced solid tumors and hematologic malignancies

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### Background

PRO1160 is a novel antibody-drug conjugate (ADC) directed to CD70, an antigen mediating immuno-suppression that is overexpressed in multiple solid tumors and hematologic malignancies, with limited distribution in normal tissues. PRO1160 comprises (1) a human monoclonal antibody specific for CD70, (2) a protease-cleavable proprietary hydrophilic linker, and (3) exatecan, a topoisomerase 1 inhibitor. Comprehensive prior work demonstrated that the hydrophilic linker confers excellent physicochemical properties and pharmacokinetics (PK) across a range of payload mechanisms and is superior to conventional linkers on these critical parameters for ADCs. In addition, exatecan is broadly active in many tumor types, is membrane permeable, and is not a substrate of multidrug resistance pumps, thus likely lending strong bystander effects and durable treatment responses. PRO1160 is highly potent in cell-derived xenograft models of renal cell carcinoma (RCC), non-Hodgkin lymphoma (NHL), and nasopharyngeal carcinoma (NPC). PRO1160 also demonstrates marked antitumor activity in patient-derived xenograft models of diverse tumor sites, histologies, molecular subtypes, target expression levels, and Epstein Barr Virus status. PRO1160 is stable in circulation and displays PK characteristics indistinguishable from the parent antibody in rats. In a GLP toxicity study in cynomolgus monkeys, the primary PRO1160-related toxicity resided in the thymus and bone marrow, was consistent with exatecan toxicities, and was reversible.

PRO1160-001 is an ongoing, open-label Phase 1/2 study to evaluate the safety, tolerability, PK, and antitumor activity of PRO1160 in patients with metastatic RCC, metastatic or relapsed NPC, or advanced relapsed/refractory NHL.

Patients must have measurable disease per the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 or the Lugano Classification for NHL. Patients must also have previously received therapies known to confer clinical benefit unless considered ineligible, refused by the patient, or not available in the region.

PRO1160 is given by intravenous infusion on Day 1 of a 21-day cycle and treatment may continue until disease progression, unacceptable toxicity, or other reason for discontinuation. The primary objectives are to evaluate the safety and tolerability of PRO1160 and to identify the maximum tolerated dose, if reached, and recommended phase 2 dose (RP2D).

This study consists of 2 parts, Part A: dose-escalation and dose-level expansion, and Part B: 3 tumor-specific expansion cohorts (metastatic RCC, metastatic or relapsed NPC, or advanced relapsed/refractory NHL) treated at the RP2D. PK, immunogenicity, and antitumor activity will also be evaluated. The study is currently enrolling at sites in the US, with future enrollment in China planned (Clinicaltrials.gov: NCT05721222).

**Keywords**

CD70, ADC, antibody-drug conjugate, exatecan, NHL, renal cell carcinoma, nasopharyngeal carcinoma

# DOD CDMRP/KCRP Awards

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## Defining Cellular and Genetic Factors for Renal Cell Carcinoma Subtypes

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Duke University

### Technical Abstract

**Background:** Renal cell carcinoma (RCC) possesses multiple histological and molecular subtypes that limit the efficacy of our current treatments and therapeutic strategies. Recent advances in biotechnology revealed heterogeneity and identified frequently altered genes in RCC patients. However, these genetic changes were only characterized at the end stage of cancer development from patient samples. Which types of cells in the kidney epithelium can initiate RCCs with the genetic alteration needs to be functionally investigated. Therefore, a thorough functional validation of the key determinants in RCC development is necessary for the discovery of therapeutic interventions to treat kidney cancer.

**Focus Areas:** (1) Conduct basic biology research to better understand etiology and cancer progression, metastatic disease, refractory disease and therapeutic resistance, genetic and environmental risk factors, and the prevention of kidney cancer and (2) Define the biology of rare kidney cancers and develop treatments to improve outcomes and reduce death.

**Hypothesis/Objective:** We hypothesized that RCC subtypes are determined by specific cellular and genetic factors. The objective is to investigate the contributions of cells of origin and to functionally validate recurrent genetic alterations in the initiation of RCC using isolated primary human kidney epithelial cells in our kidney cell transformation assay.

**Specific Aims:** (1) Determine oncogenic combinations initiating kidney cancer subtypes using a rapid and efficient human cell transformation assay; (2) Identify sub-populations of kidney cells that can initiate kidney cancer subtypes, and (3) Define essential oncogenic factors in renal cell carcinoma subtypes using a leave-one-out approach.

**Study Design:** To functionally validate cells of origin and recurrent genetic alterations for RCC initiation, Park laboratory has established a highly efficient human kidney cell transformation assay. Using this assay, we created a genetically defined kidney tumor from normal kidney cells by introducing oncogenic factors. This tumor showed the histological and molecular features of clear cell RCC. In our preliminary study, we made a list of recurrent genetic alterations in RCC subtypes

and identified cell surface proteins that can distinguish specific kidney sub-populations from recently published high-throughput sequencing datasets. In Aims 1 and 2, to identify oncogenic combinations for kidney cancer initiation, we will introduce a pool of lentiviruses expressing oncogenic factors to specific kidney cell types for initiating kidney tumors. We will insert a barcode to our lentiviral vector to deconvolute and determine lentiviral combinations in the resultant kidney tumors using sequencing techniques. The tumors will be evaluated to characterize histological and molecular features. They will be subjected to RNA-sequencing techniques to profile and compare their transcriptomes to human RCC specimens. In Aim 3, we will define key oncogenic factors for RCC subtypes by the leave-one-gene-out approach.

**Impact:** If successful, this research proposal will address two important questions in kidney cancer biology about: (1) cells of origin for kidney cancer and (2) genes essential in kidney cancer initiation and development. These studies also will create a panel of new genetically defined RCC models, which could be valuable resources for developing and exploring therapeutic targets.

**Innovation:** Understanding the functional role of recurrent genetic alterations playing in the initiation of cancer is challenging, since most cancer patients only present once cancer is fully formed. To overcome this barrier, researchers have leveraged genetically engineered mouse models (GEMMs) to identify the functional roles of specific genetic alterations in cancer research. However, to investigate combinations of 20-30 genes in GEMMs would require extraordinary effort from an institution, let alone a single laboratory. Using our kidney cell transformation assay, we can transform a small number of isolated normal human kidney cells (10,000 cells) into tumor cells by lentiviruses expressing defined genetic factors (up to 19 copies). This technical advancement will allow testing of additional genetic changes with the same tissue, removing the ambiguity that comes with using samples with different genetic backgrounds. In addition, we will apply a single-cell DNA amplicon sequencing technique with a barcoded lentiviral overexpression and/or knockdown system to define oncogenic combinations in RCC initiation. This technique will allow us to rapidly identify the functional annotation of genetic alterations and the cooperativity or mutual exclusivity of these events, which will provide substantial insight into genetic drivers and potential dependencies of RCC subtypes. Eventually, our genetically engineered human RCC models can be used for various studies to understand the mechanisms by which kidney cancer initiates and progresses as well as for drug screens to treat RCCs.

**4**

## Targeting GPNMB in Renal Tumors in Tuberous Sclerosis Complex and Translocation Renal Cell Carcinoma

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### Technical Abstract

**Background:** The MiT/TFE family of transcription factors (TFEB/TFE3/MITF) are essential, functionally redundant regulators of lysosomal biogenesis, and critical drivers of tumorigenesis in translocation renal cell carcinomas (tRCC, with underlying genomic rearrangements of TFE3 and TFEB) as well as renal tumors with loss of the TSC1/2 tumor suppressor genes (which drives TFE3/TFEB activity). GPNMB is a transcriptional target of the MiT/TFE family and a potentially oncogenic cell-surface protein which is highly amenable to therapeutic targeting using fully human and clinically tested anti-GPNMB antibodies such as glembatumumab (CR011). GPNMB expression is elevated in renal cells and tumors with PRCC-TFE3 and TSC1/2 loss, but its value as a therapeutic target in these tumors is not known. Radiotherapy with alpha-particle emitters (aRPT), is emerging as a superior form of targeted therapy with increased biological effectiveness, minimal damage to normal cells and decreased susceptibility to normal mechanisms of tumor cell resistance, but its therapeutic potential has been untested in tRCC or renal tumors with TSC2 loss.

**Hypothesis and Objective:** Herein, we will address the hypothesis that MiT/TFE-driven GPNMB expression represents a previously unrecognized and potentially targetable biomarker and driver of renal tumorigenesis in tRCC and renal tumors with TSC2 loss. We will determine whether GPNMB depletion is sufficient to decrease tumorigenesis and whether GPNMB can function as a soluble biomarker in renal tumors with high MiT/TFE activity. We will also determine if cell surface GPNMB can be targeted for aRPT using a human anti-GPNMB antibody (CR011), radiolabeled with actinium-225(225Ac), in renal tumors.

**Area of Emphasis:** This work will elucidate the mechanisms by which MiT/TFE transcription factors drive renal tumorigenesis, identify new diagnostic biomarkers for tRCC and tumors with TSC1/2 loss, and develop novel treatment strategies for these rare subtypes of kidney tumors.

**Aim 1:** Determine whether GPNMB drives tumor progression and/or serves as a functional biomarker in the setting of tRCC or renal tumors with TSC2 loss.

**1A:** Test whether GPNMB depletion inhibits tumor progression (cellular growth, invasion, and angiogenesis) in RCC cells and tumors harboring PRCC-TFE3 or TSC2 loss.

**1B:** Test whether elevated GPNMB expression modulates Wnt/beta-catenin signaling, and whether treatment with Wnt inhibitors reduces progression in renal tumors harboring PRCC-TFE3 or TSC2 loss.

**1C:** Test whether soluble GPNMB is a functional biomarker in RCC cells and tumors harboring PRCC-TFE3 or TSC2 loss.

**Aim 2:** Determine whether GPNMB can be targeted by a-radiopharmaceutical therapy (aRPT) to reduce progression of tRCC or renal tumors with TSC2 loss.

**2A:** Optimize methods to conjugate an alpha-particle emitter actinium-225 (225Ac) and its imaging surrogate indium-111 (111In) to a fully human monoclonal anti-GPNMB antibody (glembatumumab [CR011]).

**2B:** Assess the biodistribution and dosimetry of 111In-Gb in tumor-bearing and tumor-free mouse models of PRCC-TFE3 or TSC2 loss.

**2C:** Evaluate the preclinical efficacy of 225Ac-Gb in cell cytotoxicity assays and tumor-bearing mouse models of PRCC-TFE3 or TSC2 loss.

**Impact:** MiT/TFE transcription factors are drivers of tumorigenesis in tRCC and renal tumors with TSC1/2 loss; however, this finding has not yet been leveraged for diagnosis or treatment of these rare molecular tumor subtypes. Here, we will determine whether an MiT/TFE transcriptional target, GPNMB, is a biomarker or oncogenic driver in these tumors and directly test whether alpha particle-based radiopharmaceutical therapy (aRPT) using a fully human and clinically tested anti-GPNMB antibody (CR011), radiolabeled with actinium-225(225Ac), can be an effective form of treatment.

**Innovation:** We will be the first to test the hypothesis that GPNMB is an oncogenic driver and biomarker of tumorigenesis in the context of high MiT/TFE activity in tRCC or TSC1/2-deficient renal tumors. Moreover, we will be the first to develop and examine the efficacy of an alpha particle-based radiopharmaceutical therapy (aRPT) for tRCC and tumors with TSC1/2 loss, representing an entirely new and targeted treatment paradigm for these molecular RCC subtypes.

**5**

## Deep Functional Characterization of MiT/TFE Fusions in Translocation Renal Cell Carcinoma

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### Technical Abstract

**Background:** Translocation renal cell carcinoma (tRCC) is a rare and aggressive type of non-clear cell renal cell carcinoma (RCC) that represents 1%-5% of sporadic RCC in adults and 20%-75% of kidney cancers in children. Biologically, tRCC is driven by rearrangements involving a member of the MiT/TFE family of transcription factors (TFE3, TFE2, TFEC, MITF), most commonly TFE3. Currently, there are no treatments that are specifically targeted to tRCC, and the treatments approved for clear-cell RCC are less effective in patients with tRCC. A fundamental barrier to progress in tRCC is an incomplete mechanistic understanding of precisely how MiT/TFE gene fusions exert their oncogenic function. Key unknowns regarding MiT/TFE fusions include: (1) the key sequence elements/domains of the fusion that dictate oncogenicity; (2) critical co-regulators of MiT/TFE fusions that modulate their activity; and (3) downstream oncogenic effector pathways activated by MiT/TFE fusions. To date, these factors have not been systematically identified or studied.

**FY21 KCRP Areas of Emphasis:** We will address three FY21 KCRP areas of emphasis. (1) Sequence-function mapping studies (Aim 1) represent basic biology research to better understand etiology and cancer progression. (2) All aims directly address the focus area of defining the biology of rare kidney cancers. (3) High-throughput genetic screens (Aim 3) are aimed at developing novel therapeutic strategies for the treatment of kidney cancer. This work will also be led by an early career investigator and this award aligns with the KCRP strategic goal of supporting the development of the next generation of kidney cancer researchers.

**Hypothesis/Objective:** The overarching objective of this project is to uncover the mechanisms by which MiT/TFE fusions drive tRCC and to understand how these functions differ relative to wild type (WT) MiT/TFE proteins. We hypothesize that MiT/TFE fusions may acquire neomorphic, RNA-dependent interactions relative to WT MiT/TFE proteins, which may allow them to selectively activate oncogenic effector pathways. We propose detailed biochemical and functional genetic characterization of MiT/TFE fusions to elucidate the mechanisms by which they drive tRCC.

### Specific Aims

**Aim 1:** Leverage targeted and unbiased genetic approaches to identify oncogenic sequence elements within MiT/TFE fusions.

**Aim 2:** Elucidate RNA-dependent functions of MiT/TFE fusions.

**Aim 3:** Discover functionally important downstream effectors of MiT/TFE fusions.

**Study Design:** In Aim 1, we will generate a spectrum of MiT/TFE fusion constructs with mutations in functional domains and test the effects of these mutations on oncogenicity. We will then leverage CRISPR/Cas9 base editing technology to perform unbiased, gene-wide mutational scanning of MiT/TFE fusions in fusion-positive cell lines. These studies will define functionally important sequence elements of MiT/TFE fusions. In Aim 2, we will define RNA-dependent roles for MiT/TFE fusions by comprehensive biochemical and epigenomic profiling. In Aim 3, we will discover and validate oncogenic effector pathways downstream of MiT/TFE fusions via genome-scale genetic screening.

**Impact:** The proposed work is relevant to multiple FY21 KCRP focus areas and is led by an early career investigator. This proposal is consistent with the KCRP vision of eradicating death and suffering from kidney cancer, as it seeks to perform comprehensive molecular characterization of the MiT/TFE fusion, which is the singular genetic alteration in tRCC. This work will generate multiple valuable high-throughput datasets for the kidney cancer research community, including: deep mutational scanning data across a spectrum of MiT/TFE fusions, genome/proteome-wide interaction profiling, and genome-scale genetic screening data. This work will functionally characterize MiT/TFE fusions at unprecedented resolution and will be foundational for future preclinical efforts to rationally target tRCC, an aggressive subtype of kidney cancer that is currently an unmet need in kidney cancer research.

**Innovation:** This work will result in extensive biochemical and functional profiling of MiT/TFE fusions using cutting-edge technologies, including CRISPR/Cas9 base editing, genome-/transcriptome-/proteome-wide biochemical profiling, and genome-scale functional genetic screening. Additionally, this work explores the novel paradigm that MiT/TFE fusion partners may contribute to oncogenicity, which may provide opportunities for highly selective targeting of cancer cells without effects on WT MiT/TFE proteins in normal cells. Overall, this work will significantly advance our understanding of the pathways that drive tRCC and will provide valuable resources to the kidney cancer research community.

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## Identifying Novel Immune Evasion Tumor Immune Networks as Targets for ccRCC Immunotherapy

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### Technical Abstract

**Background and Rationale:** The importance of the host immune system's interaction with cancer cells within the kidney tumor microenvironment is well recognized and has led to the effective application of checkpoint immunotherapy-based regimens against this disease. Although these strategies have ushered remarkable cures for some patients with the most common subtype of kidney cancer (clear cell renal cell carcinoma, also known as ccRCC), the vast majority of patients display either de novo or acquired resistance.

These clinical findings have fueled significant interest to identify novel therapeutic strategies that harness the curative potential of immunotherapy, but through non-overlapping mechanisms of action with current strategies. Toward this end, single-cell genomic technologies (single-cell RNA sequencing; scRNAseq) provide a robust platform for therapeutic discovery by identifying "ecosystems" of diverse cell-cell interactions (CCIs) occurring within the tumor-immune microenvironment (TIME). Indeed, early efforts applying single-cell genomics to ccRCC have already uncovered novel mechanisms of immune evasion associated with disease progression, underscoring the potential of this technology for therapeutic discovery.

Though promising, scRNAseq is cost-prohibitive (~\$5000/sample) precluding the interrogation of large patient cohorts that are required to robustly associate molecular signatures capturing immune evasion CCIs in the TIME to disease progression. Additionally, scRNAseq has inherent technical limitations, most notably being the lack of spatial resolution due to a reliance on dissociation of tissues into single-cells or nuclei. This has motivated the search for cost-effective orthogonal approaches that not only capture the biological information in single-cell datasets but also overcome the shortcomings of the technology.

Here we will leverage a high-dimensional proteomics technology, imaging mass cytometry (IMC), to capture tumor-immune evasion cell networks identified from a large RCC single-cell genomics dataset generated at the University Health Network (UHN). Importantly, IMC is capable of measuring millions of cells across hundreds of patient samples at low cost (\$500/sample), allowing us to scale the biological information contained in scRNAseq data in a spatially

conserved manner across a large clinically annotated biobank of ccRCC patients at UHN.

**Guiding Hypothesis:** That tumor immune networks associated with ccRCC disease progression and their genetic drivers represent targetable vulnerabilities.

### Aims/Deliverables:

- (1) Establish a gold-standard taxonomy of spatially resolved ccRCC tumor immune networks.
- (2) Identify tumor-immune networks associated with disease progression and overall survival in ccRCC.
- (3) Model and functionally validate tumor immune networks in humanized mouse ccRCC xenografts models.

**Impact:** Overall, the short-term deliverables from this proposal will equip the scientific community with a comprehensive understanding of immune cell phenotypes and their interactions within networks inside the kidney TIME. By associating these features to patient survival, we will prioritize those that should be funnelled into therapeutic target and biomarker development programs. In turn, we envision the fundamental insights gained from our emerging data to foster new research directions in the long term that directly lead to novel immunotherapeutic strategies and their appropriate use within precision medicine-based approaches. Ultimately, through these coordinated efforts, we hope to deliver new therapies for patients with kidney cancer to improve the currently abysmal survival outcomes they face.

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## Targeting TFEB and TFE3 in Renal Tumorigenesis

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### Technical Abstract

**Focus Areas:** This proposal addressed two KCRP Translational Research Partnership Award focus areas: (1) Define the biology of rare kidney cancers and develop treatments to improve outcomes and reduce death. (2) Develop novel therapeutic strategies for the treatment of kidney cancer, such as novel drug targets, therapeutic modalities and agents, treatment combinations, and drug delivery systems.

**Background/Rationale:** The Henske lab has demonstrated that TFEB and TFE3 are constitutively nuclear in TSC, and that knockout of TFEB nearly completely reverses the kidney phenotype in mouse models of TSC. The Ballabio lab has

shown that knockout of TFEB completely reverses the kidney phenotype in a mouse model of BHD. These findings provide a novel molecular connection between TSC-associated RCC, BHD-associated RCC, and other forms of RCC characterized by hyperactivation of TFE3 and TFEB.

**Hypothesis/Objective:** Our central hypothesis is that TFEB and TFE3 drive renal tumorigenesis in TSC, BHD, linking them to translocation RCC, and that inhibition of TFEB and TFE3 will have efficacy in preclinical models of TSC, BHD, as well as other forms of RCC characterized by hyperactivation of TFE3 and TFEB. A key translational corollary of this hypothesis is that agents identified in the high throughput small molecule screen will lead to novel therapeutic strategies for several forms of RCC.

#### Specific Aims:

**Aim 1.** To determine the mechanisms underlying TFEB-dependent renal tumorigenesis in TSC and BHD

**Aim 2.** To identify genes that regulate the activity of TFEB and TFE3 in TSC, BHD, and tRCC

**Aim 3.** To identify small molecules that regulate the activity of TFEB and TFE3 in TSC, BHD, and tRCC

**Study Design:** In Aim 1, we will determine the effect of TFEB knockdown in novel tamoxifen-inducible, kidney-specific mouse models of TSC and BHD and identify transcriptional connections between TSC and BHD. In Aim 2, we will perform an siRNA screen to identify genes that inhibit TFEB-driven luciferase activity, using a novel system in which an mCherry GPNMB luciferase reporter is expressed in cells with CRISPR/Cas9 knockout of TSC2 or FLCN and GFP-TFEB expression is induced with doxycycline. Selected hits will be tested in mouse models of BHD and TSC and cellular models of tRCC. In Aim 3, we will perform a high throughput small molecule screen to identify compounds that inhibit TFEB (using the same cells as in Aim 2). Selected hits from this screen will be tested in mouse models of TSC and BHD and cellular models of tRCC.

**Innovation:** This proposal addresses innovative hypotheses, utilizes novel preclinical mouse models, and includes a state-of-the-art high throughput small molecule screen.

**Impact:** Our ultimate goal is to achieve clinical impact by identifying targeted therapies for RCC associated with hyperactivation of TFEB and TFE3.

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## The Bone Metastasis Immunological Niche: Unraveling Its Complexity and Role in Therapy Response and Resistance

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#### Technical Abstract

Clear cell renal cell carcinoma (ccRCC) represents up to 75% of renal neoplasms, with bone as a major site for distant spread (35%-40% of patients). Bone metastasis (BM) is a source of significant morbidity and increased mortality, and confers to patients treated with systemic therapies worse progression-free and overall survival compared to other metastatic sites. Dual immune checkpoint blockade (ICB) with nivolumab and ipilimumab significantly improved survival in ccRCC patients with intermediate and poor risk disease. However, despite an unprecedented success, only a subset of patients benefits from this treatment due to variable expression of targets or emerging resistance, and immune-mediated toxicity is often very high. Single-cell RNA sequencing (scRNA seq) studies have suggested that advanced ccRCC patients who progress on immunotherapy have enrichment of exhausted T cell phenotypes and an M2 macrophage population that is immunosuppressive.

A key feature of ccRCC is von Hippel-Lindau tumor suppressor mutation, consequent vascular endothelial growth factor (VEGF) over-expression, and increased angiogenesis. Tyrosine kinase inhibitors (TKIs) targeting VEGF improve survival in ccRCC patients, including axitinib, lenvatinib, and cabozantinib. Besides inhibiting tumor blood vessel formation, VEGF inhibition has shown a positive impact on host immunity, including abrogation of the immunosuppressive environment. Combining TKI and ICB resulted in improved overall survival rates and progression-free survival, similar to dual checkpoint inhibition, but with lower rates of severe immune toxicity, becoming available frontline treatment regimens.

Analysis of BM is often neglected, due to exclusion of certain patients from clinical trials, difficult specimen collection and processing, and complex underlying biology. Therefore, both the nature and role of the immune infiltrate in ccRCC BM progression and response to immunotherapy, alone or in combination with TKIs, remain largely unexplored. The immunological niche likely differs compared with that of primary tumors or other metastatic sites due to the cellular and molecular composition of the bone environment, which has been shown to mediate therapy resistance. This urges clarifications in order to define a more precise and rational strategy to intervene within bone metastatic patients.

Based on this rationale, we hypothesize that ccRCC BM establishes a unique tumor-associated immune microenvironment promoted by the reciprocal interplays with the bone host cell partners, which induces therapy resistance. Our overall goal will be achieved in the following aims:

**Aim 1.** Define the cellular composition, transcriptional states, and spatial context of the ccRCC BM immunological niche in a treatment-naïve patient population;

**Aim 2.** Identify the cellular and molecular changes induced within the ccRCC BM immunological niche to systemic treatment (TKI, ICB, TKI+ICB) to evaluate for mediators of therapy resistance;

**Aim 3.** Determine the feasibility of using core biopsy specimens for monitoring of ccRCC BM immunological niche.

By performing multimodal analyses at single-cell resolution, we plan to unravel the complexity of the BM immune ecosystem, at baseline and after treatment, in patient samples collected during surgical resections of ccRCC BM. CyTOF and scRNA seq approaches will comprehensively characterize both cellular composition and transcriptional states. These analyses will be paralleled by investigation of the topological context by means of three-dimensional multiphoton microscopy at subcellular resolution, to provide multiparametric information on the spatial distribution of tumor, resident stroma, and infiltrating immune cells. In addition, this strategy allows the potential to identify the presence of defined spatial niches of resistance. Then, we will validate single-cell profiling and topological mapping in bone biopsies.

ccRCC BM contributes to substantial morbidity, mortality, and cost. Clearly defining the immune microenvironment of ccRCC BM and the impact of ICB and TKI therapies, alone or in combination, will provide a needed framework to develop precision strategies for intervention in individual patients. In parallel, we are planning a concept for a prospective clinical study in RCC BM to be opened and pursued within the 3-year term of the grant to compare evolution of bone versus non-bone metastases after exposure to cabozantinib and then the combination of cabozantinib with ICB. Our work will allow defining a more rational clinical administration of therapies that target and modulate the bone microenvironment to improve both survival and quality of life for patients afflicted with this lethal disease, with the ultimate goal to eventually eliminate cancer and related suffering. Overall, our work is relevant to four key KCRP Focus Areas and aims to establish a new collaboration (1) to increase understanding of the biology of kidney cancer (2) in order to develop novel therapeutic strategies for its treatment (3) and improve the patient care (4).

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# Targeting a Combined VHL and 3p Chromatin Remodeler Deficit in Renal Cell Carcinoma

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## Technical Abstract

Clear cell renal cell carcinoma (ccRCC) epitomizes a heterogeneous cancer in which deletion of the short arm of chromosome 3 is an initiating genetic alteration. This results in the loss of one copy of VHL, as well as one copy of each of the chromatin remodelers SETD2, PBRM1, and BAP1, which are all co-located on 3p. A second inactivating hit in VHL is nearly universal among ccRCC tumors and is required to relieve an initial bottleneck to progression and clonal expansion. Subsequent loss of heterozygosity of SETD2, BAP1, and PBRM1 due to mutation of the remaining wild type alleles of these genes, singly or in different combinations, further drives tumor progression. Although 3p deletion appears to represent the initiating genetic driver of tumor formation in ccRCC, it is not understood how heterozygous loss of VHL and of chromatin remodeling genes contributes to disease initiation and progression. We recognize the obligate heterozygosity of these tumor suppressors early in tumorigenesis, preceding VHL loss, and propose to interrogate these 3p driver mutations in aggregate.

Recently, based on data from our labs and others, a paradigm shift in ccRCC biology has recognized new functions for the 3p SETD2, BAP1, and PBRM1 genes that are independent of their characterized functions as chromatin remodelers but dependent on regulation of the tubulin cytoskeleton. We were the first to discover that SETD2 trimethylates alpha-tubulin at lysine 40 (alpha-TubK40me3) on spindle microtubules (2), and recently showed that PBRM1 functions as a reader for this methyl mark (3). Importantly, loss of either SETD2 or PBRM1 produces an identical mitotic error-prone phenotype. In addition, BAP1 functions at the mitotic spindle to deubiquitinate gamma-tubulin and maintain centrosome integrity. Since each of these tumor suppressors regulates microtubule functions at the mitotic spindle, we will investigate the impact of their combinatorial mono-allelic loss (i.e., 3p deletion) on microtubule-dependent processes including genomic instability and cellular migration and invasion.

In this application, we propose to explore for the first time how these seemingly disparate contributors to ccRCC interconnect to acquire a precise functional signature specific to ccRCC. We address a major limitation in the field by creating a human cell culture model that mimics early deletion of 3p genes

(mono-allelic for SETD2, PBRM1, and BAP1), combined with loss of VHL. This innovative tool will enable us to establish a precise phenotypic signature of mono- or bi-allelic loss of 3p chromatin remodelers and explore therapeutics targeting this unique feature of ccRCC genetics. Thus, our overarching hypothesis is that mono-allelic 3p deletions, obligatory in ccRCC evolution, are fixed deficits that promote tumor formation and represent an opportunity for therapeutic intervention. To test this hypothesis, we propose the following Specific Aims:

**Specific Aim 1:** Develop a model for the complex 3p deletion and mutation patterns of ccRCC. In this Aim, we will use our novel human 3p deletion model to evaluate how VHL inactivation together with mono- and bi-allelic loss of each of the chromatin remodelers (SETD2, BAP1, PBRM1) alone and in combination affects cell functions and phenotypes. We will interrogate both the chromatin and cytoskeleton, including mitotic outcomes to establish individual and cooperative contributions of these genes to cancer-relevant cellular phenotypes.

**Specific Aim 2:** Test the hypothesis that haploinsufficiency of 3p chromatin remodelers coupled with VHL loss can be targeted therapeutically. Given that the vast majority of ccRCC tumors harbor deletions in 3p, we seek to specifically target this haplo-insufficient state. We will utilize a whole-genome CRISPR-Cas9 library to identify gene dependencies in our human 3p deletion model and identify specific pathways and mechanisms that drive synthetic lethality.

**Specific Aim 3:** Test the efficacy of a candidate panel of small-molecule compounds in patient derived organoids. Consistent with evaluating mechanism-based therapeutic strategies, we will use inhibitors that target specific functional deficits in cells with 3p deletion. PIK3beta;AKT inhibitors that we recently demonstrated to be synthetic lethal with SETD2-loss, and targets identified in Aim 2, will be interrogated for 3p functional dependencies, and validated in patient-derived organoid models for activity.

How obligate mono-allelic loss of 3p chromatin remodelers contribute to early disease pathogenesis is not resolved. Our studies on the convergence of 3p chromatin remodelers as regulators of microtubule functions open new avenues to understand how a combined monoallelic loss of these tumor suppressors can be targeted in ccRCC. Our studies provide a unique opportunity to identify therapies targeting the precise 3p signature in the ccRCC tumor cell itself (as opposed to current therapeutics, which are tumor cell non-autonomous) with applicability to both sporadic ccRCC and VHL disease.

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# Metabolic Demands and Determinants in the RCC Tumor Microenvironment

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## Technical Abstract

**Background:** Recent attention to the complex interactions of cells present in the microenvironment of clear cell renal cell carcinoma (ccRCC) has focused a new level of attention on tumor resident macrophages. Three pieces of evidence from studies in the clinic and in the laboratory are emerging to lead us to propose a comprehensive analysis of human tumors to better understand the roles these cells play in supporting tumors. First, although ccRCC is well known to harbor driver VHL gene mutations, which can alter cancer cell metabolism to promote glycolysis, a conundrum has faced the field, known as the PET Paradox. The paradox is that while ccRCC tumors are genetically predisposed to increase glucose uptake, they are often cold on FDG PET scans. The reason for this discrepancy is unknown. Our prior work on human tumors following MRI-FDG-PET showed that tumors that harbored any regional FDG-PET signal also displayed gene expression features associated with poor prognosis. We have subsequently used reductionist scientific approaches to trace glucose utilization, with the surprising finding that glucose is partitioned into macrophages, which are more metabolically active, and glutamine and lipid are instead preferentially distributed into the cancer cells. Finally, we used single-cell sequencing and informatic strategies to determine a population of macrophages that is specifically associated with poor outcome.

**Objective/Hypothesis:** It has become clear that to make significant inroads into further altering immune response in ccRCC will require engaging the macrophage component. We hypothesize that the distinct metabolic properties of different cell subsets in the ccRCC TME contribute to tumor metabolic heterogeneity (evidenced by mixed PET imaging signals) and that genetic alterations in ccRCC shape the TME and influence the metabolism and function of non-cancer cells present in the tumor space.

**Specific Aims:** We will (1) determine the influence of VHL mutation on macrophage residence in tumors; (2) characterize the metabolic demands of human cancer cells and immune cells harvested from tumors; and, (3) analyze the glycolytic features of cancer and immune cells in the context of intact ccRCC tumors.

**Study Design:** This study uses isogenic mouse-derived tumor cell lines to test the impact of mutations on the surrounding immune cells and then utilizes the Vanderbilt Center for Immunobiology RCC Analysis Lab repository of annotated and genetically defined human tumors, maintained in blocks and in 2D culture and 3D organoid culture, to carefully examine the cells comprising the tumor microenvironment, with a particular emphasis on functional metabolic characteristics.

**Impact:** With these data we propose to develop a comprehensive lens into the 3D space and cell metabolic interactions of ccRCC tumors to develop biomarker and therapeutic strategies. This research will generate new basic biology and clinical insight to accelerate research into therapies that utilize the heterogeneous cellular components of the tumor to make assessments of tumor behavior and to develop therapies that take advantage of the unique sets of cells that make up the ccRCC tumor microenvironment.

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## Derepression of Human Endogenous Retrovirus and Implications for Immunotherapy for Clear Cell Renal Cell Carcinoma

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### Technical Abstract

**Research Plan:** Nearly 75,000 people are diagnosed with kidney cancer per year in the United States and there are ~15,000 kidney cancer-related deaths annually. Clear cell renal cell carcinoma (ccRCC) is the most common form of kidney cancer (>70% of all cases). ccRCC has historically been viewed as an immunogenic tumor despite its low mutational burden, and some ccRCC patients benefit from immunotherapies such as immune checkpoint blockade (ICB) therapy. However, the overall response rate is well under 50% and it is unclear why some patients respond, and others do not. In this regard, recent clinical studies showed that ccRCC aberrantly express human endogenous retroviruses (hERVs) and that high hERV expression correlates with response to ICB. These observations suggest that increasing hERV expression could enhance the efficacy of ICB. hERVs are remnants of retroviruses that integrated into the genome millions of years ago. They are largely silenced by mutations or promoter hypermethylation. Nonetheless, some hERVs maintain their open reading frames and therefore retain the potential to be translated. Childs and colleagues showed that one ccRCC patient who responded to allogeneic bone marrow transplant harbored cytotoxic T cells that recognized

ERVE-4-derived peptides displayed by his ccRCC. This was the first evidence that aberrantly expressed hERV could act as neoantigens and be recognized by the adaptive immune system. Nearly 90% of ccRCCs harbor inactivating mutations of von Hippel-Lindau (VHL) tumor suppressor gene, which causes stabilization of an oncogenic transcription factor, HIF2. Childs showed that HIF2 transcriptionally activated ERVE-4. I hypothesized that ERVE-4 is simply a prototype of multiple hERVs that are directly transcribed by HIF2. My preliminary data suggest I am correct. In Aim 1, I will do a genome-wide search for hERVs, including over 3,000 annotated hERVs that are directly transcribed by HIF2 using ChIP-Seq, RNA-Seq, and SLAM-Seq. Since many hERVs are silenced by promoter hypermethylation, I will also treat ccRCC cells with DNA methyltransferase inhibitors to uncover additional hERVs that can be induced by HIF2 once their promoters are hypomethylated. The next important question is whether any of the HIF2-responsive hERVs are translated and presented on the cell surface. In Aim 2, I will use Ribo-Seq technique and HLA-immunoprecipitation (IP) followed by mass spectrometry to uncover hERV-derived peptides that are translated and presented by major histocompatibility complexes (MHCs). ERVE-4 will serve as a positive control in all the proposed experiments since I have confirmed Childs's finding that it is regulated by HIF2 and translated.

**Personnel/Research Development Plan:** I am committed to pursuing a career in kidney cancer research as an independent laboratory investigator at a top academic institution. In order to successfully transition into an independent researcher, I will improve by (1) keeping up with literature in the field, with particular focus on kidney cancer and immunotherapy; (2) having weekly meetings with my mentor, Dr. Kaelin, to discuss experimental design, troubleshooting, data interpretation, as well as "the big picture"; (3) presenting at the weekly Kaelin Laboratory work-in-progress meeting twice a year; attending the monthly DF/HCC Kidney Cancer SPORE meeting, the bi-weekly Broad Institute Cancer Program Meeting, and one national/international cancer meeting per year; (4) collaborating with experts in the field, including Stirling Churchman (Ribo-Seq) and Catherine Wu (hERV bioinformatic analysis and HLA-IP/MS). My mentor, Dr. Kaelin, is a world-renowned physician-scientist and has been awarded many prestigious awards, particularly for his contribution to the understanding of VHL-HIF biology. These findings laid the foundation for our understanding of how tissues sense oxygen and how dysregulation of this process promotes tumorigenesis in ccRCC. Dr. Kaelin's experience in successfully training over sixty postdoctoral fellows, as well as his incredible knowledge of science, especially in ccRCC biology, will be instrumental to the success of the proposed project.

**Impact:** This study should provide new mechanistic insights into how hERV are regulated genetically and epigenetically. This knowledge might allow us to pharmacologically increase

hERV expression to enhance the effectiveness of ICB. In addition, hERV-derived peptides that are presented on MHCs and recognizable by T cells could form the basis for future T-cell therapies and cancer vaccine, a step toward personalized medicine for kidney cancer patients. The knowledge gained from HIF regulated hERV could guide me to establish a career bridging basic biology of kidney cancer to novel therapy development.

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## Targeting Interleukin-1beta to Overcome Adaptive Immune Resistance in Renal Cell Carcinoma

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### Technical Abstract

**Background:** Clear cell renal cell carcinoma (ccRCC) is an immunogenic tumor with multiple immunotherapy regimens demonstrating clinical efficacy. However, not all tumors respond, making the development of new therapeutics that overcome primary and adaptive immune resistance critical unmet needs. Single-cell level characterization of the tumor microenvironment (TME) has identified diverse subsets of tumor-associated macrophages (TAMs) that play important (and sometimes opposing) roles in modulating antitumor immune responses to immune-checkpoint blockade (ICB). In preclinical models, we recently showed that blocking the innate cytokine IL1 $\beta$  reprograms TAMs to antitumor phenotypes and enhances ICB activity. We therefore initiated a clinical trial of neoadjuvant anti-IL1 $\beta$  (canakinumab) plus anti-PD-1 (spartalizumab) in localized ccRCC. As part of this trial, we are collecting fresh primary tumor tissue empowering a unique opportunity to quantify the biological activity and immunogenicity of combined IL1 $\beta$  and PD-1 blockade in ccRCC. We will first integrate data from multiplexed imaging, immune-profiling, and single-cell sequencing to elucidate treatment changes in the TME. We will then leverage computational approaches that we have previously validated in ccRCC to identify novel targets to maximize the antitumor immune response. Finally, we will evaluate the functional therapeutic impact and test novel combinatorial therapies using both murine and a novel T-cell co-culture system. Collectively, these studies will inform the optimal design of future hypothesis-driven clinical trials. The overall objective of our proposal is to determine the biological effects of combined IL1 $\beta$  and PD-1 blockade and identify mechanisms of response and resistance to immunotherapy in ccRCC.

**Hypothesis:** We hypothesize that IL1 $\beta$  blockade remodels the myeloid compartment in ccRCC creating a permissive TME for immune checkpoint inhibition. **Specific Aims:** (1) To reprogram suppressive TAMs toward a pro-inflammatory phenotype through IL1 $\beta$  blockade. (2) To identify drugs targeting master regulators (MRs) mediating therapeutic response/resistance. (3) To test the activity of predicted combination immunotherapy in mouse and organoid RCC models. **Study Design:** We will analyze tissue from an accruing, investigator-initiated trial of neoadjuvant anti-IL1 $\beta$  (canakinumab) and anti-PD-1 (spartalizumab) in localized ccRCC (N=14) compared to a cohort of untreated controls (N=14) (NCT04028245). To date, 4 of 14 patients have enrolled on the study. In aim 1 we will test our hypothesis that combination IL1 $\beta$ /PD-1 blockade decreases suppressive TAMs and increases CD8 T cells using multiplexed immunofluorescence (mIF) to quantify immune cell density and proximity. Given TAMs are comprised of significant phenotypic heterogeneity with varying degrees of pro-inflammatory and immunosuppressive function, we will also determine the effects of IL1 $\beta$ /PD-1 blockade on the broader myeloid and lymphoid compartments using single-cell RNA sequencing (scRNASeq) and multiparametric flow cytometry. In Aim 2 we will apply a novel systems biology computational approach (metaVIPER) to the scRNA-seq data obtained in Aim 1, which allows inference of protein activity and identification of rare cell subclusters. We will determine treatment-induced changes in MR protein activity driving therapeutic response/resistance. We will validate these changes at the protein level by mIF. We will then identify candidate drugs targeting MRs of interest using the OncoTreat algorithm. Finally, in Aim 3, candidate drugs will be tested in the RENCA syngeneic RCC mouse model. We will also test the effects of anti-IL1 $\beta$ /PD-1 therapy and candidate drugs using a novel high-fidelity organoid T cell co-culture system. These studies will allow us to identify novel candidate therapeutic agents for future trials. **Impact:** These translational studies address a critical problem in kidney cancer treatment, maximizing the clinical benefit of ICB. Specifically, this proposal builds on recent discoveries that the myeloid compartment mediates significant intratumoral immunosuppression and resistance to ICB in ccRCC. If the current studies demonstrate significant immunologic and clinical activity, future randomized phase 2 or 3 trials could evaluate the clinical efficacy of IL1 $\beta$ /PD-1 blockade in the neoadjuvant setting. We will also identify novel immunotherapy targets in ccRCC, which will be tested in murine models and a high-throughput organoid T cell co-culture system to rapidly accelerate the development of novel combination regimens for ccRCC. This application addresses the KCRP focus on novel therapeutic targets and strategies for kidney cancer.

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## Targeting RNA Sensing to Enhance Immunotherapy Responses in Kidney Cancer

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### Technical Abstract

**Background:** Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of metastatic clear cell renal cell carcinoma (ccRCC), yet most patients do not experience complete and durable responses. To address this significant, unmet need to increase the number of patients that will benefit from immunotherapy, we have identified the retinoic acid-inducible gene I (RIG-I) pathway as a novel immunotherapeutic target for ccRCC. RIG-I recognizes RNA with a triphosphate (3p) moiety on the 5' end (3pRNA), which triggers a potent antitumor innate immune response. Accordingly, RIG-I agonists are promising immunotherapeutics, but their clinical efficacy and utility is limited by major drug delivery barriers. We are solving this challenge through the development of lipid nanoparticles (LNPs) specifically engineered to enhance 3pRNA activity and to enrich RIG-I activation in the RCC tumor microenvironment (TME). This innovation affords a unique opportunity to harness the potential of RIG-I agonists, which have thus far not been explored in ccRCC. Our preliminary studies demonstrate that 3pRNA-loaded LNPs (3pRNA/LNPs) inhibit tumor growth and increase survival time in a mouse model of kidney cancer, the first demonstration of its kind. Building on this exciting finding, we propose to develop and advance 3pRNA/LNPs as a novel immunotherapeutic modality for ccRCC.

**Focus Area:** Develop novel therapeutic strategies for the treatment of kidney cancer, such as novel drug targets, therapeutic modalities and agents, treatment combinations and drug delivery systems.

**Objective/Hypothesis:** The objective of the proposed research is to engineer an optimized LNP platform for 3pRNA delivery that maximizes RIG-I activation in the TME and to evaluate the efficacy and safety of this new class of immunotherapy for ccRCC. We hypothesize that optimized LNPs will enhance the immunopharmacological properties of 3pRNA to potently activate RIG-I signaling at tumor sites, resulting in increased tumor immunogenicity and de novo priming and activation of a robust and long-lasting endogenous antitumor CD8+ T cell response that eliminates ccRCC tumors and increases response rates to ICIs.

**Specific Aims:** (1) Engineer LNP properties to maximize the therapeutic window of RIG-I agonists. (2) Investigate the effect of RIG-I signaling on the TME and antitumor immunity in RCC. (3) Establish combinatorial immunoregimens for RCC via synergy between RIG-I activation and ICIs.

**Study Design:** In Aim 1, we will optimize LNPs as carriers for systemic administration of 3pRNA that maximize RIG-I activation in the RCC TME. We will develop a new 3pRNA/LNP formulation by re-engineering of the LNP with a "smart" poly(ethylene glycol) (PEG) corona that is removed in response to the hypoxic TME of RCC tumors. We will modulate LNP properties to optimize 3pRNA/LNP pharmacokinetics, RIG-I activation in the TME, antitumor efficacy, and safety in mouse models of RCC.

In Aim 2, we will demonstrate that RIG-I activation can reprogram the RCC TME to a more inflammatory milieu that promotes induction, infiltration, and activation of antitumor T cells that eliminate RCC tumors. We will comprehensively profile the effects of 3pRNA/LNP on innate and adaptive immune responses in both mouse and ex vivo human models of ccRCC.

In Aim 3, we will evaluate 3pRNA/LNPs in combination with clinically approved ICIs. We will determine optimal combinational immunoregimens of 3pRNA/LNPs with ICIs and evaluate the effect of combination therapy on the TME and antitumor immunity in multiple mouse models of RCC.

**Impact:** This work will have both short- and long-term scientific and translational impact that will accelerate progress toward preventing deaths from kidney cancer through the development of an innovative nanomedicine that reinvigorates endogenous mechanisms of antitumor immunity in ccRCC. Our studies have direct potential to expand the immunotherapeutic armamentarium for patients with ccRCC and other kidney cancer subtypes, including service men and women who are at increased risk for kidney cancer.

**Innovation:** The primary conceptual and technological innovations proposed include: (1) the first evaluation of a RIG-I agonist as an immunotherapy for RCC; (2) development of a clinically advanced LNP technology that is designed specifically to enhance 3pRNA activity and efficacy in RCC; (3) an investigation into how RIG-I activation influences the immune contexture of RCC, including its effects on both innate and adaptive arms of antitumor immunity; (4) a new paradigm for reinvigorating dysfunctional T cell responses in RCC based on de novo priming of antitumor CD8+ T cells that re-populate RCC tumors; and (5) evaluation of novel, rationally designed combination immunotherapies for ccRCC with high translational potential.

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## A Novel STAT3 Antisense Oligonucleotide-Based Immunotherapy for Renal Cell Carcinoma

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### Technical Abstract

Recent advancements in the treatment of renal cell carcinoma (RCC) using immune checkpoint inhibitors (ICI) against PD1 or CTLA-4 receptors have improved survival rates in patients. However, a proportion of RCC patients do not respond to anti-PD-1/CTLA-4 combination immunotherapy. We postulate widespread immune suppression may underpin patient responses to therapy and a treatment modality approach combining T cell directed ICIs with STAT3 targeting is required to overcome this suppression.

In our preliminary studies, we characterized immunosuppressive myeloid cell populations, T cell subsets, and immune biomarkers in blood samples from RCC patients with advanced stage IV disease, undergoing anti-PD-1/CTLA-4 combination therapy. Results of our multicolor flow cytometry and plasma analysis suggest an elevation of inflammatory cytokines IL-17A and IL-6 in patients with no clinical response to ICIs and furthermore that ICI therapy is associated with elevated activated Signal Transducer and Activator of Transcription 3 (pSTAT3) with a significant increase in its downstream target Arginase-1 between cycle 1 and cycle 8 of treatment ( $P=0.0008$ ). The pSTAT3/ARG-1 signaling is known for promoting T cell suppression and tumor immune evasion, thus strongly suggesting that immature PMN-MDSCs are potentially involved in limiting outcomes of ICI therapy in RCC patients, similarly shown before in other genitourinary cancers such as prostate and bladder cancers. Our lab has recently developed a strategy to target

STAT3 selectively in tumor-associated myeloid cells using STAT3 antisense oligonucleotide (STAT3ASO) conjugated to immunostimulatory CpG oligodeoxynucleotides acting as targeting moiety. Our preliminary in vitro studies demonstrate CpG-STAT3ASO treatment can reverse patient-derived CD15+ granulocyte immunosuppression on healthy T cells and restore T cell proliferation. In initial efficacy studies, we assessed the activity of three versions of CpG-STAT3ASO conjugates with various chemical modifications, such as 2'-O'methyl- or locked nucleic acid, in a syngeneic bladder tumor model (MB49). MB49 cancer cells were subcutaneously injected into two flanks of male C57BL/6 mice and treated every second day with 5mg/kg of various CpG-STAT3ASO injected intratumorally into one of the tumor sites. All CpG-STAT3ASOs inhibited tumor cell growth in both treated and distant tumors in comparison to controls. The immunohistochemical analysis indicated an increase in the percentage of CD8+ cells within CpG-STAT3ASO treated tumors in comparison to controls, suggesting activation of CD8 T cell-mediated antitumor immunity. We also tested IV modes of CpG-STAT3ASO treatment and observed efficacy within the same MB49 mouse model. Therefore, with a demonstrated efficacy in bladder cancer, we suggest targeting STAT3 signaling in the RCC-associated myeloid cells using CpG-STAT3ASO may provide a potential novel strategy for augmenting immune checkpoint therapies.

Thus, our aims in the study are to (1) characterize immune alterations in patients with RCC receiving combination ICI and correlate this to responses to therapy. This will allow us to understand the role of STAT3 signaling in patient responses to ICIs. (2) Assess the efficacy of novel CpG-STAT3 ASO administration with or without PD1/CTLA-4 blockade in syngeneic models of RCC in mice. The proposed project will ultimately benefit patients with advanced RCC and raise objective response rates. Finally, the proposed project will provide me with training under a research scientist and a clinical scientist treating patients with RCC that will combine the fields of oncology, molecular biology, chemistry, and immunology to prepare me for a career at the forefront of kidney cancer research.